CHAPTER 13

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# Alport Syndrome, Familial Benign Hematuria, Nail-Patella Syndrome, Type III Collagen Glomerulopathy, and Pierson Syndrome



## ALPORT SYNDROME

In 1927, Alport (1) reported the association of deafness with a form of "hereditary familial congenital hemorrhagic nephritis" occurring in successive generations of one family. He observed that "the male members tend to develop nephritis and deafness and do not as a rule survive," whereas "the females have deafness and hematuria and live to old age." The main features of the disease were established, and an X-linked dominant inheritance, the usual mode of inheritance, was clearly suggested. Subsequently, many additional families have been reported from various parts of the world, and the clinical spectrum and genetic basis of Alport syndrome (AS) have expanded to include other extrarenal defects and variants. The estimated frequency of AS is 1:5000. It accounts for 1% to 2% of end-stage renal disease (ESRD) in Europe, India, and the United States.

The pathogenesis of AS has been elucidated by advances in pathology, biochemistry, genetics, and molecular biology. The primary defect in AS involves type IV collagen, the main component of the basement membranes. AS is genetically heterogeneous, and different mutations in the genes encoding the α3, α4, or α5 chain of type IV collagen are responsible for the various forms of disease. X-linked inheritance is the most common mode of transmission. The phenotypic heterogeneity of the disease can be explained by the molecular heterogeneity: nearly every family has its "private" mutation. Benign familial hematuria may represent a benign variant of type IV

collagen diseases and the heterozygous form of autosomal recessive AS. Animal models of AS now provide opportunities for investigating pathogenesis of the disease and developing new therapeutic approaches.

## Pathologic Findings

### Gross Appearance

No specific macroscopic changes have been reported in the kidneys obtained at autopsy of patients dying from ESRD. The kidneys are shrunken and atrophic with a finely granular cortical surface (2). Atrophy predominates in the cortex, in which yellow linear streaks are sometimes visible (corresponding to lipid-filled foam cells). More severe contraction is observed in the kidneys obtained after several years of dialysis.

### Light Microscopy

#### Glomeruli

Light microscopic changes have been described in numerous reports (2–7).

In renal biopsies obtained early in the course of the disease, the glomeruli appear normal or show minimal changes, hypertrophy of podocytes, rigidity of glomerular capillary walls, irregular enlargement of mesangial stalks, and focal thickening of the capsular basement membrane (Fig. 13.1A and B). Focal and segmental thickening and lamellation of the glomerular capillary walls, better seen on silver stains, become visible with progression of the disease, and they are usually associated with marked widening of the mesangial stalks. Segmental lesions of the tuft, producing a pattern of focal and segmental glomerulosclerosis (FSGS), develop in an increasing number of glomeruli; they are caused by marked thickening of the glomerular basement membrane (GBM) and the mesangial stalks, associated with hyaline deposits and collapse of the capillary loops (Fig. 13.1C and D). The end point is complete sclerosis or hyalinosis of the glomerulus. Correlation between the severity of glomerular changes and the age at renal biopsy has been observed by several groups (4,6).

#### Tubules

The tubules are initially normal, except for the presence of occasional red blood cell casts (Fig. 13.1E). The foci of nonspecific tubular lesions then develop. These lesions actually may precede significant light microscopic glomerular changes but subsequently develop alongside the glomerular lesions.

#### Interstitium

Interstitial fibrosis is usually absent before the age of 10 years. Then, interstitial fibrosis and tubular atrophy develop and progress in correlation with declining glomerular filtration rate (GFR) (3). Focal areas of interstitial fibrosis may be seen before the appearance of overt glomerular changes.

Lipid-laden foam cells have been considered a marker of AS because of their abundance and distribution (Fig. 13.1F). When present, they are concentrated at the corticomedullary junction or they occur as long, linear rows throughout the cortex. However, they are inconstant and are rarely found in early biopsy specimens. They are abundant at the stage of heavy proteinuria, and their number may decrease with progression to renal failure. They are not specific and are observed in patients with abundant proteinuria, regardless of the type of glomerular involvement.

#### Blood Vessels

No significant vascular changes are observed early in the course of the disease. Lesions of arteriolosclerosis may develop in hypertensive patients.

### Immunofluorescence Microscopy

Immunofluorescence microscopy of renal tissue using antibodies to immunoglobulins and complement components is usually negative (6,7). However, faint deposits of IgG, IgM, and the third component of complement (C3) are occasionally observed (3–5,7) and may mimic immune complex–mediated glomerulonephritis in a few patients (8). Scattered granules of C3 distributed haphazardly throughout the glomerular tuft and the arterioles are not uncommon, but their presence is not a marker of any specific disease and their absence does not exclude the diagnosis of AS. With progression of the disease, focal deposits of IgM, C3, or C1q are observed in hyalinized segments of glomeruli.

### Electron Microscopy

Ultrastructural studies demonstrated the basic lesion of AS (5,9–11), subsequently observed by many investigators (3,4,6,7,12,13). This finding was of the utmost importance because it provided a useful morphologic marker for AS and gave the first indication of the primary involvement of the GBM in the disease.

The typical lesion is characterized by thickening of the GBM (800 to 1200 nm), with splitting and fragmenting of the lamina densa into several strands forming a basket-weave pattern. Small, electron-dense granules are usually visible within the lucent zones delineated by the thin interwoven layers of lamina densa (Figs. 13.2 and 13.3). The inner and outer contours are irregularly festooned and are lined by hypertrophied podocytes on the exterior. The lesion is often widespread, at least in adults, involving more than 50% of capillary loops. It seems to become more extensive on serial biopsy specimens (5,14). However, GBM changes may be patchy, alternating with segments of normal or reduced thickness. In fact, in younger patients, a second type of lesion, the thin basement membrane (100 to 200 nm), is the prevalent change (4,6,7,12–14), and focal ruptures of the GBM with repair by newly formed basement membrane material may be seen (4). On the whole, the most striking feature in children is the irregular appearance of the GBM, resulting from the close alternation, even within the same loop, of thick, split, and extremely thin GBM segments (Fig. 13.4). The GBM lesion develops early in life; it has been found in several patients younger than 2 years. It is observed in male and female patients, but as described by Rumpelt (14) in a quantitative ultrastructural analysis, and confirmed by White et al. (13), male patients have more splitting than do female patients, and splitting increases with age in male patients but not in female patients. Similar findings have been reported in the Samoyed dog, an animal model of X-linked AS: The GBM is normal in thickness at birth in both sexes; then, extensive thickening and splitting of the GBM appear in the males, whereas only focal areas of multilaminar splitting occur in females (15).

In some patients, the typical GBM thickening is absent (9,12) and widespread thinning of the GBM is observed, whatever the age of the patient and the type of the mutation (Figs. 13.5 and 13.6) (6,7,12,16–18). In a series of 105 patients

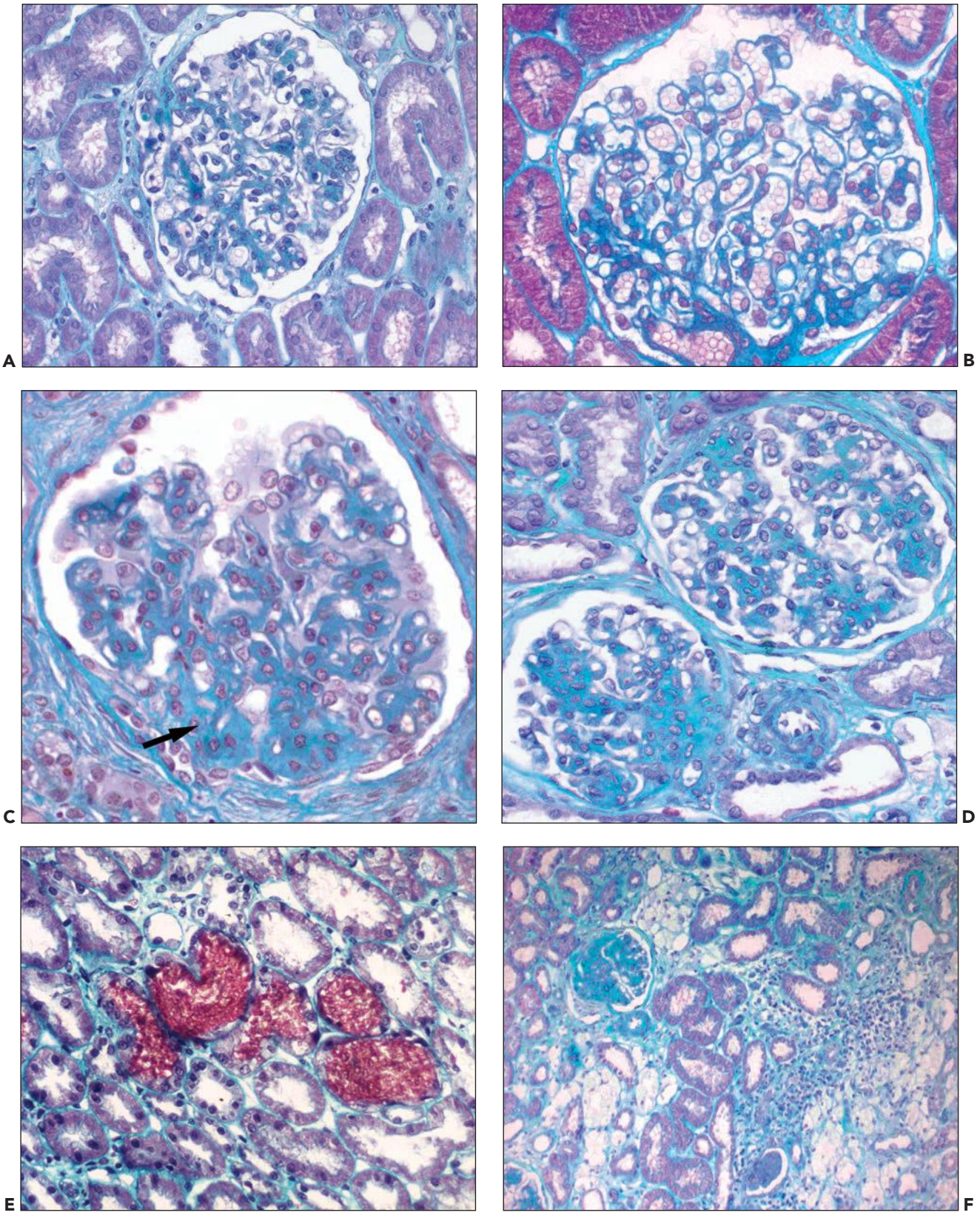



**FIGURE 13.1** **Alport syndrome.** Light microscopy. **A:** Normal glomerulus in a 6-year-old boy. (×250.) **B:** Rigidity and moderate thickening of the GBM in a 12-year-old boy. (×330.) **C:** Irregular thickening of the basement membrane, marked podocyte hypertrophy, and focal segmental glomerulosclerosis (*arrow*) in a 14-year-old boy. (×330.) **D:** Multiple lesions of focal segmental glomerulosclerosis in a 17-year-old boy. (×250.) **E:** Presence of red blood cell casts. (×350.) **F:** Clusters of interstitial foam cells. Same patient as in **(D)**. (×200.). (**A–F**, trichrome light green.)

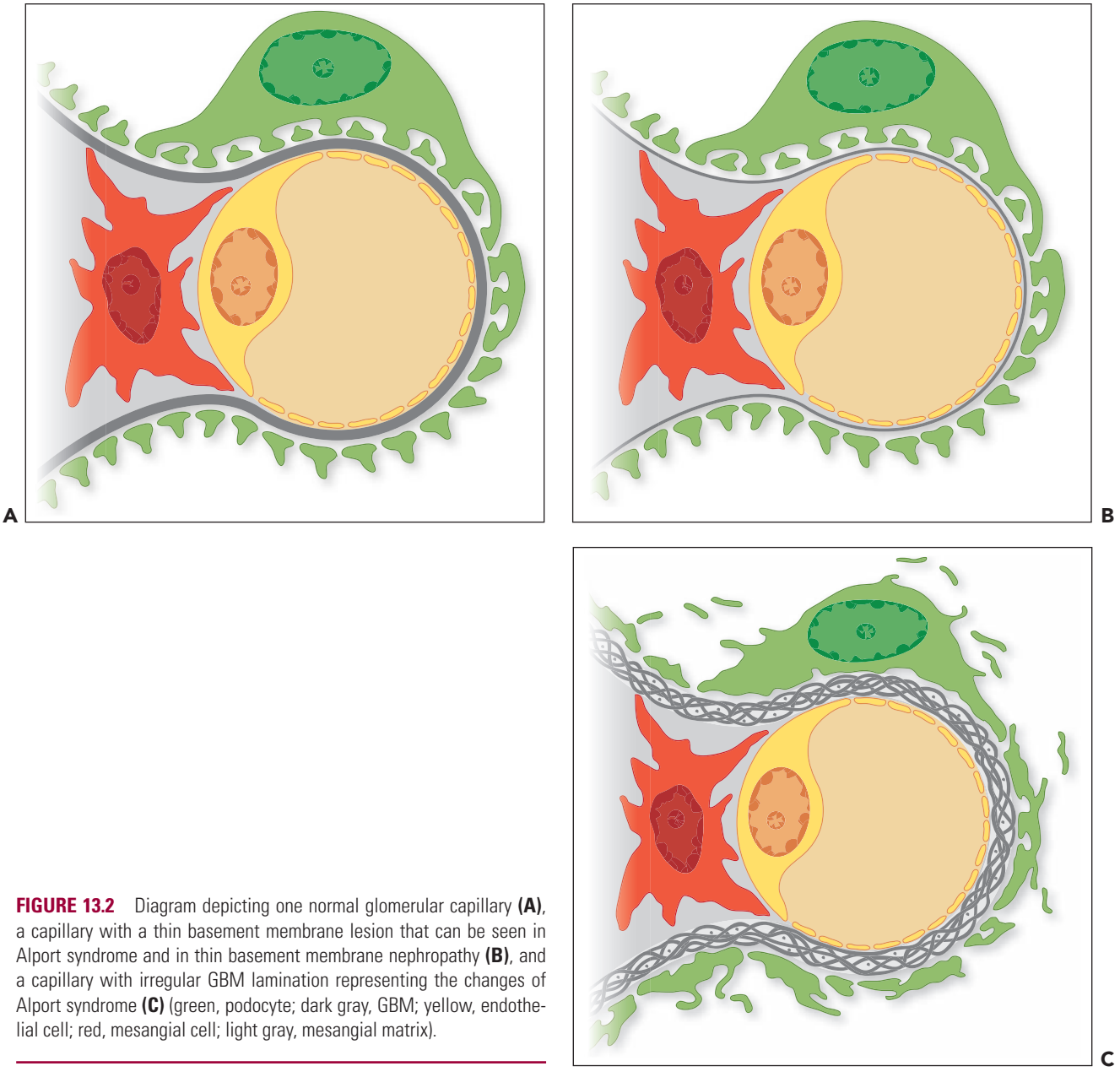



**FIGURE 13.2** Diagram depicting one normal glomerular capillary **(A)**, a capillary with a thin basement membrane lesion that can be seen in Alport syndrome and in thin basement membrane nephropathy **(B)**, and a capillary with irregular GBM lamination representing the changes of Alport syndrome **(C)** (green, podocyte; dark gray, GBM; yellow, endothelial cell; red, mesangial cell; light gray, mesangial matrix).

affected with progressive hereditary nephritis and studied by electron microscopy, we observed diffuse attenuation of the GBM in 30; 19 of these patients had classic AS, and 11 had progressive hereditary nephritis without deafness. Intrafamilial concordance of ultrastructural changes is usually observed, and thickening of the GBM with fragmentation of the lamina densa is both a marker of certain families and an index of severity. However, rare discordances have been reported (12,19).

Other lesions have been described. Frequently, podocytes are enlarged, contain vacuoles, and show focal or diffuse effacement of foot processes. The mesangium is initially normal, followed by progressive increase in mesangial matrix, focal mesangial hypercellularity, and mesangial expansion along the subendothelial aspect of the GBM. The mesangial material appears heterogeneous, containing dense granules of various sizes, clear areas, and curvilinear membranous structures. Finely granular dense deposits can be found focally in severely involved GBM segments (Fig. 13.7). Focal thickening of the Bowman capsule or tubular basement membrane (TBM) is frequently associated with the GBM lesions (Fig. 13.8). These thickenings are characterized by marked bulging and splitting of the basement membranes delineating clear areas containing vesicular structures or dense laminated particles having the appearance of lipid deposits. Accumulation of lipid droplets within capsular epithelial cells is rarely observed, whereas it is frequent in tubular and interstitial cells.

The specificity of the GBM pathologic features has been questioned. According to Hill et al. (20), thickening and splitting of the GBM closely mimicking hereditary nephritis can be seen in poststreptococcal glomerulonephritis, FSGS and IgA nephropathy, and GBM attenuation may be observed in most types of glomerulopathies. Actually, widespread

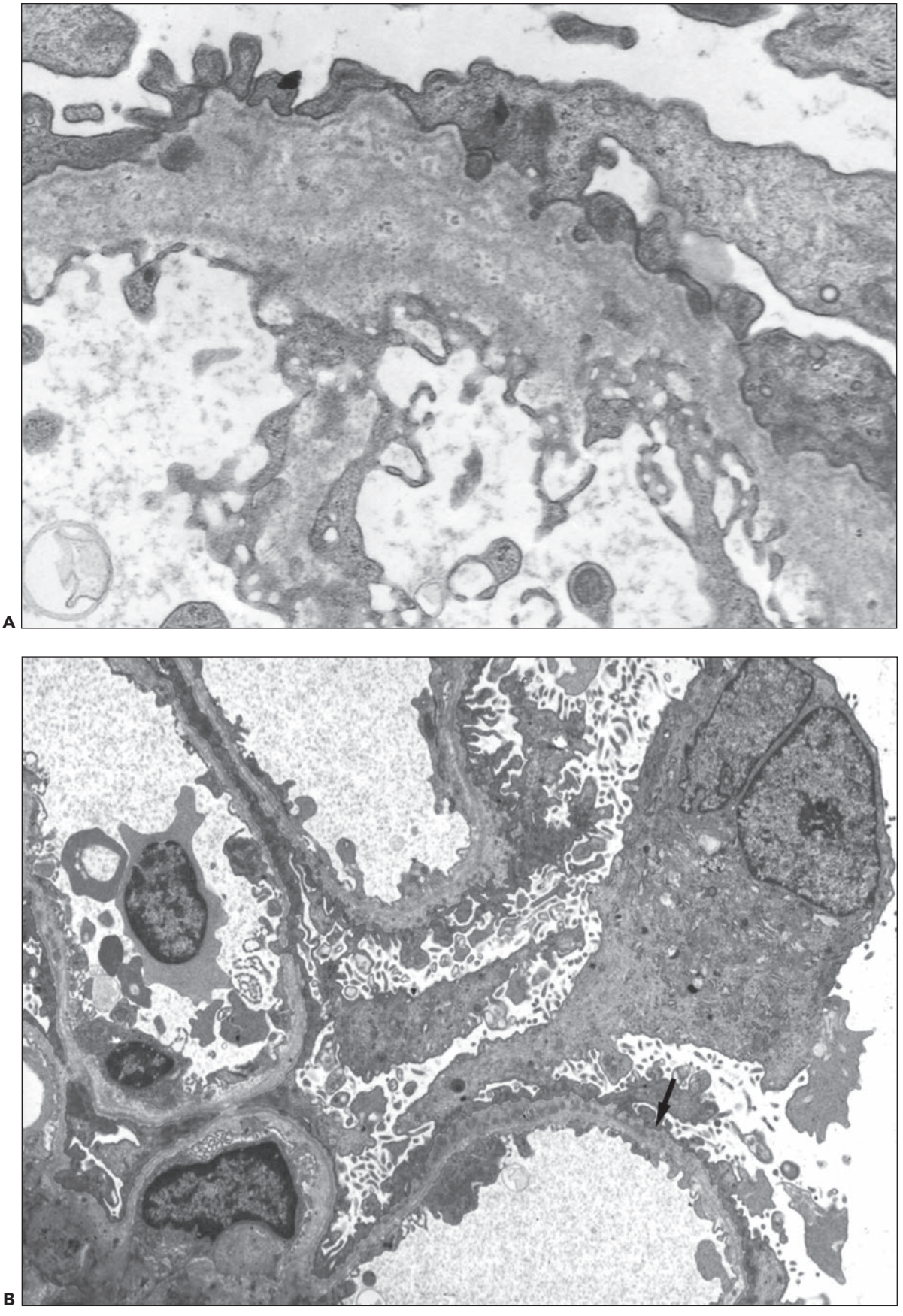


**FIGURE 13.3** **A:** Electron microscopy from a 12-year-old boy with X-linked Alport syndrome. Note thickening and irregular contours of the glomerular basement membrane, splitting of the lamina densa, and small electron-dense granules. (Uranyl acetate-lead citrate, ×22,500.) **B:** Electron microscopy from the same 12-year-old boy showing the irregular distribution of GBM lesions with nearly diffuse foot process effacement and focal presence of granular deposits (*arrow*) distributed along a thick GBM segment. (Uranyl acetate-lead citrate, ×7800.)

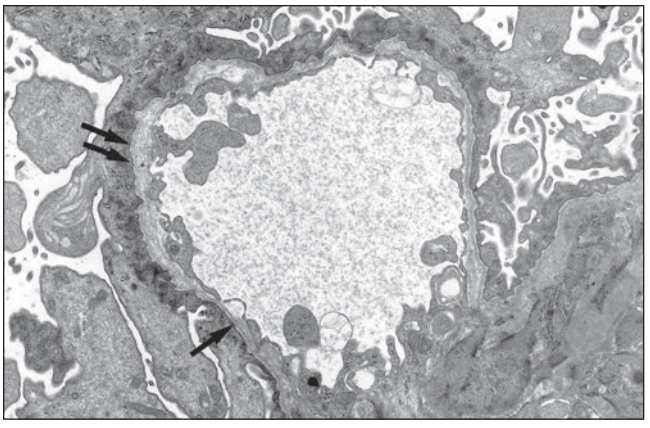


**FIGURE 13.4 Electron microscopy from a 10-year-old boy with X-linked Alport syndrome.** Note the alternation of thick and split (*double arrows*) and of thin (*single arrow*) glomerular basement membrane segments. (Uranyl acetate-lead citrate, ×13,200.)

thickening and splitting of the GBM in the proper clinical setting strongly support a diagnosis of AS, but occasional areas of splitting clearly do not have the same diagnostic specificity. In our experience, the finding of extensive GBM changes in 30 of 50 hematuric children, in whom the diagnosis of IgA nephritis had been ruled out by immunofluorescence, allowed the diagnosis of AS, which was confirmed by further family investigations, follow-up data, or the finding of type IV collagen mutations. Characteristic ultrastructural changes of the GBM have been observed in patients with no family history of renal or hearing impairment (5). These observations are important because they strongly suggest that these patients are affected with de novo AS, a hypothesis easily confirmed by genetic studies. Very rare cases of widespread GBM lesions mimicking AS pathology have been described in young children presenting with isolated sporadic or familial steroid-resistant nephrotic syndrome (21), in a few cases of Denys-Drash or Frasier syndromes linked to *WT1* mutation (22), in the rare Pierson syndrome linked to mutations in the *LAMB2* gene (see section on Pierson syndrome), or in nephrotic syndrome associated with hypomelanosis of Ito (23).

## Extrarenal Tissues

Only a few studies have been devoted to nonrenal tissues. Unique pathologic changes have been demonstrated by postmortem light and electron microscopic examination of the inner ear. These include a zone of separation between the basilar membrane and the basement membrane of cells of the organ of Corti, and the presence of cells filling the tunnel of

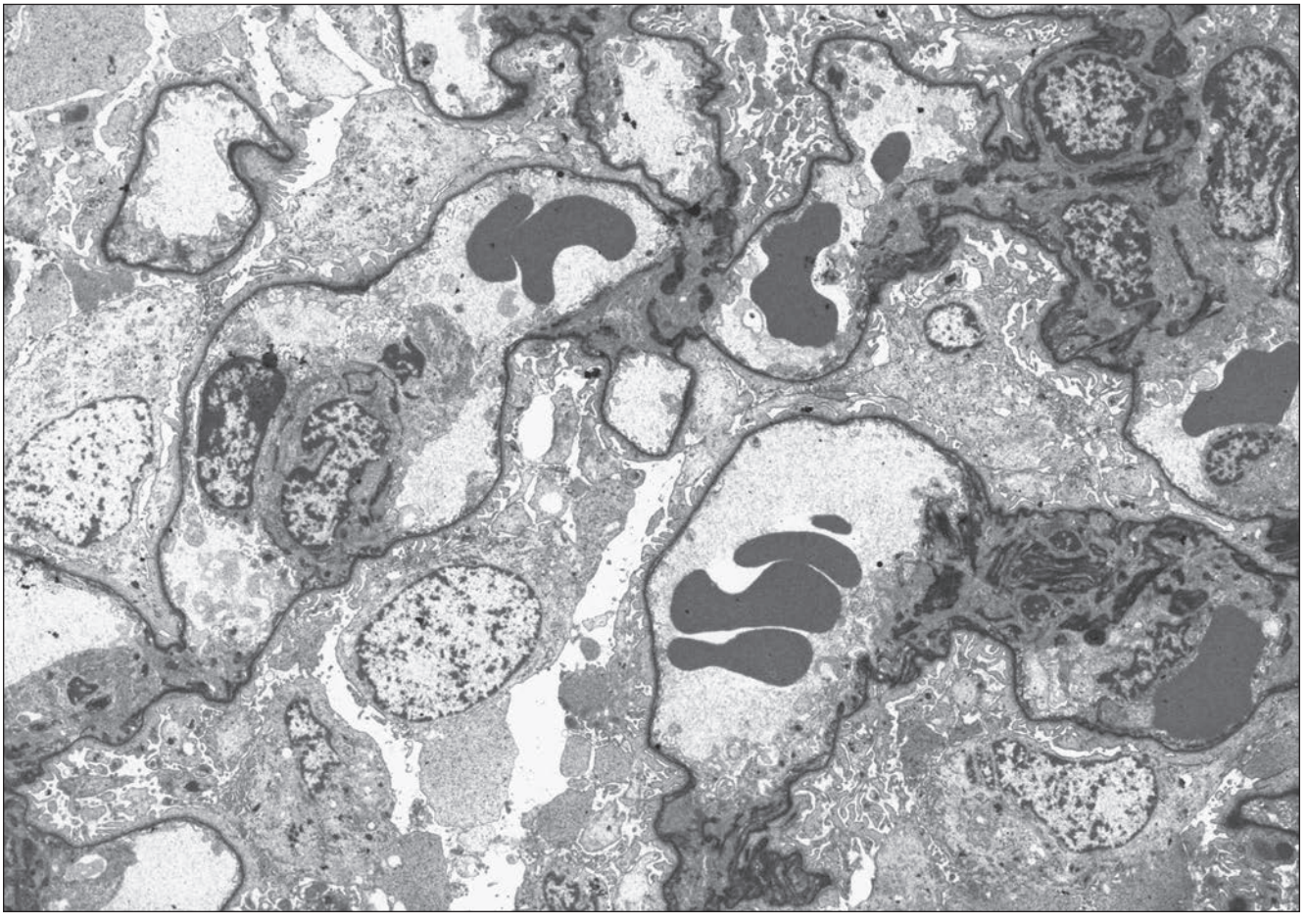


**FIGURE 13.5 Electron microscopy from a 26-year-old male patient with X-linked Alport syndrome who developed ESRD at the age of 37 years.** Note the widespread attenuation of the GBM. (Methenamine silver staining ×2500.)

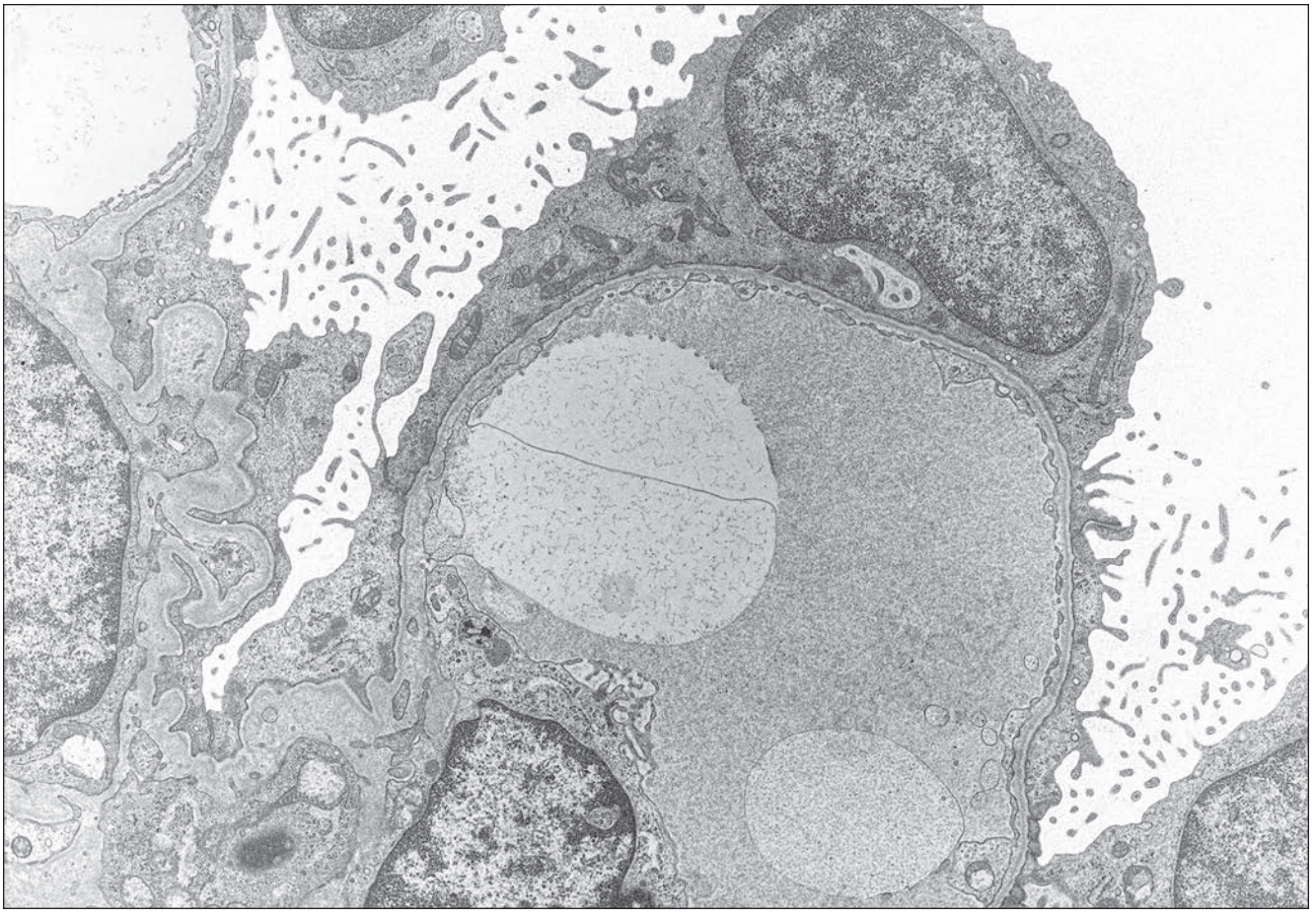


**FIGURE 13.6 Electron microscopy from the same 26-year-old patient.** Extremely thin GBM with regular contours and diffuse effacement of foot processes. (Uranyl acetate-lead citrate, ×8000.)

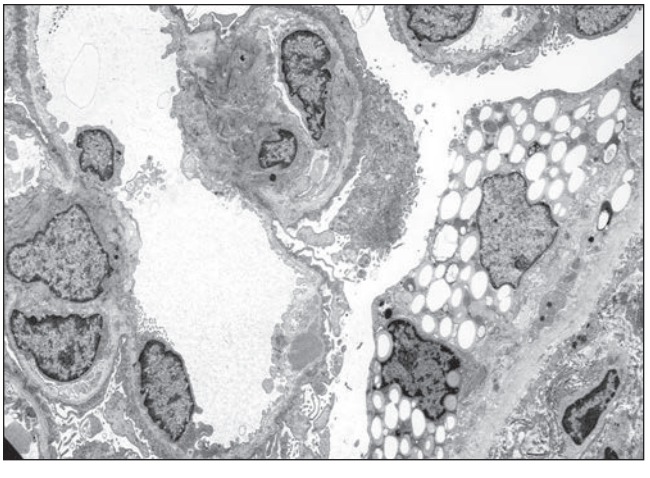


**FIGURE 13.7 Electron microscopy from a 12-year-old boy with Alport syndrome showing the widespread alteration of the GBM with focal subendothelial and subepithelial deposits.** There are diffuse effacement of foot processes, Bowman capsular thickening, and a foamy appearance of the capsular epithelial cell. (Uranyl acetate-lead citrate, ×4500.)

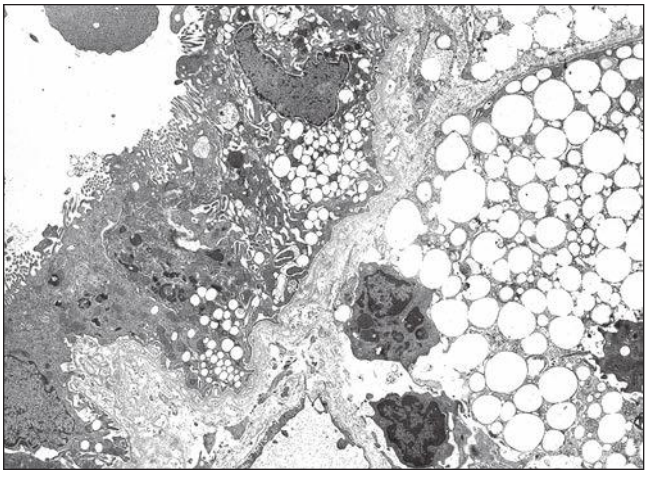


**FIGURE 13.8 Electron microscopy from a 15-year-old boy with Alport syndrome showing marked TBM thickening and splitting.** Lipid droplets are present in tubular epithelial cells. They massively involve the interstitial cell cytoplasm. (Uranyl acetate-lead citrate, ×4500.)

Corti, morphologically similar to supporting cells (24). In the eye, the anterior capsule of the lens is thinned, and vertically oriented fractures have been seen (25,26). The retina may have thinning of the internal limiting membrane/nerve fiber layers and of the retinal pigment epithelial basement membrane of the Bruch membrane (27). Contrary to initial observations, electron microscopic examination of the epidermal basement membrane (EBM) failed to disclose any significant ultrastructural abnormalities (28,29, personal observations).

## Type IV Collagen

Type IV collagen is the major structural component of all basement membranes. Like other members of the collagen family, type IV collagen is a multimeric protein composed of three α chains coiled around one another to form a triple-helical molecule or protomer (30). Each chain comprises a long 350-nm collagenous domain and, in contrast to interstitial collagens, a noncollagenous (NC) globular domain at the 3′ end of the molecule and a short NC domain at the 5′ end. The collagenous domain, which contains about 1400 amino acid residues, is characterized by the repeated Gly-X-Y triplet sequence, in which every third amino acid is a glycine. The presence of glycine in every three residues is crucial for proper triple-helix formation, because glycine is the only amino acid small enough to fit into the center of the triple helix. Twenty-one to twenty-six short interruptions of the Gly-X-Y sequence are presumed to give flexibility to the molecule. The C-terminal NC1 domain, of about 230 amino acid residues, is a globular structure consisting of two symmetrical subdomains, each containing six cysteine residues, the positions of which are highly conserved between hemi-NC subdomains and between chains. These cysteines are the basis for intrachain and interchain disulfide bonds. Cysteine residues are also present in the short NC sequence located at the N-terminal part of the chains.

Six genetically distinct but closely related α(IV) chains have been identified (Fig. 13.9A). Each contains about 50 exons. Because of the large size of their introns, they are large genes, with a size between 100 and more than 425 kilobases (kb) (31). They are located pairwise in a head-to-head fashion on three different chromosomes. The structural similarities between the different chains suggest that the genes diverged from a common ancestor that was initially duplicated on the same chromosome and then eventually triplicated. *COL4A1* and *COL4A2* were the first to be localized (13q34). They are separated by 130 base pairs (bp) and share a bidirectional promoter (32). The genes *COL4A3* and *COL4A4* were assigned to 2q35-37, and their genetic structure was determined (33–35). The genes encoding the α5 and α6 chains of type IV collagen were localized to chromosome Xq22 and characterized. They are separated by a short 450-bp intergenic region (36–39).

Many type IV molecules could be potentially formed. However, only three protomers have been discovered: α1.α1.α2(IV), α3.α4.α5(IV), and α5.α5.α6(IV) (Fig. 13.9B) (31). They associate to form interwoven networks providing a scaffold for other basement membrane glycoproteins: two protomers bind via their NC1 domains to form dimers; four molecules associate via disulfide cross-links in the 7S domain to form tetramers (Fig. 13.9C). Three distinct networks have been recognized: α1α1α2(IV)–α1.α1.α2(IV), α3.α4.α5(IV)–α3.α4.α5(IV), and α1.α1.α2(IV)–α5.α5.α6(IV). Lateral side-by-side interactions, especially numerous between α3.α4.α5(IV) protomers, make the network tighter and more resistant to proteolysis (40).

The three networks are not equally distributed within the basement membrane. The most abundant, α1.α1.α2(IV) is ubiquitous and found in all basement membranes. However, in the glomerular tuft, the strong mesangial expression of the α1(IV) and α2(IV) chains contrasts with their faint GBM distribution, limited to the subendothelial aspect of the basement membrane (Fig. 13.10). The other networks have restricted and specific distributions. In the mature kidney, the α3.α4.α5(IV) network is present within the GBM, the distal tubule basement membrane, and focally in the Bowman capsule basement membrane (7,31,41,42). However, in rat as in human GBM development, there is a switch from the exclusive α1.α1.α2(IV) network detected at the early capillary loop stage to the α3.α4.α5(IV) network present in the mature glomerulus where it is synthesized by the podocyte (43). The α3.α4.α5(IV) network is also present in the alveolar basement membrane and the specialized basement membrane of the eye and the cochlea (44–48). The α1.α1.α2(IV)–α5.α5.α6(IV) network is expressed in the Bowman capsule and collecting duct basement membranes, in the epidermal and smooth muscle cell basement membranes of the urinary bladder, uterus, esophagus, and fundus of the stomach, and in the vascular smooth muscle cell basement membrane of the aorta (49). The α6 chain is consistently absent from the GBM.

AS is genetically heterogeneous (50). Mutations in *COL4A5* result in X-linked dominant AS, whereas mutations in *COL4A3* or *COL4A4* lead to autosomal recessive or dominant AS. In most patients, the resulting chain defect (absence or abnormal structure) impairs the protomer assembly and the formation of the normal collagen IV network, a consequence readily observed by immunohistologic staining of the basement membranes using specific antibodies against the different α(IV) chains. Importantly, *COL4A3* or *COL4A4* mutations in the heterozygous state are also associated with benign familial hematuria.

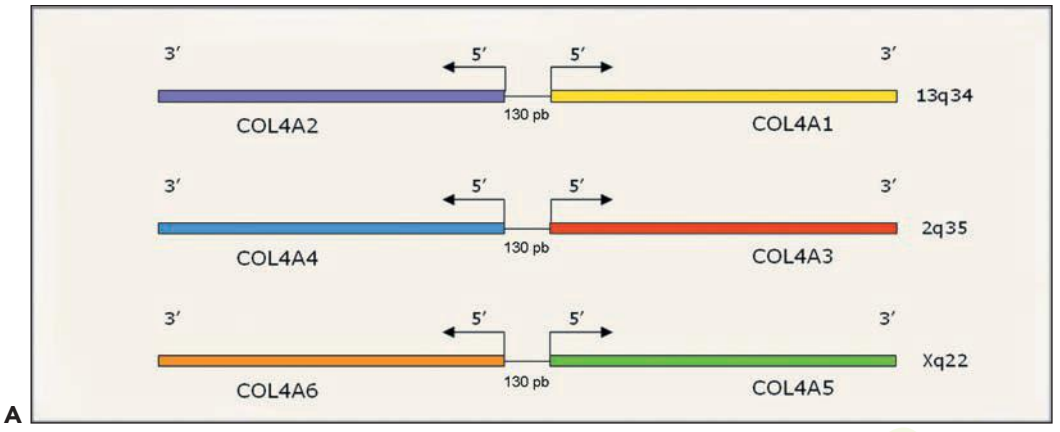
## X-Linked Dominant Alport Syndrome

X-linked dominant transmission is the mode of inheritance in 80% to 85% of AS families.

### Clinical Features

Classic X-linked AS is characterized by the familial occurrence of hematuric nephritis progressing to ESRD, at least in male patients, and of hearing loss, which is also progressive. Hematuria is the cardinal symptom. Macroscopic or microscopic, it is usually detected in childhood, in infancy, or even at birth (2,4,11,51). In a series of 58 patients observed in our Department of Pediatric Nephrology, the age at discovery of hematuria was less than 6 years in 74% (4). However, in some patients who do not manifest macroscopic hematuria, microscopic hematuria may be detected only in adulthood, either isolated or associated with proteinuria, hypertension, or chronic renal failure.

Evolution and prognosis differ according to sex. In male patients, microscopic hematuria is universal and usually persistent. Its presence is a necessary criterion for diagnosis. Single or recurrent episodes of macroscopic hematuria precipitated by exercise or upper respiratory tract infections are observed in about 60% of the patients younger than 15 years old, but these episodes are exceptional in adults. Proteinuria may be absent, mild, or intermittent in young patients. It increases

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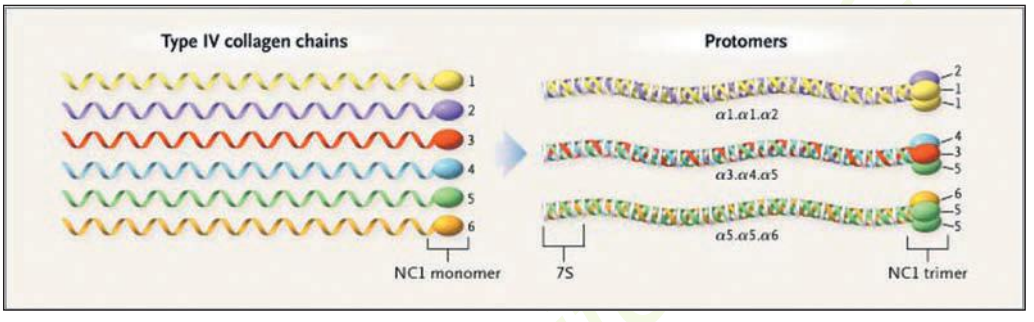


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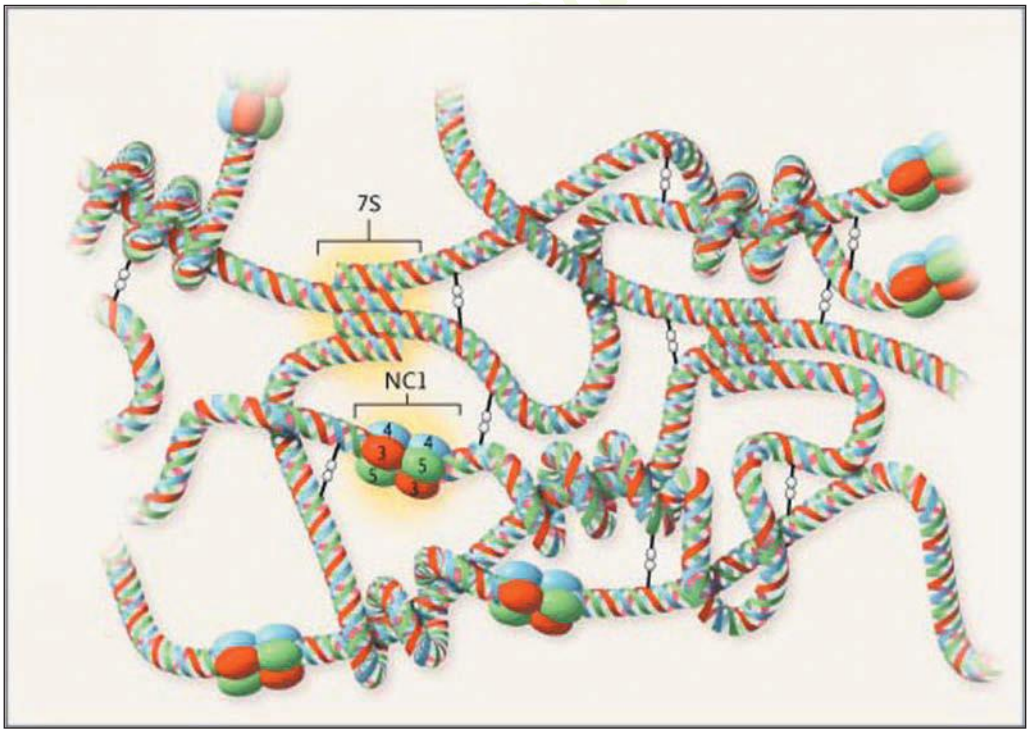



C

**FIGURE 13.9** **A:** The genes *COL4A1* to *COL4A6* are located in pairs, head to head, on three different chromosomes. **B:** They encode six different type IV collagen α chains. Each of them comprises a long collagenous domain, a noncollagenous globular domain at the C terminus, and a 7S domain at the N terminus. They are arranged into three triple-helical protomers that differ in their chain composition. **C:** Protomers create basement membrane networks with other protomers by uniting two NC1 domains at the C terminus and by uniting four 7S domains at the N terminus. Here is shown the α3.α4.α5(IV)–α3.α4.α5(IV) network, with disulfide cross-links between triple-helical domains. (Modified from Hudson BG, Tryggvason K, Sundaramoorthy M, et al. Alport's syndrome, Goodpasture's syndrome, and type IV collagen. *N Engl J Med* 2003;348:2543.)

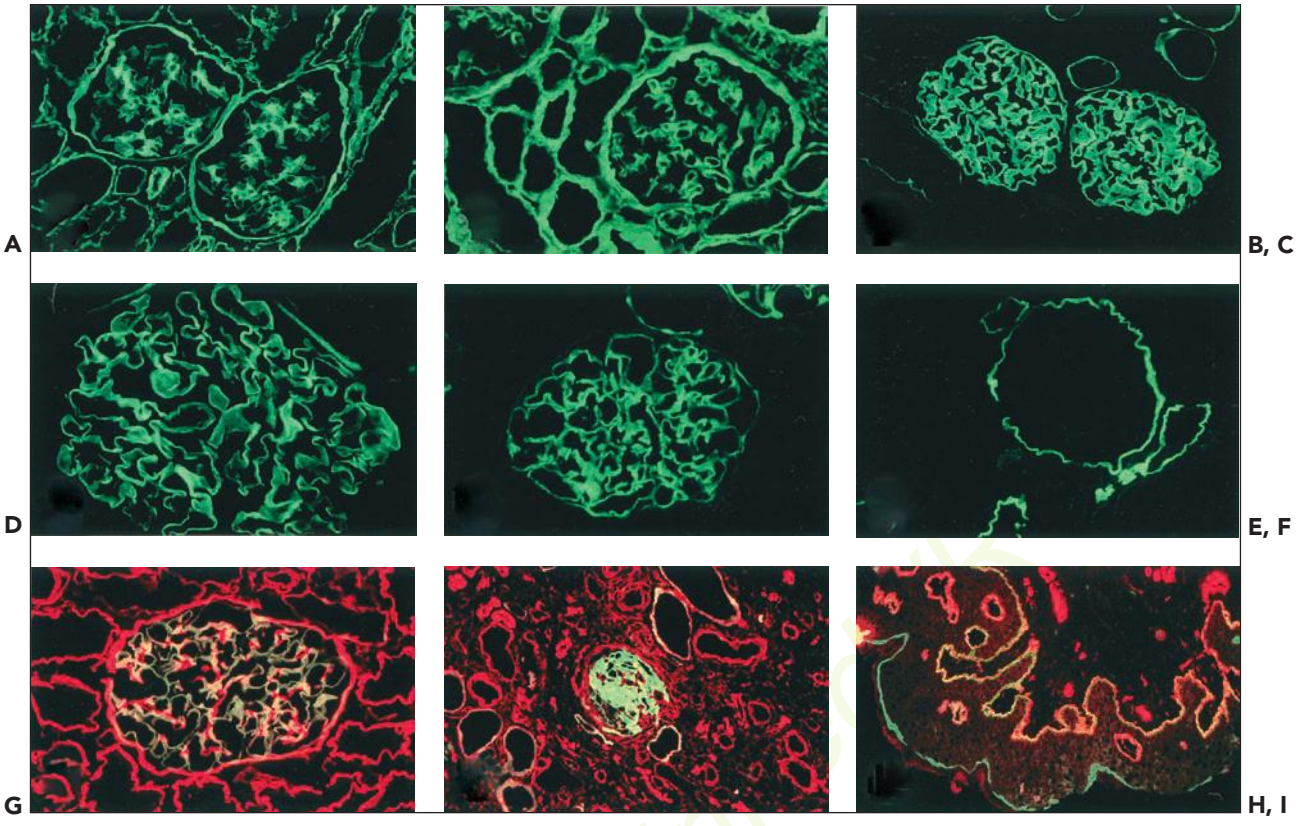



**FIGURE 13.10** **Immunofluorescence. A–F:** Normal renal distribution of the α1–α6 type IV collagen chains. **A and B:** The α1 and α2 chains are codistributed in all basement membranes, but their expression is faint in the GBM. **C–E:** The α3, α4, and α5 chains are codistributed in the GBM and the basement membranes of distal tubules; they are absent in the mesangial matrix. **F:** The α6(IV) chain is absent in the GBM and strongly expressed in the Bowman capsule and the distal tubule basement membranes. **G–I**: Double immunolabeling with anti-α2 (*Texas red*) and anti-α5(IV) (fluorescein) antibodies showing the respective localization of the two chains in a normal kidney specimen **(G)** and the strong persistence of the α5(IV) chain in the sclerotic glomerulus from a non-AS patient **(H)**. In the skin **(I)**, both chains are present in the epidermal basement membrane (*yellow fluorescence*).

steadily with age, with possible development of the nephrotic syndrome (4). Proteinuria was observed in 95% of the patients belonging to the European Community cohort of 195 AS families (51). All male patients progress to ESRD at ages ranging from 8 to older than 60 years. Hypertension is usually absent before the stage of chronic renal insufficiency. According to the rate of progression to ESRD, two types of the disease have been distinguished: (a) a "juvenile" type characterized by a highly stereotypical course within a given family and by the occurrence of ESRD in men around the age of 20 years and (b) a "nonprogressive" or "adult" type in which the age at ESRD is higher, approximately 40 years, and the course much more variable. In the latter group, precise prognostication in the individual case is impossible.

Hematuria is observed in nearly all female patients, but it may be intermittent or detected only in adulthood. It was present in 95.5% of patients (309 of 323) in the European series (52). Completely asymptomatic carriers are rare. Proteinuria may be absent; it is usually mild or intermittent (4,5,52) and was observed in 75% of patients in the European Study (52). It increases with age in some patients at risk of progressing to ESRD. The prognosis of renal involvement is impossible to predict from family history, as shown by the clinical course of affected female members of the family initially reported by Alport: three of them developed ESRD between 12 and 24 years of age, whereas three others had a normal life span of 69 to 90 years (53). Gross hematuria in childhood, progressive increase in proteinuria, nephrotic syndrome, diffuse GBM thickening, and hearing loss are associated with an adverse outcome (54). Random X inactivation (the process known as lyonization) may account for the variable clinical course of the disease in female patients. On the whole, according to Jais et al. (52), the risk of developing ESRD before the age of 40 years was 12% in girls and women versus 90% in boys and men, but the risk of progression in women appears to increase after the age of 60 years.

Bilateral sensorineural hearing loss affecting high and middle frequencies is not congenital, but it may be detected over the first decade, especially in boys (4). In children, serial audiologic tests show progressive hearing loss in most boys and some girls, whereas hearing impairment is generally stable in adults. The true prevalence of hearing loss within AS-affected families is difficult to assess in the absence of systematic audiologic evaluation. Some kindreds with progressive hereditary nephritis without hearing loss are AS variants. They represent nearly 20% of families with *COL4A5* mutations in the study of Jais et al. (51).

Several types of ocular changes involving the lens, retina, or cornea have been reported. Anterior lenticonus is a conical protrusion of the anterior aspect of the lens that develops progressively over the years in male patients (55) and is exceptional in female patients (4,53,56). Its presence is predictive of early renal failure (56). Retinal changes are characterized by the progressive appearance of asymptomatic perimacular yellowish flecks (57,58). Both types of lesions are specific and are observed in about one third of these patients. Nonspecific lesions of the cornea have been reported to occur frequently in patients with AS: posterior polymorphic dystrophy was detected in 11 of 17 patients from Thailand (59), and recurrent corneal erosions were observed in about 20% of patients with AS and renal failure (60).

Other different anomalies have been reported in patients with X-linked AS. First described by Garcia-Torres and Orozco (61), diffuse esophageal leiomyomatosis, also involving the tracheobronchial tree and the female genital tract, has now been reported in 69 patients from 33 families, all affected with the severe, juvenile type of AS. The main symptoms, dysphagia, postprandial vomiting, and recurrent episodes of bronchitis, usually appear before 10 years of age. Congenital cataracts occur in one third of these patients. Unlike AS, diffuse leiomyomatosis is fully penetrant and is completely expressed in female patients (62). Peculiar deletions of the *COL4A5-COL4A6* genes have been demonstrated in these patients (63). Aortic abnormalities including dissections and aneurysms occurring between 13 and 36 years of age have been reported in eight male patients. All of them had a severe renal disease requiring renal replacement therapy between 10 and 22 years (64). The presence of the α1.α1.α2(IV)–α5.α5.α6(IV) network in the smooth muscle cell basement membrane of the digestive tract and the aorta can explain these manifestations. On the other hand, the rare association of AS (A), mental retardation (M), midface hypoplasia (M), and erythrocyte elliptocytosis (E) has been recognized as another X-linked contiguous gene deletion syndrome (AMME) (65).

## Immunohistologic Study of Renal, Epidermal, and Other Extrarenal Basement Membranes

As early as 1972, Spear and Slusser (10) speculated that the primary defect in AS could involve basement membrane collagen. This hypothesis was subsequently confirmed by a series of immunohistochemical, biochemical, and genetic studies. McCoy et al. and Olson et al. observed that glomeruli from patients with AS failed to bind human anti-GBM antibodies from patients with Goodpasture (GP) syndrome, a finding suggesting that an antigen normally present in the GBM was absent in these patients (66,67). This observation was confirmed by different studies using the same type of sera or monoclonal or polyclonal antibodies directed against the GP antigen later identified as the NC1 domain of α3(IV) (31). Overall, most male patients showed absent or reduced binding of the antibodies to the GBM, but this phenomenon was not universal, a finding confirming the heterogeneity of AS. Intrafamilial concordance of immunolabeling in male patients was consistently observed (66), and lack of GBM labeling was correlated with the severity of renal disease and GBM splitting (68). Variable results have been found in female patients, with absent, reduced, or even normal immunolabeling in female patients whose affected male relatives lacked the GP antigen (68).

Parallel to the studies on the GP antigen, the Minneapolis group showed that the serum (FNS1) of an AS patient who had developed anti-GBM antibodies after renal transplantation recognized an antigen more widely distributed than the GP antigen and notably present in the epithelial basement membrane (EBM) (28). This antigen was shown to be absent in the GBM and EBM of most AS patients and to have a mosaic distribution in related heterozygous female patients, suggesting that it belongs to a new type IV collagen chain, the possible substrate of AS. This hypothesis was confirmed by the demonstration that FNS1 recognizes the NC1 domain of the α5 chain of type IV collagen.

With the modern availability of specific monoclonal or polyclonal antibodies, immunohistologic analyses of the basement membrane distribution of the different chains of type IV collagen have been performed in numerous AS patients (7,12,29,40,42,48,51). Within the kidney, abnormal distribution of the α5(IV) chain, the product of the mutated *COL4A5* gene, is observed in approximately 75% of the patients: the antigen is absent in the GBM and the distal TBM in male patients and has a reduced or discontinuous distribution in related female patients (Fig. 13.11). This mosaic pattern of GBM distribution of the α5(IV) chain in female patients is consistent with the X-linked transmission of the disease. Random inactivation of one of the two X chromosomes in heterozygotes may explain different degrees of severity of the disease, in correlation with normal, absent, or discontinuous expression of the antigen along the GBM. Interestingly, the α5(IV) defect is associated with the co-absence of the α3 and α4(IV) chains that normally participate in the formation of the α3.α4.α5(IV)–α3.α4.α5(IV) network. Similarly, the α6(IV) chain, normally part of the α5.α5.α6(IV) network, is not detected in the Bowman capsule and collecting duct basement membranes. Exceptions to this typical pattern have been reported in a few patients, characterized by the absence of detectable α5(IV) contrasting with the persistence of discrete α3(IV) and α4(IV) GBM expression (12,29). In about one third of X-linked AS patients, no significant changes in the renal distribution of the α(IV) chains are detected by conventional immunohistologic methods. Results of immunolabeling are concordant within families, and correlation of abnormal labeling with the severity of the clinical and pathologic phenotype is usually observed.

In contrast to the glomerular loss of the α3.α4.α5(IV) network, the α1(IV) and α2(IV) chains, normally confined to the mesangium and the subendothelial aspect of the GBM, are present throughout the entire width of the GBM where they are codistributed with collagen types V and VI and laminin α2 (see Fig. 13.11A) (29,48,69).

In most X-linked AS, the EBM distribution of α5(IV) (and α6[IV] when performed) is abnormal, being absent in male and segmental (or discontinuous) in female patients (see Fig. 13.11H and I) (7,29,42,70,71). GBM and EBM changes are always concordant in male patients, a finding confirming the high diagnostic value of immunohistologic examination of the skin, a technique that had become the initial diagnostic approach in several laboratories. However, approximately 25% of male patients have normal α5(IV) staining of the skin (and the kidney) when examined by standard fluorescence microscopy, and normal results in female patients do not allow any definitive diagnostic conclusion because of the segmental distribution of the antigen.

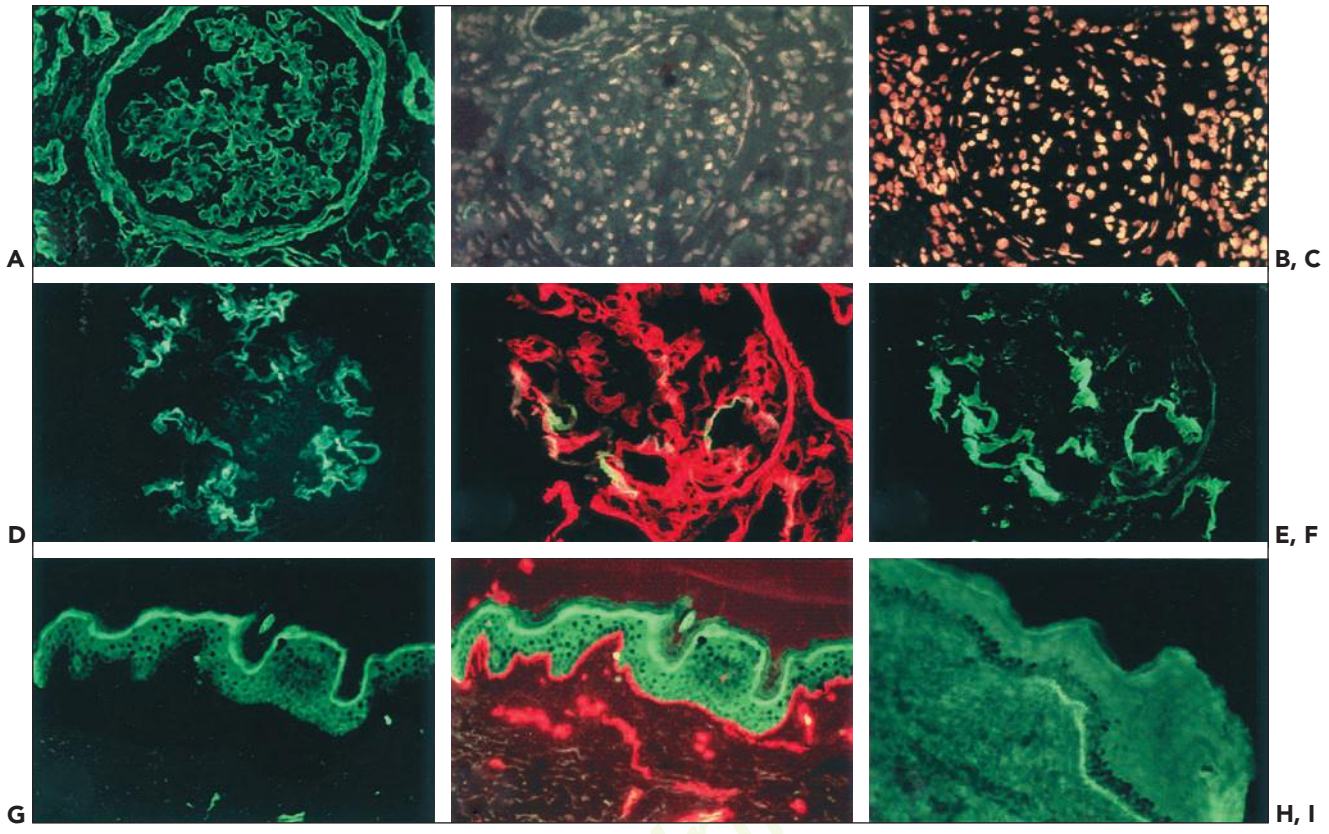



**FIGURE 13.11 X-linked Alport syndrome.** Immunofluorescence distribution of type IV collagen chains in the renal **(A–F)** and epidermal (**G–I**) basement membranes of patients affected with X-linked Alport syndrome. In a male patient **(A–C)**, no α3 or α5(IV) labeling **(B, C)**, contrasting with the strong linear α1(IV) labeling of the GBM **(A)**. In a female patient **(D–F)**, discontinuous α5(IV) GBM labeling **(D, F)**, with strong α2(IV) labeling of α5(IV)-negative GBM segments **(E)**. No EBM α5(IV) staining in a male patient **(G)**, with preservation of α2(IV) labeling **(H)**. Segmental α5(IV) labeling in a female patient **(I)**. (α1, α3, α5: FITC; α2: *Texas red*.)

The EBM expression of α5(IV), used as a marker of X inactivation, has been found to be correlated with the severity of the renal disease in female patients and proposed as a marker for predicting patient outcome (72). However, this finding was not confirmed by the study of Massella et al. (73), in which no correlation was observed between the severity of the glomerular involvement in female patients and the extent of EBM α5(IV) staining. Accordingly, X inactivation can vary widely between different tissues from the same individual (74).

Few studies have focused on the extrarenal basement membrane other than the EBM, because the involved tissues in the eye, ear, and esophagus are not readily accessible. Lens capsules from three patients with AS with anterior lenticonus were examined. The distribution of the α3(IV) to α5(IV) chains was normal in one patient, whereas all three chains (as well as α6[IV] in one case) were absent in the others (26,44). The α3(IV) and α5(IV) chains were also found to be absent in the basement membrane overlying the basilar membrane in two X-linked AS patients (24). The α5(IV) and α6(IV) chains, which are normally expressed in the esophageal smooth muscle cell basement membrane, are absent in esophageal tumors from patients with AS and diffuse esophageal leiomyomatosis (75). Curiously enough, a *COL4A6* transcript was strongly expressed by the tumor cells. An additional striking feature was the absence of the β1 chain of laminin in tumor BM and the lack or uneven expression of the α5 integrin subunit by tumor cells (75).

Abnormal distribution of the α(IV) chains has also been observed in the kidney of dog and mouse models of X-linked AS (15,76,77). Despite the absence of clinical extrarenal involvement in the Samoyed dog, the same anomaly is present in the basement membrane of the lens, retina, and inner ear (46).

## Genetics

After decades of discussion, reexamination of large pedigrees led to the conclusion that AS was genetically heterogeneous, with high frequency of X-linked dominant transmission (50). Linkage studies using polymorphic markers located on chromosome X allowed the localization of the gene at Xq22 (78). As soon as it was identified by two groups in Finland (36,37), the gene *COL4A5* became the candidate gene for X-linked AS and the first *COL4A5* mutations were identified in three Alport kindreds (79). Today more than 500 different mutations have been reported (31,51,80,81) and collected in a database (82). They range from small or large rearrangements or deletions to small point mutations, dispersed all over the gene. Most of these rearrangements are unique. According to different groups, de novo mutations occurred in 11% to

15% of patients. No mutations have been detected in only the *COL4A6* gene (i.e., sparing the *COL4A5* gene) located in tandem on the same chromosome, which is not surprising since the chain α6(IV) is not expressed in the GBM.

Gross *COL4A5* rearrangements (mainly deletions) have been identified in 5% to 15% of the families. The size and location of the deletions vary from case to case, involving from one exon to the entire length of the gene. They are intragenic or extend to nearby regions of the chromosome, possibly involving the adjacent *COL4A6* gene. In some intragenic deletions, an open-reading frame is conserved, allowing the synthesis of a shortened *COL4A5* protein, whereas frameshift deletions lead to more severe truncation of the protein. Insertions, duplications, or complex rearrangements of the gene have also been described.

Many small mutations in the *COL4A5* gene have been reported. They are distributed throughout the gene. Single base mutations leading to amino acid substitution represent about 40% of the small mutations. Most of them are missense mutations in glycine codons in the collagenous domain of *COL4A5*. They are considered pathogenic because they disrupt the Gly-X-Y repeats, altering the folding of the collagen triple helix and leading to posttranscriptional modifications of the chains. Other missense mutations affect conserved amino acid residues, especially cysteine, in the NC domain of the α5 chain. Nonsense mutations, small deletions or insertions, and splice-site mutations leading to frameshift and premature stop codons result in truncated proteins that lack the carboxy terminal NC domain of the chain.

No "hot spot" of mutation has been found. However, a few mutations have been detected in more than one family. For example, one point mutation has been identified in nine independently ascertained AS kindreds in the United States. However, genealogic studies and haplotype analysis demonstrated that affected patients are descendants of a common Mormon ancestor (83). Similarly, the high frequency of AS in French Polynesia is caused by a founder mutation, a tandem duplication of 35 *COLA5* exons (17).

The single-strand conformation polymorphism (SSCP) method used for screening the 51 exons of the *COL4A5* gene allowed the identification of mutations in 45% to 65% of families (17,80). The mutation detection rate reaches 80% to 90% when more sensitive methods are used: direct DNA sequencing or reverse transcriptase-polymerase chain reaction (RT-PCR) and direct sequencing using mRNA from leukocytes, hair roots, or cultured skin fibroblasts (84–87). New-generation sequencing protocols will allow rapid and less expensive identification of mutations (88).

## Phenotype-Genotype Correlations

Two large European and the U.S. cohorts assembling 195 and 175 families, respectively, have been studied to establish phenotype-genotype correlations in male patients (401 and 681 in respective cohorts) affected with X-linked AS (51,81). Both studies demonstrated the uniform severity of large rearrangements of the *COL4A5* gene, nonsense mutations, and deletion or insertions that change the reading frame of the gene, resulting in the synthesis of a truncated α5(IV) chain without the NC1 domain. These mutations confer on affected male patients a probability of developing ESRD before the age of 30 years of 90%, with a 50% renal survival rate at 20 years (51), and an average age at onset of ESRD of 25 years (81). In contrast, phenotypic heterogeneity is seen in the group of patients with missense mutations. In them, the probability of developing ESRD before the age of 30 is 50%, with a 50% survival rate at 32 years, and a mean age at ESRD of 37 years. In other words, both juvenile-type and adult-type AS may be observed in patients with missense mutations, and intrafamilial variability in the rate of progression of the disease is possible. In patients with splice-site mutation, the severity of the renal disease is intermediate with a risk of developing ESRD before the age of 30 of 70% and an average age at ESRD of 28 years. A meta-analysis of phenotype-genotype correlations based on 44 publications suggested a more severe renal phenotype in patients with 3′compared with 5′glycine missense mutations and in patients with splice donor compared with splice acceptor mutations (80). This correlation was not observed in the US cohort, in which mutations positioned at the 5′ end of the gene were found to be associated with earlier age at ESRD onset (81).

Similarly, missense mutations are associated with a lower and later incidence of hearing impairment (51,81). They confer a 50% risk of hearing defect at age 20, whereas all other types of mutations lead to the same risk at age 10. Absence of deafness is observed in some kindreds with *COL4A5* missense mutations; this finding shows that "hereditary nephritis without deafness" is a variant of AS. Interestingly, Samoyed dogs with a nonsense mutation in *COL4A5* leading to the absence of the α5(IV) chain have no detectable hearing defect. In these studies, no significant correlation was observed between the finding of maculopathy and the type of mutation. Conversely, the frequency of lenticonus is significantly higher in patients with large deletions or small mutations, resulting in premature stop codons (51).

In girls and women, no significant correlation was observed between genotype and phenotype, and no correlation was established between the phenotype in girls and women and the severity of the renal disease in male relatives or offspring (52). Early prognosis of the disease in AS carriers is not possible.

An interesting observation has been made in patients suffering from AS associated with diffuse esophageal leiomyomatosis. They all have large deletions encompassing the 5′ end of both *COL4A5* and *COL4A6* genes and the regulatory intergenic region; the deletions, in *COL4A6*, are limited to exons 1, 1′, and 2 (63). Interestingly, AS patients with larger deletions in *COL4A6* do not develop tumors (63).

## Autosomal Recessive Alport Syndrome

In 1985, the genealogic analysis of 41 kindreds by Feingold et al. (50) suggested that AS was transmitted as an autosomal recessive trait in about 10% of cases, a frequency confirmed by further studies. Homozygous or compound heterozygous mutations in *COL4A3* or *COL4A4* have now been reported in several kindreds (34,35,89–91).

Clinical features are identical to those observed in X-linked AS. Autosomal recessive inheritance is suggested by the severity of the disease in young female patients, consanguinity in the family, or the presence of microscopic hematuria in the father of a male patient. Progression to ESRD is constant in both sexes and usually occurs before the age of 30. A few patients reach ESRD around 10 years of age or as late as 44 years. Hearing impairment is nearly constant, and ocular lesions may or may not be associated. Ultrastructural

changes of the GBM identical to those in X-linked AS are present. Immunohistochemical distribution of type IV collagen chains may be normal, but a peculiar pattern of renal and skin distribution of the α3(IV) to α6(IV) chains is observed in most patients (92). It is characterized by the co-absence of the α3, α4, and α5 chains in the GBM, replaced by the α1.α1.α2(IV) network, whereas the α5(IV) and the α6(IV) chains are present in the Bowman capsule and collecting duct basement membranes, and in EBM, as normally seen (Fig. 13.12). Taking into account the normal distribution of the type IV collagen networks, these findings indicate that defects in *COL4A3* or *COL4A4* result in the defective expression of the α5(IV) chain only in those basement membranes in which the three chains are associated within the α3.α4.α5(IV)–α3.α4.α5(IV) network.

There is a broad spectrum of phenotypes in heterozygous carriers. Some are completely asymptomatic, whereas others present with persistent or intermittent microscopic hematuria associated with the thin GBM in the few cases examined (34,35), that is, with the typical clinicopathologic features of "familial benign hematuria" (93). Conversely, progression to renal failure has been observed in a few heterozygous carriers (35). This raises the question of the advisability of using relatives as living donors for kidney transplantation in recessive AS.

A naturally occurring autosomal recessive AS has been described in English Cocker Spaniel dogs (94), and several transgenic models of the disease have been generated in mice (95,96). The GBM of affected dogs and mice shows the characteristic basket-weave transformation and, by immunofluorescence, the GBM lacks the α3(IV), α4(IV), and α5(IV) chains. Homozygous animals progress to ESRD. Progression is more rapid in mice with deletion involving both the *Col4a3* and *Col4a4* genes than in *Col4a3* null mice.

## Autosomal Dominant Alport Syndrome

Autosomal dominant inheritance, characterized by male-to-male transmission and similar severity in men and women, has been observed in a few families (50,97–102). The clinical phenotype is milder than in the X-linked form: progression to ESRD is inconstant and usually occurs after 40 to 50 years of age (98–101). Hearing defect is also a late symptom (99,100), and no ocular involvement has been reported. Ultrastructural abnormalities of the GBM seem to be constant (97,99,101). They consist of thickening and splitting of the GBM or diffuse thinning that is the most frequent finding in our experience. No change in the distribution of the α(IV) chains has been reported. Heterozygous mutations in the *COL4A3* or *COL4A4* gene have now been identified in about 20 unrelated families and the high inter- and intrafamilial variability of the phenotype in autosomal dominant AS has been established.

Canine models of autosomal dominant AS have been described (103,104). Affected dogs have hematuria and progressive renal disease causing renal failure at various ages. Some of these animals have anterior lenticonus, but hearing loss is not associated. Ultrastructural changes of the GBM of the AS type are present, but the immunohistochemical distribution of the α3(IV) and α5(IV) chains of collagen is normal. Moreover, the expected role of *COL4A3* and *COL4A4* genes has been excluded in bull terriers by linkage studies (104). The gene defects responsible for these diseases have yet to be identified.

## Alport-Like Diseases: The Epstein and Fechtner Syndromes

In the past, the Epstein and Fechtner syndromes were regarded as AS variants (105,106). They are autosomal dominant disorders characterized by the association of hereditary nephritis,

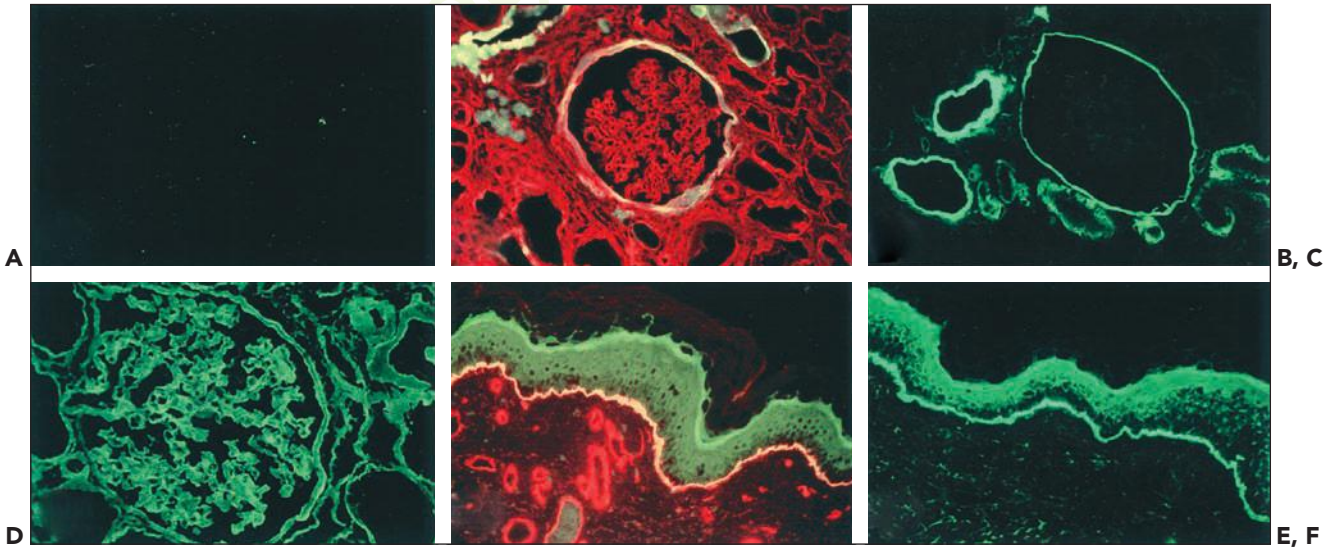



**FIGURE 13.12 Autosomal recessive Alport syndrome.** Immunofluorescence distribution of type IV collagen chains in the renal **(A–D)** and epidermal **(E, F)** basement membranes of patients affected with autosomal recessive Alport syndrome. Within the kidney, there is co-absence of the α3 and α5 chains in the GBM **(A, B)** contrasting with the persistent expression of α5 and α6 in the Bowman capsule and distal convoluted TBMs **(B, C)**, and the strong expression of α1 in the GBM **(D)**. In the skin, the α5 and α6 are normally expressed in the EBM **(E, F)** and coexpressed with the α2 chain as shown by double immunolabeling **(E)**. (α1, α3, α5, α6: FITC; α2: *Texas red*.)

deafness, and macrothrombocytopenia in the Epstein syndrome, with cataracts and small blue leukocyte inclusions in the Fechtner syndrome. Patients present with hematuria and proteinuria and develop ESRD, generally after the fourth or fifth decade of life. Renal histology shows variable and nonspecific abnormalities. Ultrastructural changes of the GBM reminiscent of the GBM lesions observed in AS have been described in a few patients. They have mostly a focal distribution, and except in rare cases (107), they are not widespread as in AS. Hearing loss is frequent and occurs early in the course of the disease, especially in female patients, before or in the absence of any renal involvement.

It is now understood that Epstein and Fechtner syndromes are not type IV collagen diseases (and therefore not forms of AS). They are linked to mutations in the *MYH9* gene encoding the nonmuscle myosin heavy chain IIA (108,109). *MYH9* is also involved in two dominant syndromes lacking renal involvement, the May-Hegglin anomaly, and the Sebastian syndrome (association of macrothrombocytopenia and leukocyte inclusions). Mice with *Myh9* mutations reproduce the human renal and extrarenal phenotype (110). Interestingly, it had been proposed that the high rate of kidney diseases in African Americans was linked to *MYH9* sequence variants but further studies demonstrated that it was more closely associated with variants in the neighboring *APOL1* gene (111,112).

## Genetic Counseling

Clinical and pathologic criteria of AS in a hematuric patient are (a) positive family history of hematuria with or without progression to ESRD, (b) progressive high-tone sensorineural hearing loss, (c) characteristic ocular changes (lenticonus and/or maculopathy), (d) typical ultrastructural changes of the GBM, and (e) abnormal GBM or EBM distribution of type IV collagen chains. Because of the demand for genetic counseling, determination of the mode of transmission is mandatory. This determination may be easily made in large families with several affected patients. Conversely, in sporadic AS, or in children belonging to small families in which only women are affected with isolated hematuria, the mode of transmission cannot be determined by clinical evaluation. Immunohistologic analysis of type IV collagen chain expression in the renal and/or skin basement membrane may be informative if anomalies consistent with X-linked or autosomal recessive mode of transmission are observed, but normal staining is noninformative. Finally, the mode of transmission may be determined by linkage analysis or molecular analysis of the different type IV collagen genes, an approach made easier by the recent development of new sequencing protocols. In addition, in identified AS families, genetic analysis is important for the detection of the asymptomatic carriers for prenatal diagnosis or before kidney donation.

## Consequences of Mutations on Type IV Collagen Network: Data From Patients and Animal Models

A striking feature observed in most human X-linked and autosomal recessive AS, as well as in animal models, is the absence of all three α3(IV), α4(IV), and α5(IV) chains within the GBM although only one of these chains is actually mutated. This suggests that transcriptional, translational, and/or post-translational events link the expression of the different type IV collagen chains. Actually, normal or slightly increased expression of *Col4a4* and *Col4a5* was observed in *Col4a3*–/– mice (95), and normal *COL4A3* and *COL4A4* expression, assessed by RT-PCR of the patient's kidneys (113) or in situ hybridization (114), was seen in X-linked AS in humans. Moreover, the α3(IV) chain was shown to be retained in the patient's podocytes (Fig. 13.13) (114). The presence of both the transcripts and the protein indicates that the absence of the α3(IV) chain in the GBM results from events downstream of transcription, RNA processing, and protein synthesis. The α3(IV), α4(IV), and α5(IV) chains of the normal GBM coexist within a triple-helical protomer, and the NC1 domain of all three chains is required in vitro for the assembly of a hexamer complex, representative of the native network. Together, these findings suggest that a mutation in one chain can prevent the incorporation of the other two chains into the triple-helical complex. According to this hypothesis, in Col4a3$^{-/-}$ mice, made transgenic for the human *COL4A3-COL4A4* locus, the human α3(IV) restores the expression of and co-assembles with the mouse α4(IV) and α5(IV) chains specifically at sites where the human α3(IV) is expressed, demonstrating that the expression of all three chains is required for network assembly (115). The hybrid network leads to normal assembly eliminating the Alport phenotype, providing further evidence that defective assembly of the α3.α4.α5(IV) protomer is the pathogenic mechanism responsible for the disease. Similarly, transfer of the α5(IV) gene to bladder smooth muscle cells restores in vivo the expression of the α6(IV) chain in the smooth muscle basement membrane in a canine model of X-linked AS (116).

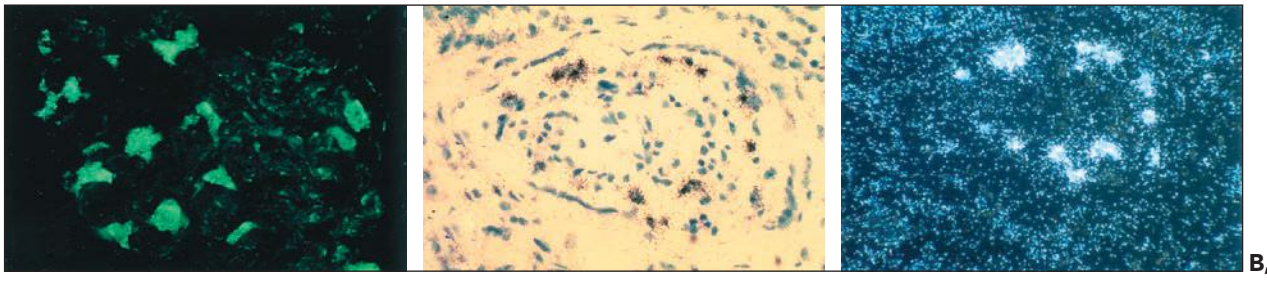



**FIGURE 13.13 Podocyte expression of the α3(IV) chain in a patient with X-linked AS and no GBM expression of the α3, α4, or α5(IV) chain. A:** Immunofluorescence: strong expression of the chain within the podocytes. **B and C:** In situ hybridization of a kidney section from the same patient: strong podocyte expression of *COL4A3* transcripts. (Light- and dark-field examination).

These observations suggest that gene therapy of AS by in vivo transfer of a corrected type IV collagen alpha chain gene into renal glomerular cells could be possible in the future (117). Bone marrow–derived stem cell therapy following irradiation has also been tested in Col4a3$^{-/-}$ mice (118,119). Significant glomerular morphologic improvement has been observed suggesting the possibility of stem cell renewal of glomerular epithelial cells and basement membrane architecture, but no data on renal survival have been reported. Surprisingly, irradiation per se has been shown to prolong survival in mice, although the mechanisms are unknown (120).

In the GBM of most AS patients or animal models, the extensively cross-linked α3.α4.α5(IV) network is absent and replaced by the embryonic α1.α1.α2(IV) network. The persistence of this network, more susceptible to proteolytic attacks by collagenases and cathepsins, may explain the progressive GBM degradation (40). In addition, up-regulation of the expression of matrix metalloproteinases (MMP) has been observed in the kidneys of X-linked AS dogs, of Alport alpha3$^{-/-}$ mice, and also of humans (121,122). However, the rate of progression of the disease is not modified in AS mice deficient for the MMP-9 gene (123), perhaps because of compensatory up-regulations of other MMPs (124). Ablation of enzymatic activity, if performed early, before the occurrence of proteinuria, has been shown to attenuate disease progression (122,124). Additional mechanisms could be involved in disease progression in Col4a3$^{-/-}$ mice: TGF-β1, a growth factor promoting accumulation of extracellular matrix is induced in Alport mouse kidneys, and its inhibition by specific antibodies prevents GBM thickening (125). Conversely, BMP-7 improves renal function in these mice (125,126). Chemokine receptor 1 blockade or HMG-CoA reductase inhibition also have a beneficial effect on the renal function of this mouse model (127,128). Until now, none of these approaches have been tested in humans affected with AS.

## Treatment

Results of two different pharmacologic approaches, initially tested in animal models, have been reported in small series of human patients. Cyclosporine slows the progression of the renal disease in X-linked AS dogs (129). Similarly, reduction in proteinuria and slower progression to renal failure have been reported in a series of eight male AS patients who did not develop signs of cyclosporine nephrotoxicity after 7 to 10 years of treatment (130). However, this favorable effect was not confirmed in two other studies using the same protocol: reduction in proteinuria was temporary in one study (131), whereas severe histologic lesions of cyclosporine nephrotoxicity developed rapidly in the other (132).

Angiotensin-converting enzyme inhibition (ACEI) reduces proteinuria and prolongs survival in Samoyed dogs with X-linked AS and in *Col4a3* knockout mice, especially if the treatment is started early in the course of the disease (133,134). Short-term reductions in proteinuria have been reported in a small series of 10 AS children receiving enalapril for a 5-year period (135). Similar evolution of proteinuria was observed in a larger study including adult patients. However, in this last study, ACEI treatment was shown to delay renal failure, especially if patients were treated early before the occurrence of proteinuria and impaired renal function (136). Further large control trials are needed to confirm the benefit of renin-angiotensin system blockade in AS patients and to determine the optimal protocol.

## Renal Transplantation

The outcome of renal transplantation in patients with AS is overall excellent (51,137,138) even if the incidence of posttransplant asymptomatic linear IgG fixation along the GBM is much higher in AS than in other renal diseases (137,139). However, a serious complication is the occurrence, in 2% to 5% of AS patients, of rapidly progressive anti-GBM glomerulonephritis. More than 30 cases have now been reported (reviewed in 138). Patients developing anti-GBM glomerulonephritis are usually male, deaf, and affected with juvenile-type AS with typical ultrastructural changes of the GBM. The presenting symptoms are hematuria and rapid deterioration of renal function, occurring within the first year after renal transplantation, sometimes within the first month. Stabilization of renal function has been observed in a few cases, possibly as the result of appropriate management including plasma exchange therapy, but this complication usually leads irreversibly to graft loss. The diagnosis is based on the findings of crescentic glomerulonephritis with linear fixation of IgG along the GBM and circulating anti-GBM antibodies. Rapid recurrence of anti-GBM nephritis in subsequent allografts is nearly constant (140).

The pathogenesis of posttransplant glomerulonephritis appears to be linked to the introduction, via the grafted kidney, of α(IV) chains for which the recipient has not established immune tolerance (66). Accordingly, most patients who develop this complication are males with X-linked AS owing to *COL4A5* deletion or mutations resulting in the absence of the α5(IV) protein. Anti-GBM antibodies are directed against the NC1 domain of the α5(IV) chain (28,138,141,142). In some patients with a complete *COL4A5* deletion, immunization against the NC domain of α3(IV) has also been observed (141–143). Male patients with missense *COL4A5* mutation and female patients with X-linked AS who express the α(IV) chains in their native kidney do not develop posttransplant anti-GBM nephritis. Conversely, anti-GBM nephritis may occur in male or female patients with autosomal recessive AS linked to large *COL4A3* deletions (142,144,145). In this situation, antibodies are directed against the NC domain of the α3(IV) chain (142,145). Interestingly, anti-GBM antibodies responsible for GP syndrome/de novo anti-GBM nephritis are also directed against the α345NC1 hexamer, but the precise targets of the auto- and alloantibodies are different (141,146). In AS patients who develop posttransplantation anti-GBM nephritis, the antibodies bind to α3NC1 and/or α5NC1 epitopes exposed at the surface of the intact cross-linked hexamer, and the antigenicity is lost after dissociation of the domain due to perturbation of the quaternary structure. Conversely, GP antibodies do not recognize the native hexamer but react only with epitopes normally hidden and unmasked by dissociation of the hexameric structure. Although patients having *COL4A5* or *COL4A3* truncating deletions are at risk of developing posttransplant anti-GBM nephritis, only a few patients with major deletions develop this complication (51,138). In addition to the genetic defect, other factors, including the immune response of the host, are likely involved. In

one of the families reported by Kashtan et al. (147), two male patients developed anti-GBM nephritis in the renal allograft, whereas two others have functioning allografts 11 and 13 years after renal transplantation.

## FAMILIAL BENIGN HEMATURIA

Familial benign essential hematuria, also named familial benign hematuria (FBH), is characterized by persistent or recurrent glomerular microscopic hematuria transmitted as an autosomal dominant trait. Hematuria is usually detected in childhood by routine urinalysis. Macroscopic hematuria is a rare event. There is no significant proteinuria, no hypertension, no progression to renal failure, and no extrarenal symptoms of AS (148). According to Rogers et al. (148) who studied a large four-generational family, the constant morphologic change was a uniformly thin GBM. Diffuse GBM thinning was observed in the five members of the family, aged 21 to 51 years, who underwent renal biopsy. Since this publication, the finding of thin GBM has been reported by several authors, mostly in children but also in adults with isolated familial or no familial hematuria (149–152).

### Pathologic Findings

By light microscopy, the renal tissue is usually normal (Fig. 13.14). Minor mesangial increase may be seen, but usually no significant glomerular changes are observed. In some individuals, a delicate uniform thinning of the GBM is appreciated even by light microscopy using well-performed periodic acid-Schiff and Jones methenamine silver stains on 2-µm-thick paraffin sections, but this impression must be confirmed by electron microscopy (153). Red blood cells may be present in the Bowman space or within tubules. Vessels are normal in children, whereas nonspecific vascular changes are observed in adult patients.

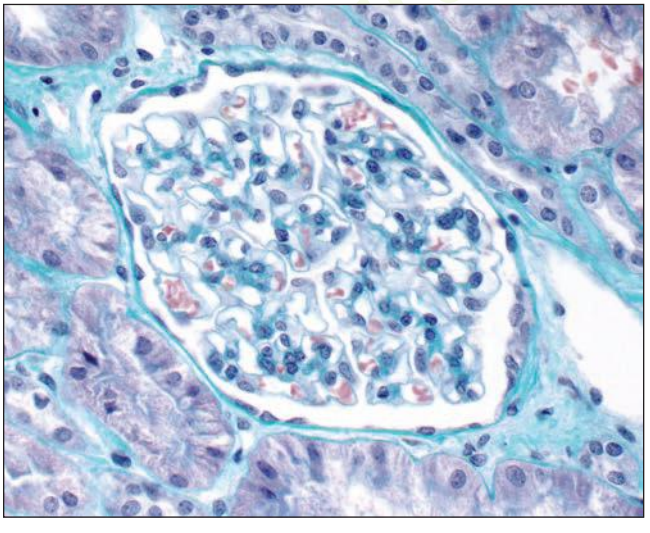


**FIGURE 13.14 Light microscopy.** Renal biopsy from a 13-year-old girl with familial benign hematuria. Normal-appearing glomerulus with podocyte hypertrophy. (Trichrome *light green*, ×250.)

Conventional immunofluorescence is usually negative. However, scattered granular deposits of C3 may be focally seen in the glomerular tuft. They may or may not be associated with vascular deposits of C3 (16). The expression of type IV collagen chains is normal (16,151,152). In typical cases, the attenuation of the GBM is extreme, regular, and involves all of the capillary loops; however, more focal involvement, with thin segments lying adjacent to normal ones, may also be observed (Fig. 13.15). The structure and contours of the GBM are normal (Fig. 13.16). Effacement of epithelial foot processes is focally observed, and GBM gaps are occasionally described in patients affected with FBH (148). Red cells traversing the GBM are rarely seen ultrastructurally.

### Genetics

In 1996, Lemmink et al. (93) established that FBH with the thin GBM may be due to type IV collagen mutations: they identified a heterozygous mutation in *COL4A4* segregating with isolated hematuria in one Dutch family. Subsequently, heterozygous mutations in either *COL4A3* or *COL4A4* were described in relatives of patients with autosomal recessive AS, presenting with microhematuria and, sometimes, documented thin GBM (34,35,154), as well as in families affected with FBH without any case of AS (155–158). Segregation with *COL4A3*/*COL4A4* has been shown in 40% to 50% of FBH families. Thus, in these families, hematuric patients are carriers for the autosomal recessive form of AS, and as previously indicated, heterozygous *COL4A3* and *COL4A4* mutations are also involved in autosomal dominant AS.

On the other hand, FBH is probably a heterogeneous condition since linkage to the *COL4A3-COL4A4* locus has been excluded in some kindreds (159,160). However, it must be pointed out that linkage analysis, based on the phenotype of family members, is particularly difficult in such a disorder. The absence of hematuria on urine analysis is not a definitive argument for assuming that an individual is unaffected because hematuria can be intermittent or even absent in individuals carrying heterozygous mutations (34,35). Renal biopsy is not performed in all hematuric individuals within a family, and hematuria could result from coincidental unrelated causes in some patients. Linkage to *COL4A5* has been suggested, based on the study of small families with a reduced follow-up (161,162). In the Slovenian series, the same missense *COL4A5* mutation was found in one AS family with delayed progression to ESRD and in five small families with the clinical presentation of FBH, but without long-term follow-up (161). No linkage to the *COL4A1*/*COL4A2* locus and no mutations in the genes were identified by screening 23 unrelated hematuric individuals/families with thin GBM, indicating that *COL4A1*/*COL4A2* does not represent a major genetic locus for this disorder (163). Actually, mutations in these two genes have been shown to be the cause of hereditary porencephaly and, for *COL4A1*, of the HANAC syndrome (hereditary angiopathy with nephropathy, aneurysms, and muscle cramps) (164–166).

### Diagnosis

The clinical diagnosis of FBH is based on the presence of persistent microhematuria and the exclusion of proteinuria, impaired renal function, and extrarenal symptoms of AS, in

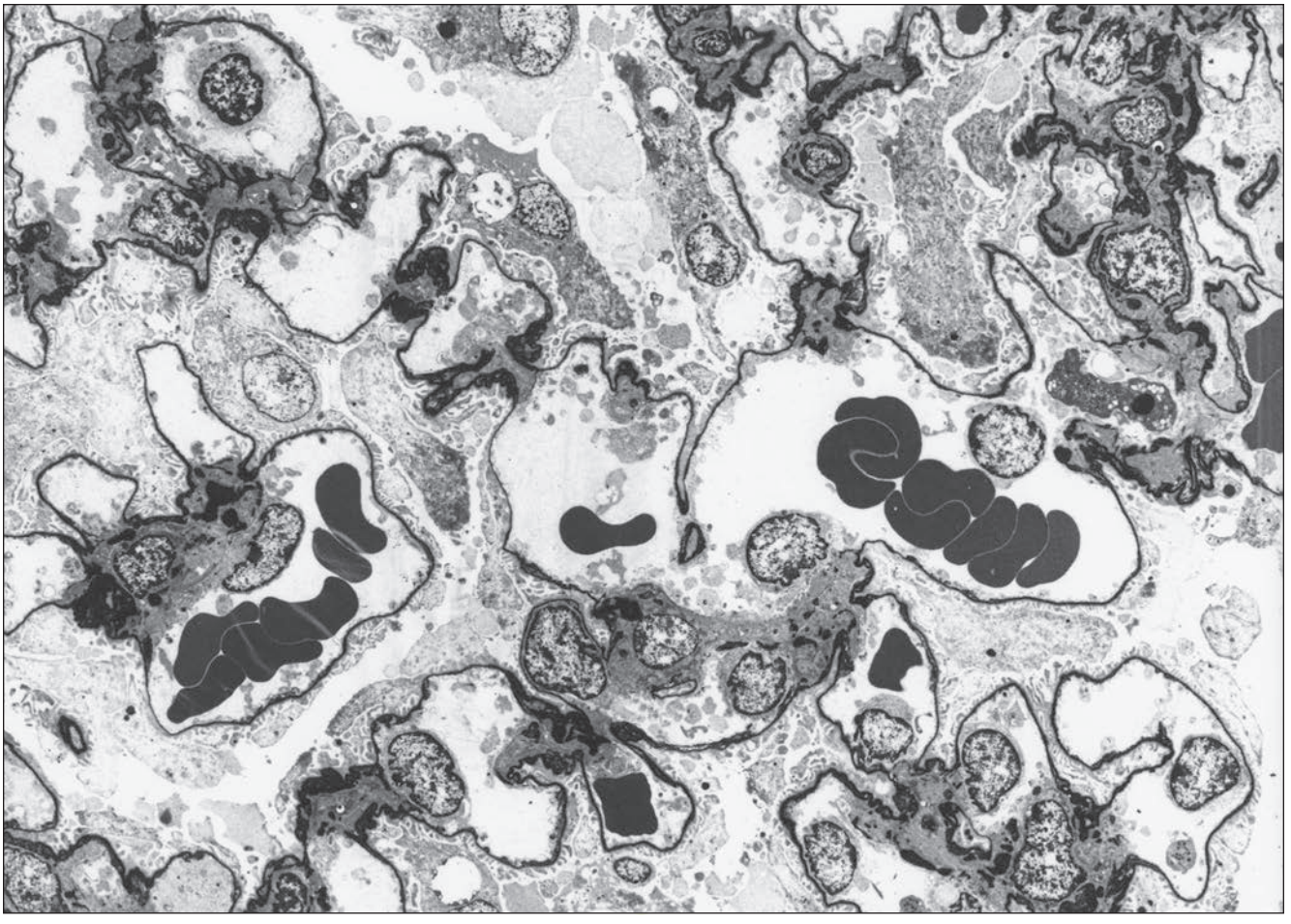


**FIGURE 13.15 Electron microscopy from the same 13-year-old girl.** Widespread thinning of the GBM. (Methenamine silver stain ×2500.)

the proband as in other family members. In the absence of large multigenerational families including hematuric males, the diagnosis may be difficult and possible only after long-term follow-up examination of patients. The occurrence of any additional symptom leads to reconsidering the diagnosis. The ultrastructural finding of thin GBM is suggestive of, but not conclusive for, FBH. Normal renal expression of type IV collagen genes does not exclude AS. Even the identification of *COL4A3* or *COL4A4* mutations in the heterozygous state does not necessarily predict a favorable course as the same mutations may be observed in autosomal dominant AS. From a practical point of view, regular (annual) follow-up examination of patients is a reasonable option, especially in the absence of large pedigrees.

## SIGNIFICANCE OF THE THIN GLOMERULAR BASEMENT MEMBRANE LESION

### Definition and Incidence

No universal agreement exists concerning the normal GBM thickness—distance between the endothelial and epithelial cell membranes—and consequently the definition of thin GBM. Different techniques have been applied to GBM thickness measurements, including the introduction of latex beads of known diameter onto the tissue sections, the orthogonal intercept method that superimposes a grid of perpendicularly oriented lines of known dimension onto the electron micrograph, and the use of digital cameras equipped with appropriate morphometric software in the modern era (reviewed in 153,167). GBM measurements are made on at least two to three glomeruli of glutaraldehyde-fixed epoxy resin–embedded renal tissues, at intervals where the GBM is cut in cross section around the free peripheral capillary loops. According to different reports, normal GBM width varied from 320 ± 35 nm in that of Aarons et al. (168), 350 ± 43 nm in that of Tiebosch et al. (169), and 330 to 460 nm in that of Dische et al. (170). The GBM thickness is greater in males than in females: 330 ± 50 nm in males versus 305 ± 45 nm in females according to an analysis of renal biopsies (171), 350 ± 50 nm versus 300 ± 40 nm in another analysis of renal biopsies (153), and 370 nm versus 326 nm in a study of living donors for kidney transplantation (172). During infancy and early childhood, the GBM thickness increases progressively from 100 to 150 nm at birth to 200 nm at 1 year of age, until reaching adult values by 11 years of age (173).

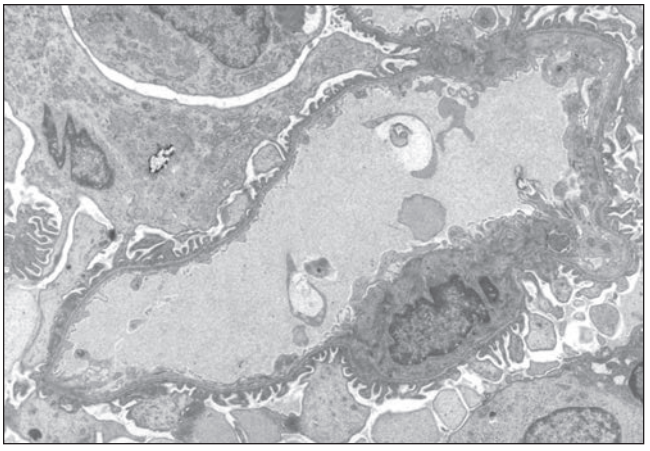


**FIGURE 13.16 Electron microscopy.** Renal biopsy from a 15-year-old boy with familial benign hematuria. Widespread thinning of the GBM. (Uranyl acetate-lead citrate ×5800.)

These discrepancies underscore the difficulties and the limits of the diagnosis of thin GBM and the absolute necessity for each laboratory to establish its own standards. In the initial description of FBH, the mean thickness of the GBM in hematuric members of the family was 150 nm (148). In our analysis of the thin GBM lesion in hematuric children, GBM thickness was less than 200 nm in 30 of 31 patients regarded as having abnormally thin GBM (16). The mean GBM thickness was also less than 200 nm in the series of Haas (171). GBM thickness less than 250 nm has been the criterion accepted in most pediatric (18,149–151) and adult series (18,152,169), but in others, the mean GBM thickness that was considered abnormal was around 300 nm (170). In those biopsies with insufficient glomeruli in glutaraldehyde-fixed tissue and where paraffin-embedded tissue is used as a salvage technique for electron microscopy, there is typically artifactual thinning of the GBMs caused by the tissue reprocessing. Therefore, it is not possible to diagnose GBM thinning reliably in deparaffinized formalin-fixed tissue (174).

The incidence of thin GBM in hematuric patients is difficult to ascertain because its demonstration depends not only on the definition of abnormally thin GBM but also on the indication for renal biopsy and the availability of electron microscopy, which is necessary for diagnosis. Despite these reservations, the thin GBM abnormality appears to be more frequent than initially thought. Its incidence in the general population should be between 5.2% and 9.2% (170). It has been found in 11% of nontransplant renal biopsies in the series of Aarons et al. (168), in 1.9% in that of Haas (171), but only in 0.8% in that of Abe (152). It has been observed as frequently as IgA nephropathy in a series of adult patients with persistent hematuria (169). Perhaps because the management of patients with isolated microscopic hematuria is different, in our pediatric department, the finding of thin GBM is five times less frequent than that of IgA nephritis.

### Clinical Significance

Since the description of "familial benign essential hematuria" by Rogers et al. (148), the finding of thin GBM has often been regarded as the hallmark of a benign hematuric disease (150,152). And the terms "thin membrane nephropathy (TBMN)," " thin basement membrane disease," "thin GBM nephropathy," or "thin basement membrane syndrome" have been widely applied to apparently nonprogressive familial or sporadic hematuric conditions, even in the absence of biopsy confirmation. Moreover, proteinuria, hypertension, and renal failure have been reported in TBMN (16,18,168,169,175,176) showing that thin GBM may have different diagnostic and prognostic significances. And, as discussed previously, diffusely thin GBM may be the only change observed in patients affected with typical X-linked, autosomal recessive or dominant AS. It is especially frequent in children and in females with X-linked AS but has been observed in up to 10% of males with X-linked AS and proven *COL4A5* mutations (51). Actually, the finding of *COL4A3* or *COL4A4* mutations in the heterozygous state, in patients with thin GBM who developed FSGS and progressed to ESRD (176), has to lead to the diagnosis of autosomal dominant AS. Neither thin GBM nor FSGS is a marker of specific disease entities. FSGS is a nonspecific glomerular lesion that occurs during the course of many conditions including AS (177). Thin GBM is a structural abnormality observed in several progressive or nonprogressive hematuric disorders (102,178,179). Even if the clinical course of adult hematuric patients with thin GBM is frequently benign, it must be kept in mind that thin GBM can be the only structural abnormality in AS patients whatever their age, and the diagnosis can be difficult, particularly in sporadic cases of persistent hematuria (Fig. 13.17).

## CONCLUSIONS REGARDING TYPE IV COLLAGEN DISEASES

In conclusion, a broad spectrum of phenotypes (ranging from AS to the complete absence of urinary symptoms and including FBH and some intermediate forms of nephropathy) is associated with *COL4A3-COL4A5* mutations. Whereas *COL4A5* mutations and homozygous or compound heterozygous mutations of either *COL4A3* or *COL4A4* consistently result in a severe phenotype AS, the clinical and morphologic consequences of *COL4A3-COL4A4* heterozygous mutations are highly variable (102,178,179). This is why the term "collagen type IV(α3–α4) nephropathy" has been proposed to group the clinical entities classified as FBH, autosomal dominant AS, and autosomal recessive AS carriers (178). This complexity makes genetic counseling and risk ascertainment difficult in individual patients. So far, no correlation has been established between the type of *COL4A3-COL4A4* heterozygous mutation and the renal phenotype, and even the same mutation can result in different phenotypes (179). Genetic or environmental factors surely contribute to the phenotypic heterogeneity between and within families. Accordingly, in the study of Voskarides et al. (180), the severe clinical course observed in some members of families with *COL4A3* or *COL4A4* heterozygous mutations (and also of hematuric patients affected with familial C3 glomerulopathy associated with *CFHR5* mutations) was linked to the presence of the *NPHS2-R229Q* podocin polymorphism in the heterozygous state. This finding has to be confirmed by the analysis of additional populations.

## NAIL-PATELLA SYNDROME

The nail-patella syndrome (NPS), also called hereditary osteo-onychodysplasia (HOOD), is a rare hereditary disorder characterized by the curious association of nail hypoplasia or

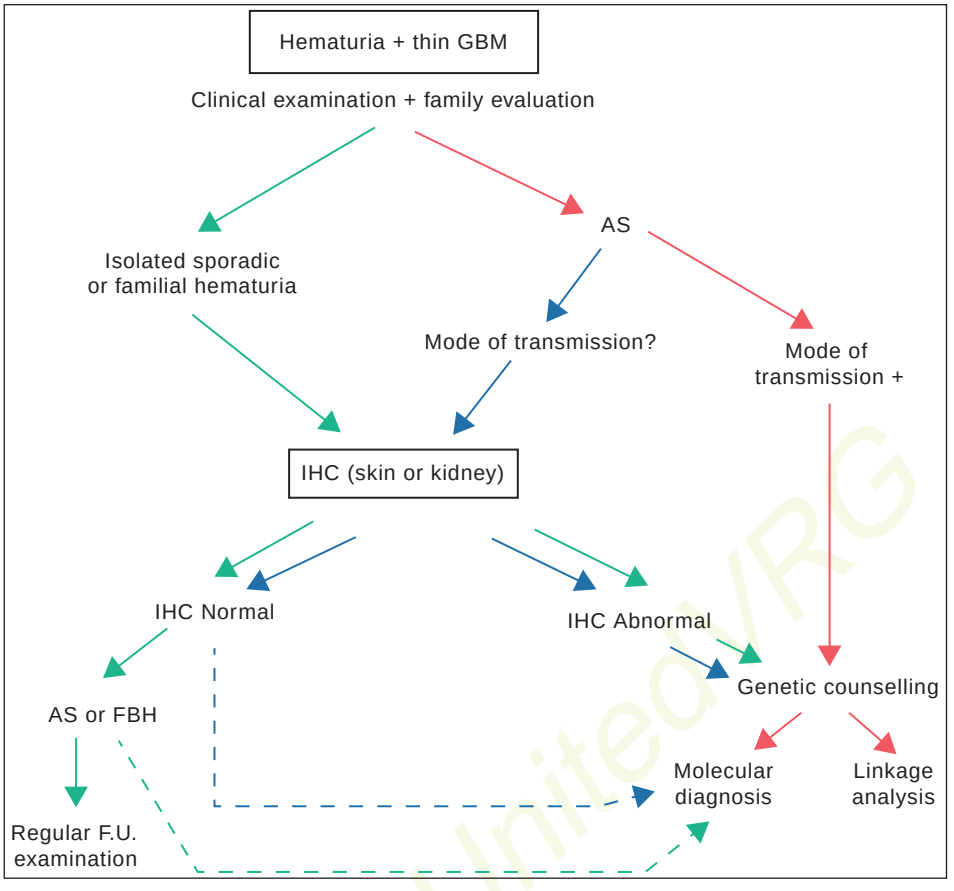



**FIGURE 13.17 Diagnostic approach for sporadic or familial hematuria with the thin GBM.** In some patients (*red line*), clinical investigation leads to the diagnosis of AS. The mode of transmission may be determined by family evaluation allowing genetic counseling. Identification of asymptomatic carriers and prenatal diagnosis are possible, based on linkage analysis or molecular diagnosis. Conversely, in patients with isolated sporadic or familial hematuria, the significance of the thin GBM remains to be determined, especially in children (*green line*). Immunohistochemical analysis of the renal or epidermal basement membranes may detect abnormal distribution of α(IV) chains, specific for AS, and direct toward one or another mode of transmission of the disease. Here again, genetic counseling is possible. The same process applies to sporadic or familial AS when the mode of transmission cannot be determined by family investigation (*blue line*). In both situations, normal immunohistochemistry does not exclude AS, and research of type IV collagen mutation should theoretically be performed. From a practical point of view, regular follow-up examination is a reasonable option. AS, Alport syndrome; FBH, familial benign hematuria; GBM, glomerular basement membrane; IHC, immunohistochemistry.

dysplasia and bone abnormalities. Little (181), an orthopedist interested in congenital absence or delayed development of the patella, was the first to mention a family of "four generations of which 18 persons had no patella and no thumbnails." Renal symptoms, described many years later, are inconstant and seem to develop in about 35% to 40% of patients (182–184). The incidence of the disease is estimated at 1 per 50,000 live births (184). As indicated in the analysis of 151 cases from the literature by Meyrier et al. (182), the disease has been observed all over the world in kindreds of all geographic and ethnic origins.

## Pathologic Findings

Light microscopy is nonspecific (Fig. 13.18). Renal tissue appears initially normal and then mild and focal glomerular lesions may be seen. These lesions consist of an irregular increase in GBM thickness and enlargement of the mesangial matrix. FSGS lesions or glomerular obsolescence develops with disease progression. At this stage, foci of tubular atrophy and interstitial fibrosis are seen, as well as nonspecific arteriolar hyalinosis. Immunofluorescence is usually negative or shows focal and segmental deposits of IgM or C3 within FSGS lesions.

Electron microscopy reveals distinctive lesions of the GBM. In 1970, Del Pozo and Lapp (185) observed prominent thickening of the GBM with the presence of irregular, sharply defined, electron-lucent areas within the thickened GBM segments. These investigators suggested that the primary defect could involve the GBM. Ben Bassat et al. (186), in 1971, identified dark fibrillar material with the periodicity of interstitial collagen in the electron-lucent zones within the GBM. Similar material was also present in the mesangial matrix. Further studies confirmed these findings and indicated that staining with phosphotungstic or tannic acid is often necessary to disclose the fibrillar bundles, whereas with standard staining techniques, the GBM has the mottled

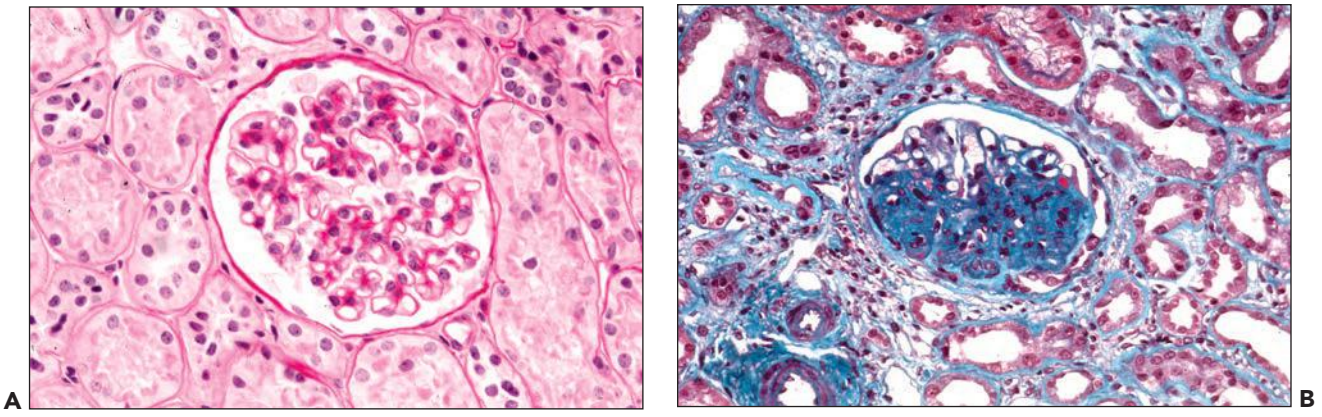



**FIGURE 13.18 Light microscopy. A:** Normal-appearing glomerulus in the renal biopsy from a 13-year-old boy with NPS and persistent mild proteinuria. (PAS ×250.) **B:** Focal segmental glomerulosclerosis in the renal biopsy from a 19-year-old female patient with NPS and nephrotic syndrome. (Trichrome *light green*, ×250.)

appearance described by Del Pozo and other investigators (Figs. 13.19 and 13.20). Fibrils are usually localized to the midportion of the GBM, but in some patients, they are found in the subepithelial or subendothelial space (Figs. 13.21 and 13.22). The extent and distribution of GBM lesions vary widely from case to case, without any correlation with the age of the patient or the severity of renal symptoms. For example, in a 63-year-old man with nephrotic-range proteinuria who developed FSGS GBM lesions were practically absent, whereas collagen fibrils were present in the mesangial areas

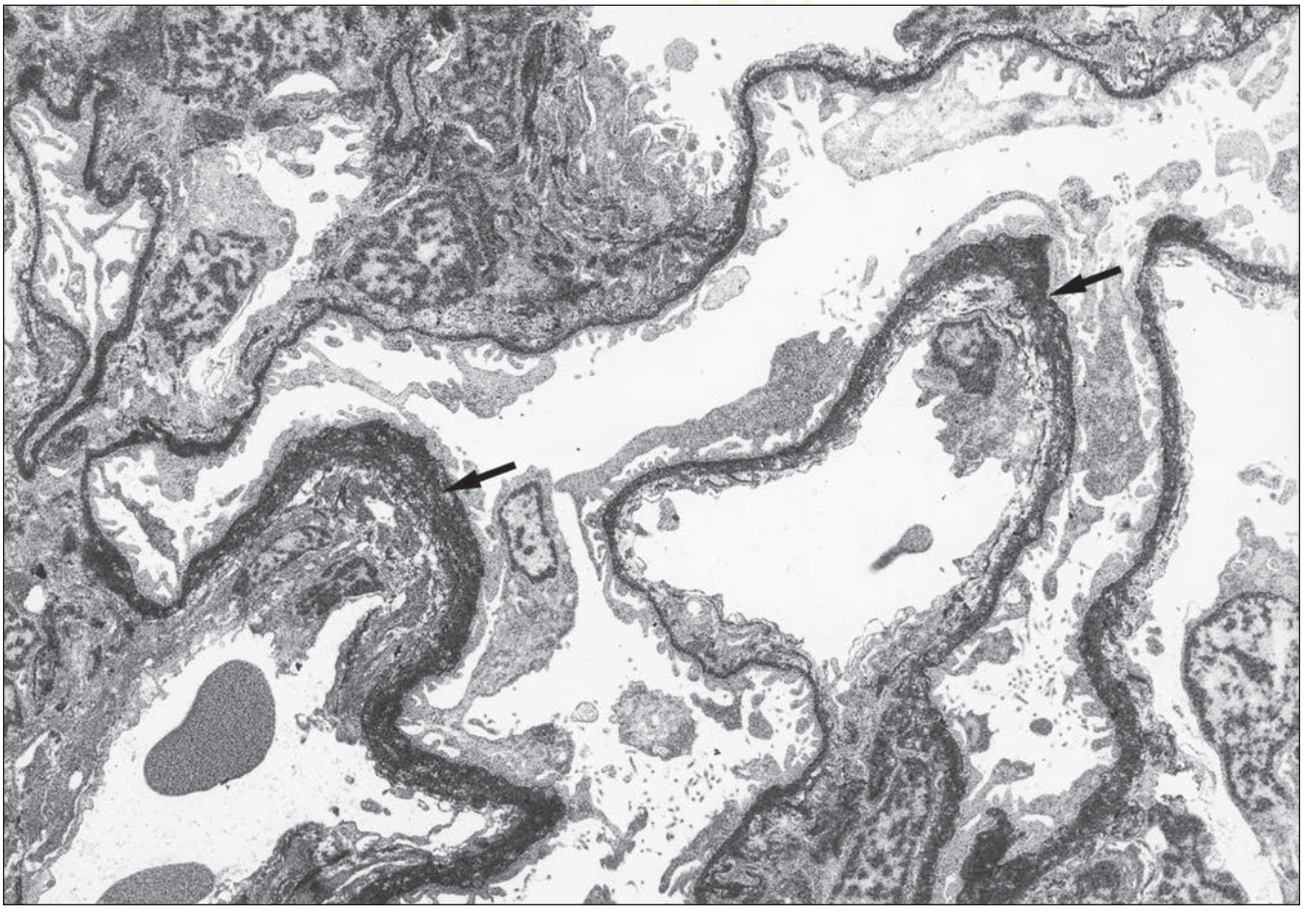


**FIGURE 13.19 Electron microscopy.** NPS. Methenamine silver staining showing the irregular distribution of GBM lesions: some GBM segments look normal, whereas variable degrees of thickening with presence of lacunae are observed in others (*arrows*). (×3300.)

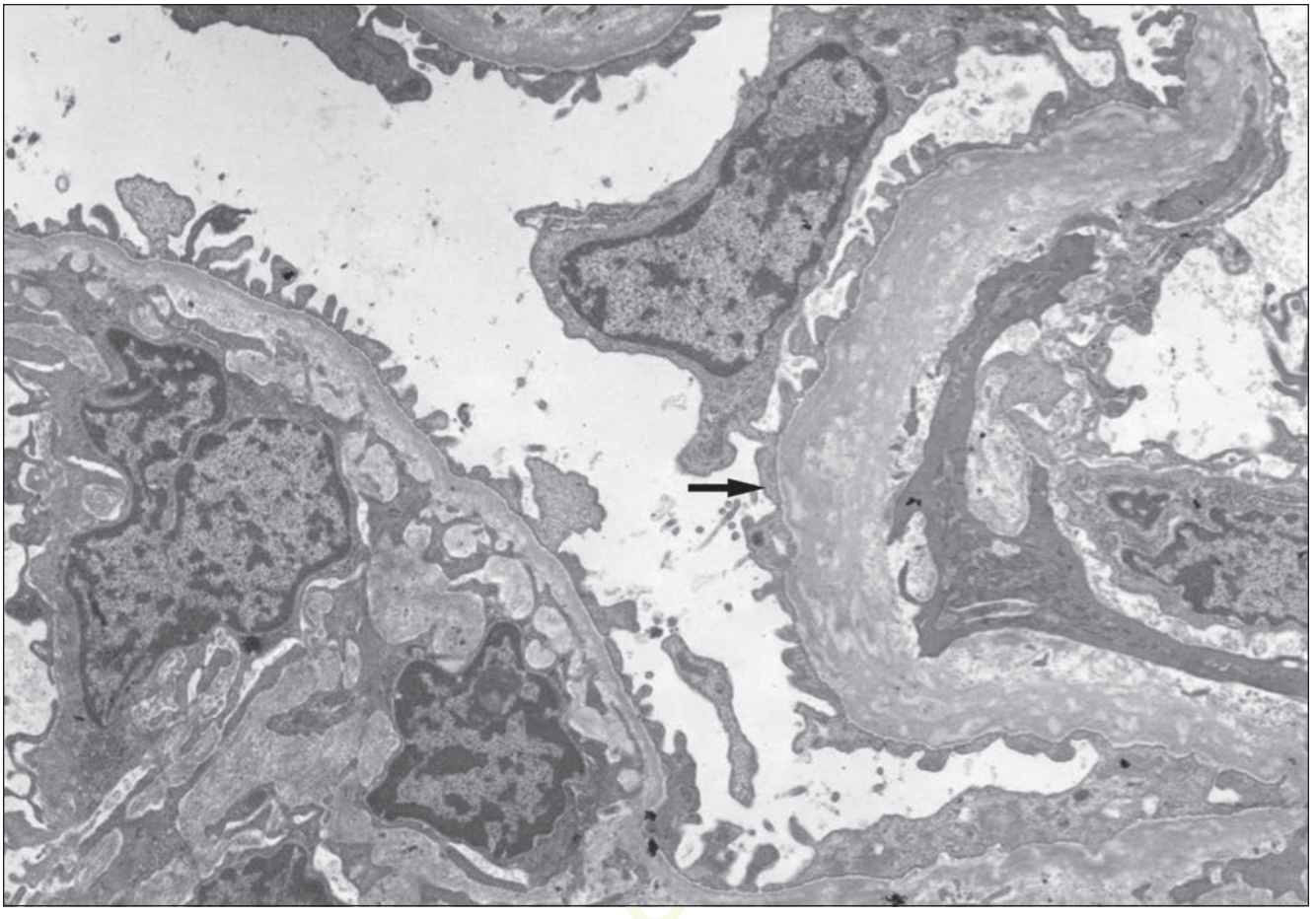


**FIGURE 13.20 Electron microscopy.** NPS. There is irregularity in GBM involvement, with moth-eaten appearance of the thickened segment (*arrow*). (Uranyl acetate-lead citrate, ×7650.)

(Fig. 13.23). GBM lesions have been found in NPS patients in the absence of clinical evidence of renal involvement. Using immunofluorescence and immunogold techniques, we showed that fibrils were specifically labeled by anti–collagen type III antibodies (Fig. 13.24). However, as described in the following section, the glomerular lesions of NPS differ histologically from those of type III collagen glomerulopathy in their irregular distribution and their tendency to produce FSGS lesions, rather than GBM double contours with subendothelial widening.

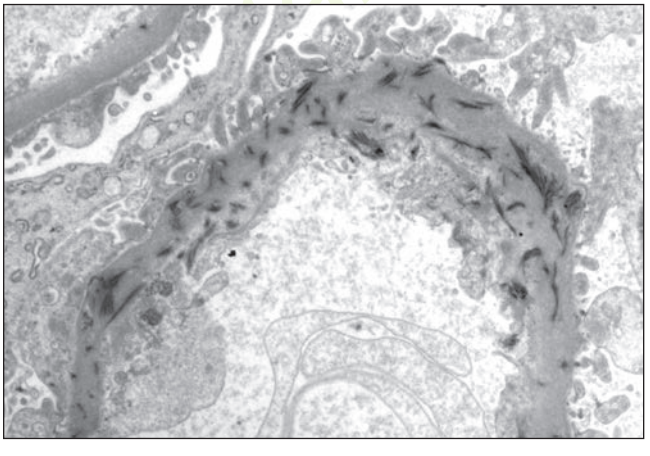


**FIGURE 13.21 Electron microscopy from a 39-year-old man with NPS.** Collagen fibrils are present in the whole thickness of the glomerular basement membrane. (Phosphotungstic acid, ×13,000.)

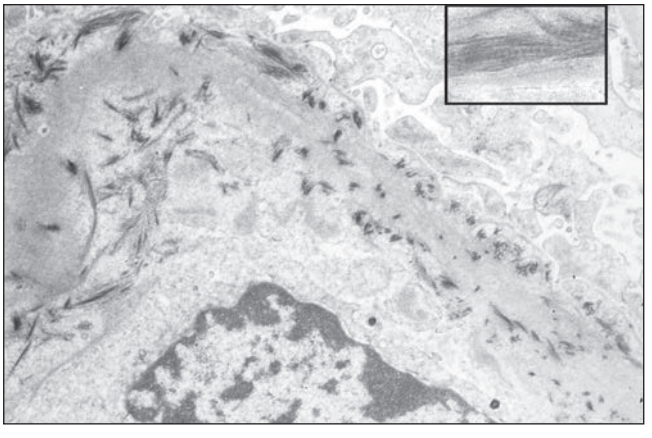


**FIGURE 13.22 Electron microscopy.** In another NPS patient, collagen fibrils predominate in the subepithelial and the subendothelial space. (Phosphotungstic acid, ×13,500.) **Inset:** Periodicity is demonstrable within the cross-striated fibrils. (Phosphotungstic acid, ×54,000.)

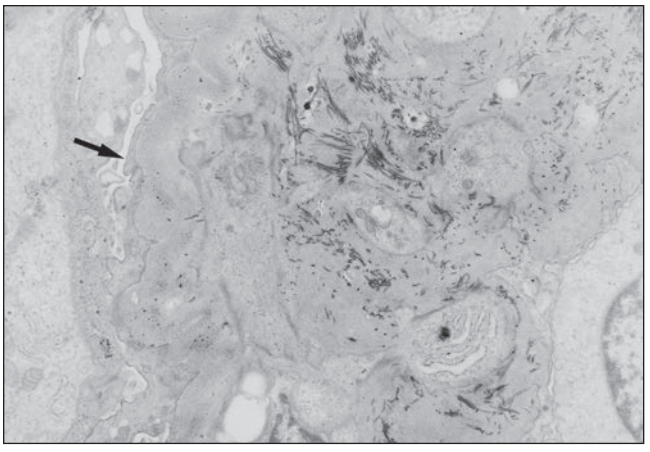


**FIGURE 13.23 Electron microscopy of the renal biopsy of a 63-year-old patient with typical NPS and nephrotic-range proteinuria.** Abundant bundles of type III collagen in the mesangial areas are detected by phosphotungstic acid staining. The GBM covering the mesangial area is free of fibrillar collagen (*arrow*). (×9500.)

## Clinical Features

NPS is a pleiotropic condition affecting mostly the nails, the knees, the elbows, and the pelvis, thus forming the classic tetrad (182–184). These anomalies are bilateral and symmetrical. In some patients, they are severe enough to be recognized at birth, whereas in others, they must be identified by careful examination. Nail aplasia or dysplasia, always predominating on the hands and particularly on the thumbs, is nearly constant. Hypoplasia or dysplasia of the patella, frequently associated with dysplasia of other structures of the knee, is also observed in about 95% of the patients. Elbow dysplasia with posterolateral subluxation of the radial head is responsible for limitation of extension of the forearm. Iliac horns are pathognomonic, and they are discovered on plain radiography of the pelvis in about 70% of the patients. In young children, unilateral or bilateral clubfoot may be the major initial abnormality. Recently, open-angle glaucoma and ocular hypertension have been recognized as additional features of the disease.

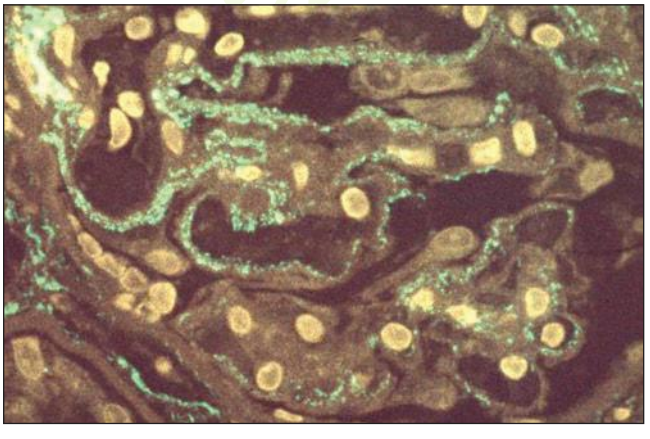


**FIGURE 13.24** Immunofluorescence showing the distribution of type III collagen in the GBM of a 15-year-old patient with NPS and mild proteinuria. (×500.)

The prognosis of the disease depends on the presence and severity of renal involvement (183,187,188). Some families do not develop renal symptoms, whereas in others, renal disease is frequent (187). Persistent and moderate proteinuria, associated with microscopic hematuria in some patients, is the most frequent presenting symptom. Nephrotic syndrome has been observed in a minority of patients and has an ominous significance. Progression to renal failure occurs in about 30% of the patients with renal symptoms, usually many years after discovery of proteinuria, but more rapidly, in less than 10 years in some patients (187,189). A puzzling feature is the unpredictable course of the glomerulopathy, even within a given family, as exemplified by a family studied by Meyrier et al. (182). One patient died at 25 years of age from ESRD, whereas his proteinuric brother has functioning kidneys at 64 years. Superimposed nephropathies, such as membranous glomerulonephritis, IgA nephropathy, GP syndrome, and necrotizing angiitis, have been observed in patients with NPS.

## Genetics and Nature of the Defect

The autosomal dominant mode of transmission suggested by the initial observation of Little (181) was confirmed by the analysis of additional pedigrees. Genetic linkage between NPS and the ABO blood group was established as early as 1955 by Renwick (190). Linkage to the adenylate kinase (*AK1*) locus was subsequently demonstrated and was followed by the localization of the *NPS* gene to chromosome 9, at the 9q34 locus (191).

Based on renal ultrastructural changes, it was initially presumed that the primary defect in NPS could affect one of the GBM components. However, the mutated gene is *LMX1B*, an LIM-homeodomain transcription factor playing a key role in limb development (192–195). LMX1B establishes dorsoventral patterning of the limb where it is expressed in a temporally and spatially restricted manner in the mouse and the chicken. More than 150 different *LMX1B* mutations have now been reported in NPS patients; some of them are recurrent (192–196). They are located in the LIM or homeodomain regions and are thought to result in a loss of function of the mutated protein—that is, in haploinsufficiency. Some of them have been shown to disrupt sequence-specific DNA binding. No correlation has been found between the type of mutation (missense, nonsense, frameshift, deletion) and the severity of renal or extrarenal symptoms. Individuals with an *LMX1B* mutation located in the homeodomain appear to have significantly more frequent and higher value of proteinuria compared to subjects carrying mutations in the LIM domain according to one study (197), but this correlation was not confirmed in the Pittsburg study (188).

The role of LMX1B in determination of dorsoventral patterning in the developing limb provides a satisfactory explanation for the occurrence of nail and skeletal anomalies in NPS patients. The link between the *LMX1B* mutation and the GBM alterations was not initially evident. However, the gene *Lmx1b* is expressed in the mouse kidney, specifically in the podocytes, from the S-shaped body stage onward, and expression persists in postnatal mature podocytes (193,198,199). *Lmx1b* null mice have a severe podocyte phenotype and die at birth. The development of podocytes is retarded without

formation of foot processes and slit diaphragms (193,200). Reduced expression of *Col4a3* and *Col4a4* transcripts, with a stronger effect on *Col4a4*, is associated with diminished GBM expression of the α3 and α4 chains of type IV collagen (198). The expression of podocin, which is mutated in a subset of children with steroid-resistant nephrotic syndrome (201), and of CD2AP, a podocyte protein also required for normal glomerular function (202), was also found to be severely reduced in *Lmx1b* null mice (203,204). Overall, these findings indicate that LMX1B regulates the expression of several podocyte genes critical for podocyte differentiation and function. They strongly suggest that defects in GBM type IV collagen and changes in podocyte phenotype could contribute to the glomerular disease in NPS. However, NPS is due to heterozygous *LMX1B* mutations, and *Lmx1b*$^{+/-}$ mice have no renal symptoms and no morphologic abnormalities, even after 1 year of life. Moreover, in NPS humans affected with a severe glomerular disease, no changes in the glomerular expression of type IV collagen chains, podocin, or CD2AP have been detected (189). Interestingly, preservation of podocin and type IV collagen chains is also observed in constitutive podocyte-specific *Lmx1b* knockout mice that develop massive proteinuria and die from renal failure approximately 2 weeks after birth (199).

## COLLAGEN TYPE III GLOMERULOPATHY

Accumulation of fibrillar collagen within the glomerular extracellular matrix has been reported in several patients without any other symptom of NPS (205–215). It is a rare disease; approximately 40 cases have been described, mainly observed in Japan (206,207,211,212). It was initially considered a clinical variant of NPS, but morphologic changes are different, and clinical findings and family studies have excluded this hypothesis.

### Pathologic Findings

In contrast to NPS glomerulopathy, diffuse glomerular changes are universally observed by light microscopy. They consist of marked enlargement of the glomerular tuft resulting from both expansion of the mesangial matrix and thickening of the capillary walls (Fig. 13.25). On periodic acid-Schiff stain, the thickening is owing to the presence of poorly stained subendothelial material associated with mesangial interposition. The lesion may mimic diffuse thrombotic microangiopathy, or it may resemble type I membranoproliferative glomerulonephritis if mesangial hypercellularity is associated. With progression of the disease, severe narrowing of the capillary lumens is observed, and the lesion may mimic diabetic glomerulopathy or amyloidosis. Conventional immunofluorescence is negative (211,212) or shows focal nonspecific deposits of immunoglobulins (mainly IgM) and complement components (205–209).

The hallmark of the disease is the presence of type III fibrillar collagen in the glomerular extracellular matrix. But using standard electron microscopy, the lesion is not readily visible. Double-contoured appearance of the GBM is observed after methenamine silver stain (Fig. 13.26). After classical uranyl acetate-lead citrate staining, the mesangial matrix and the subendothelial aspect of the GBM are enlarged and have a clear, mottled appearance, and endothelial cells are hypertrophied (Fig. 13.27). Bundles of fibrillar collagen are revealed by phosphotungstic or tannic acid treatment of the sections (Fig. 13.28A). Accumulation of collagen fibers is often massive in adult patients, mostly of Asian origin, but unlike the lesions observed in NPS, the lamina densa is usually preserved. In two children, however, bundles of collagen fibers have been seen in the lamina densa and the lamina rara interna and externa, mimicking the NPS lesions. Immunohistochemistry at the light or electron microscopic level shows mesangial and peripheral capillary loop staining with anti–type III collagen (Figs. 13.28B and 13.29). It was associated with type V collagen deposition in one patient (211). The huge accumulation of type III collagen, normally absent in the glomerular extracellular matrix, is different from the focal distribution detected in about one third of the patients with various types of glomerulopathies (216).

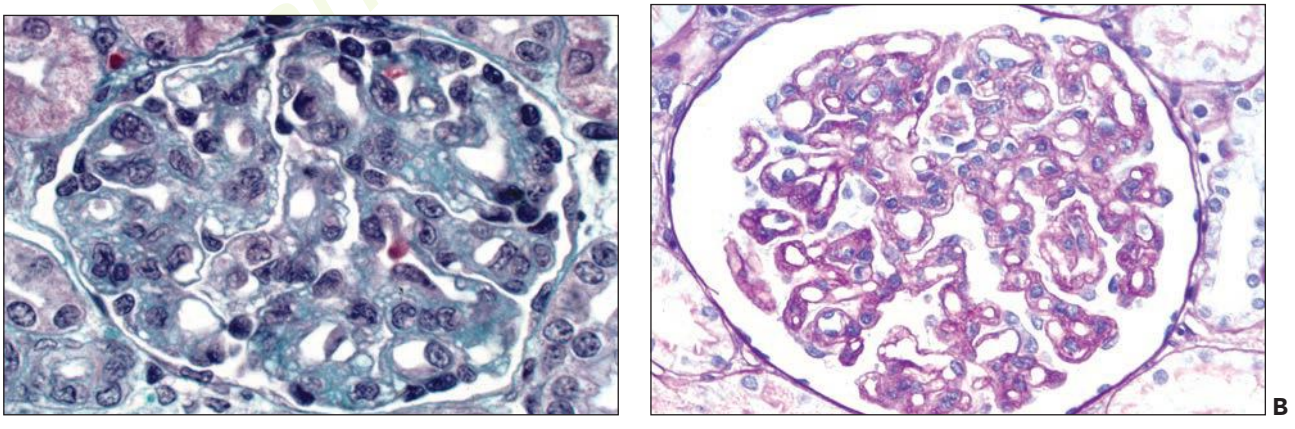



**FIGURE 13.25 Light microscopy.** Collagen type III glomerulopathy in a 9-year-old boy. **A:** Note the diffuse thickening of the glomerular capillary walls by a clear subendothelial material, the parietal expansion of the mesangial matrix, and the endothelial hypertrophy. (Trichrome light green, ×450.) **B:** There is a double-contoured appearance of the glomerular capillary wall due to focal mesangial interposition and the presence of a clear PAS-negative material. Podocyte hypertrophy. (PAS, ×450.)

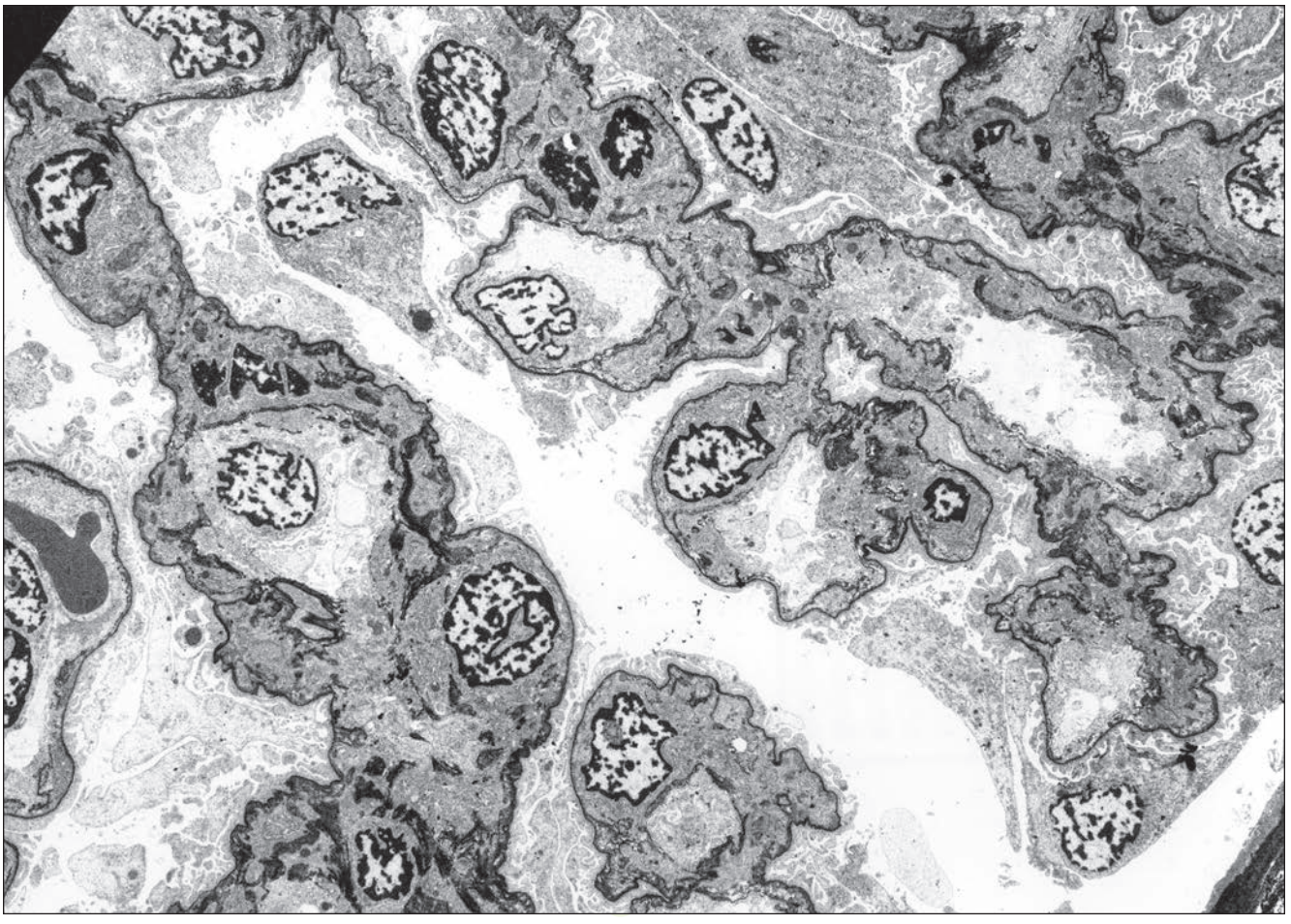


**FIGURE 13.26 Electron microscopy from a 10-year-old boy with collagen type III glomerulopathy.** Irregular thickening of the glomerular capillary wall is mostly due to mesangial interposition. Focal enlargement of the subendothelial aspect of the GBM is also present, as well as endothelial cell hypertrophy. (Methenamine silver stain ×2250.)

## Clinical Features

Collagen type III glomerulopathy affects both sexes. The first symptoms are detected between infancy and late adulthood. Extrarenal symptoms, such as those seen in NPS, are absent in the patients and their family members. According to the geographic origin and the age at presentation, two types of evolution have been observed. In Japanese and in a few Caucasian patients, symptoms are first detected in adulthood, mostly after 40 years of age. They usually consist of persistent proteinuria, without hematuria, with or without hypertension. Proteinuria and serum creatinine concentration increase slowly, suggesting that renal dysfunction is a late event. The disease is usually sporadic, but it has been observed in one pair of siblings in two families (211).

The natural history of the disease is more severe when first symptoms occur in early childhood (205,209,210). Progressive increase in proteinuria, eventually leading to nephrotic syndrome, early occurrence of severe hypertension, and progression to renal failure are observed in most children. In some of them, abrupt precipitation to ESRD was attributable to superimposed hemolytic-uremic syndrome (209). Anemia of the hemolytic type or unexplained respiratory symptoms has been observed in some patients (209). A major feature of the disease in children is the familial occurrence of the nephropathy, suggesting an autosomal recessive transmission of the disease: (a) of the 12 patients reported, 7 belonged to pairs of siblings; (b) they were first cousins in two families; and (c) parents were unaffected (205,209). Interestingly, collagen type III glomerulopathy has been reported in one patient presenting with inherited factor H deficiency (210), and this association was also observed in our laboratory in a young patient from consanguineous parents.

## Nature of the Defect

The nature of the primary defect(s) is still unknown. It may differ according to the ethnic origin, the age at onset of the disease, and the family history. Actually, analysis of reported cases suggests that collagen type III glomerulopathy includes two different disease entities. In one affecting mostly adults from Asia, the disease is slowly progressive despite the massive accumulation of type III collagen leading to severe narrowing of the capillary lumens, and most cases are sporadic. In the second affecting children with a possible autosomal

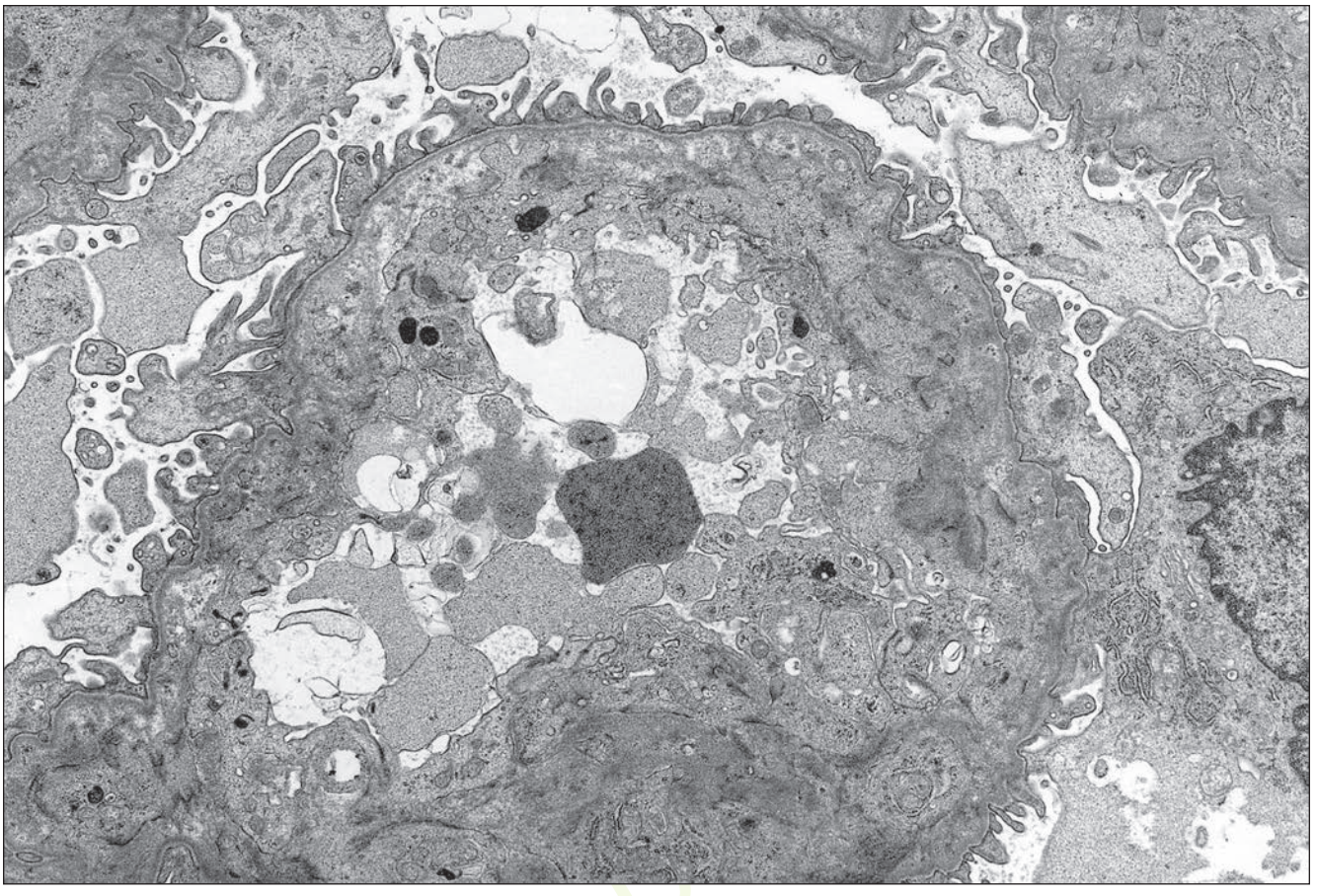
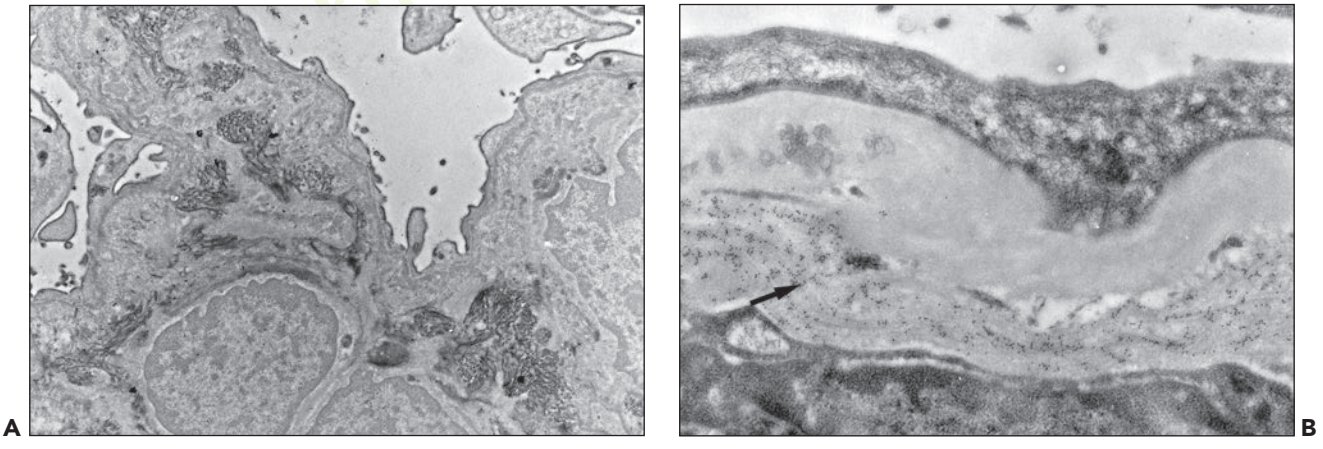


**FIGURE 13.27 Electron microscopy from a 7-year-old boy with collagen type III glomerulopathy.** There is marked thickening of the capillary wall due to both mesangial interposition and the presence of a clear fluffy material in the subendothelial space. Foot processes are preserved. Endothelial cells are hypertrophied. (Uranyl acetate-lead citrate, ×10,000.)



**A** **B**

**FIGURE 13.28 Electron microscopy in collagen type III glomerulopathy. A:** Bundles of collagen fibrils are present in the mesangial matrix and the capillary wall. (Phosphotungstic acid, ×12,000.) **B**: Immunogold staining shows that the subendothelial material is labeled with anti–type III collagen antibodies (*arrow*). (×21,000.)

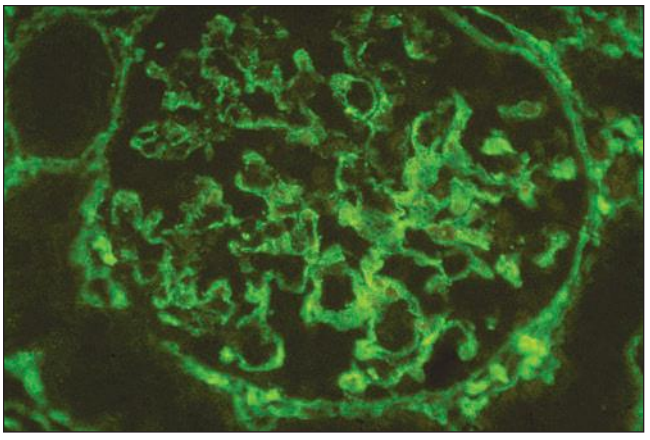


**FIGURE 13.29 Collagen type III glomerulopathy.** Immunofluorescence showing the strong mesangiocapillary labeling with anti–type III collagen antibodies. (×330.)

recessive inheritance, rapid progression to renal failure is observed, whereas type III collagen deposition is not as prominent as in adult cases. Further studies are necessary to clarify the possible link between the glomerulopathy and the defect in the complement system occasionally observed in children.

The origin of type III collagen accumulated in the glomerulus is not known. It may be due to local production or to systemic disturbance in type III collagen metabolism. Marked elevation of the serum concentration of procollagen III peptide has been observed in all patients studied (206,207,211). Serum elevation of this peptide, which is the N-terminal sequence of the procollagen molecule, is an indicator of stimulation of type III collagen synthesis. Its precise pathogenic significance in type III collagen glomerulopathy remains to be elucidated, but from a practical point of view, determination of the serum procollagen III peptide is a useful and noninvasive marker for the disease.

## PIERSON SYNDROME

In 1963, Pierson et al. (217) reported the curious association, in siblings, of eye abnormalities and congenital nephrotic syndrome progressing rapidly to ESRD. This association was further observed in a few neonates. In 2004, the analysis of two large unrelated consanguineous families, with several affected children, and the review of previously published cases allowed the delineation of a distinct entity and the identification of the genetic defect that affects the *LAMB2* gene encoding the β2 laminin chain (218,219). Laminins are important basement membrane components, involved in cell adhesion, migration, proliferation, and differentiation. They are large cross-shaped heterotrimeric glycoproteins composed of three different chains α, β, and γ. At least 15 different isoforms have been identified, based on their chain composition. Laminin-521 (for α5β2γ1) is the major laminin in the GBM. It is also expressed in ocular BM and at the neuromuscular junction.

### Pathologic Changes

By light microscopy, glomerular changes are usually severe, even in early specimens obtained soon after birth or at autopsy of fetuses after termination of pregnancy (Fig. 13.30) (218–223). They are classified as "diffuse mesangial sclerosis" but are often atypical. They are characterized by irregular thickening or thinning of the GBM with or without increase in mesangial matrix, progressing to retraction, and sclerosis of the glomerular tuft. There is no mesangial proliferation. By contrast, epithelial proliferation leading to exuberant crescent formations is frequent. Tubules are dilated, contain protein casts, and are lined by a flat epithelium. No specific immune deposits are observed. In a few patients, with delayed-onset nephrotic syndrome, lesions are initially less severe, ranging from minimal glomerular changes in one patient (224) to FSGS in others (225,226). Sequential examination of renal tissue shows rapid worsening of glomerular lesions with increase in the number of epithelial and fibrous crescents (227). Electron microscopy, performed in a few patients, showed diffuse alterations of the GBM, which appeared irregular with alternating thick and thin zones, splitting of the lamina densa, and irregular outer contours that mimicked AS GBM lesions (Figs. 13.31 and 13.32) (226,227).

### Clinical Features

Congenital/infantile nephrotic syndrome, with massive proteinuria and rapid progression to ESRD leading to death in the neonatal period, has been described in most patients (review in 223). Moreover, prenatal renal involvement was demonstrated in three siblings in whom renal enlargement and hyperechogenicity or hydrops fetalis were detected as early as 20 weeks of gestation (218). Conversely, childhood-onset nephrotic syndrome has been observed in a few patients (223,226,228–230), and two of them had not progressed to renal failure at 6 and 14 years, respectively (226,230).

Ocular abnormalities were present in all cases except in two patients in one family (225). The most characteristic lesion is microcoria (extremely narrow, nonreactive pupils). But other anomalies have been described. They included enlarged or large-appearing cornea, in some cases suggesting buphthalmos, lens abnormalities, chorioretinal pigmentary changes, retinal detachment, iris hypoplasia, and glaucoma, resulting in blindness in some children (221,229). Neuromuscular symptoms may develop in some surviving patients. They consisted in hypotonia, myasthenic syndrome, and psychomotor retardation (220,222).

### Genetics and Nature of the Defect

Based on homozygosity mapping in two consanguineous families, a candidate region encompassing the *LAMB2* gene was localized on chromosome 3p (218). *LAMB2* was considered a strong candidate because of the known renal and extrarenal expression of laminin β2 (previously named S-laminin) and the consequence of *lamb2* disruption in mice: they develop massive proteinuria and have abnormal retinal and neuromuscular differentiation (231,232).

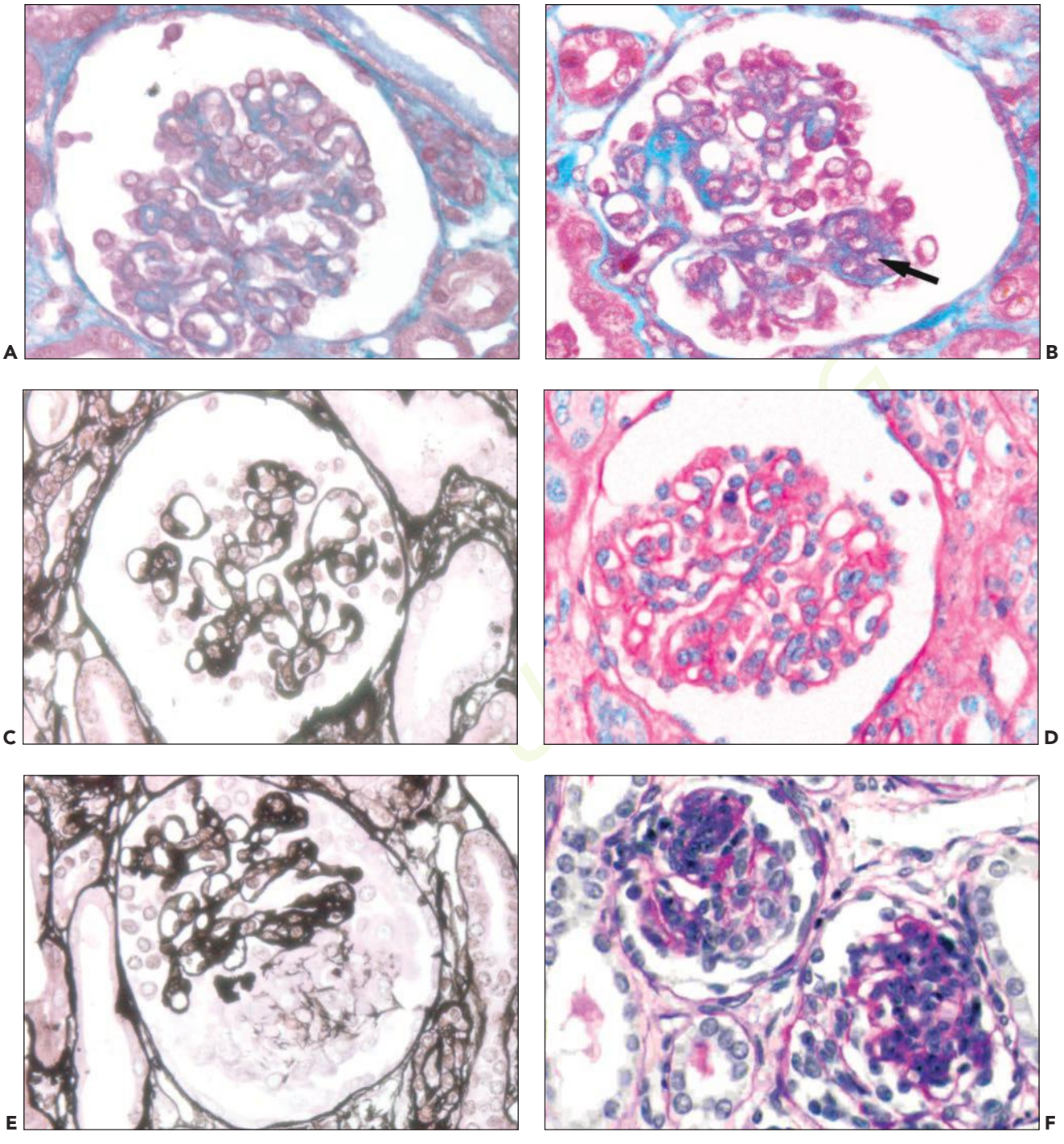



**FIGURE 13.30** **Light microscopy.** Various types of glomerular changes observed in infants 1 to 6 months of age affected with Pierson syndrome. **A:** Increase in the mesangial matrix and podocyte hypertrophy. **B:** Same features with the presence of a small lesion of focal segmental glomerulosclerosis (*arrow*). **C:** Irregular increase in mesangial matrix and GBM thickness. **D:** Rigidity and thickening of the GBM. In **A–D,** the Bowman space is enlarged, partly due to some retraction of the glomerular tuft. **E–F:** Cellular crescents superimposed on the glomerular tuft changes. (**A, B:** trichrome *light green*, ×330; **C, E:** *methenamine silver*, ×330; **D:** PAS, ×330; **F**: PAS, ×200.) . (Courtesy of Dr. Raymonde Bouvier.)

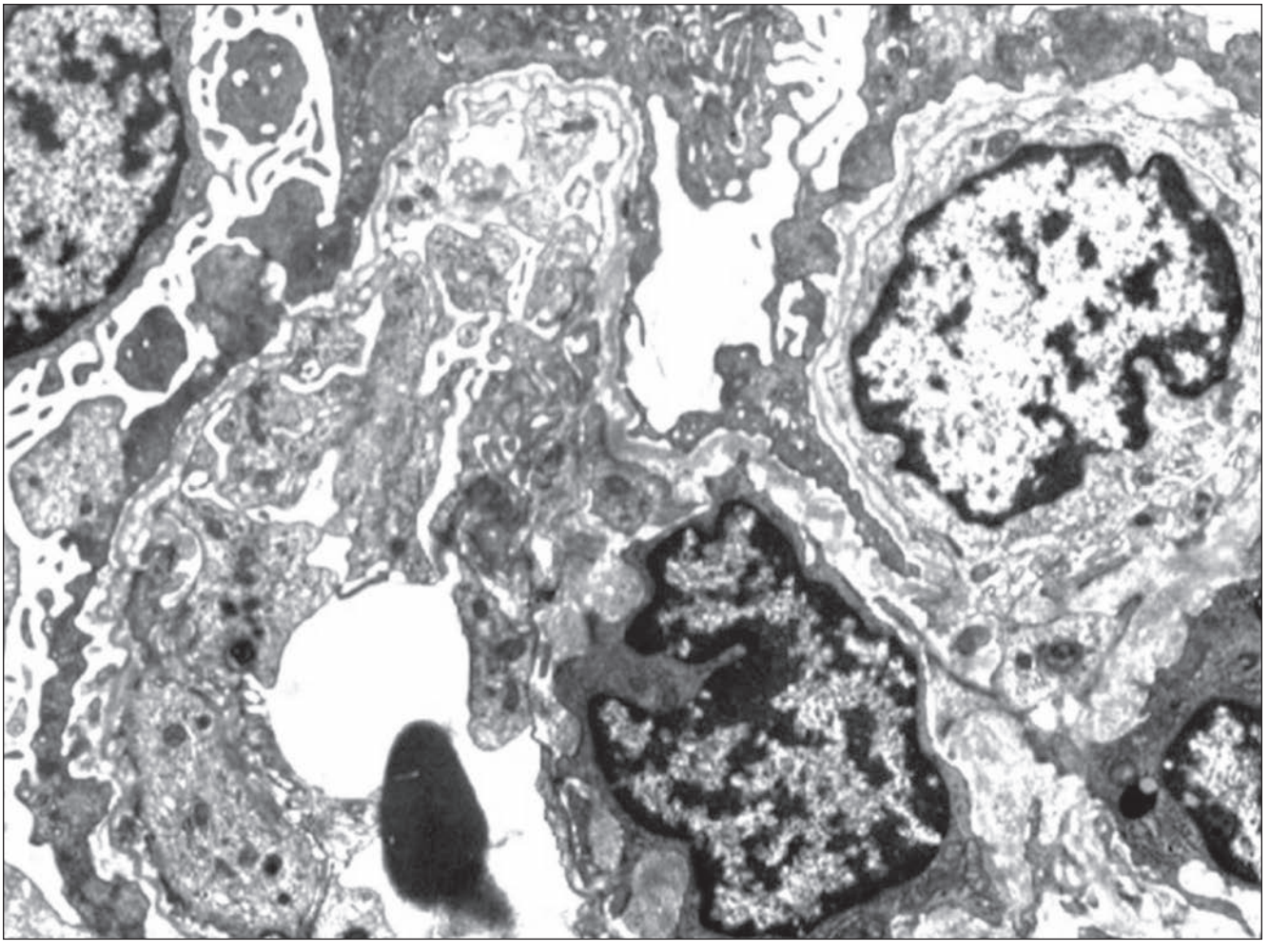


**FIGURE 13.31 Pierson syndrome.** Electron microscopy from a 1-month-old baby with congenital nephrotic syndrome. There are irregular thickening and duplication of the GBM, effacement of foot processes, and endothelial cell hypertrophy. (Uranyl acetate-lead citrate, ×6000.). (Courtesy of Dr. Raymonde Bouvier.)

At this time, more than 50 different *LAMB2* mutations in the homozygous or compound heterozygous state have been identified in 42 unrelated families (223,228–230). The detection rate reaches 98% to 100% in typical cases indicating that Pierson syndrome is genetically homogeneous (223). All types of mutations have been observed, but most of them are truncating leading to a severe phenotype and the absence of renal, muscle, and ocular expression of the β2 chain (219,222,223). Less severe mutations (missense or small in-frame deletions), localized in the laminin domain of the β2 chain important for interaction with α and γ laminin chains, were detected in patients with congenital or delayed renal disease and milder extrarenal features (223,228–230). Residual expression of laminin β2 may be seen in the GBM (230). In a mouse model of Pierson syndrome linked to the R246Q mutation, laminin-521 secretion was shown to be impaired, and improvement was obtained by increasing the expression of the mutant protein (233). Similarly, forced expression of the laminin β1 chain in mice lacking the β2 chain improves laminin secretion, glomerular structure, and permselectivity (234).

## CONCLUSION

The list of hereditary nephropathies linked to GBM defects is probably not yet complete. At this time, there is no known human nephropathy linked to perlecan, agrin, or entactin/nidogen defect. It has been shown that glomerular filtration is normal in mice lacking both perlecan and agrin in their GBM (235); however, GBM permselectivity properties are altered in transgenic mice lacking entactin-1/nidogen-1 (236). Moreover, homozygous mutation in tetraspanin (CD151), which forms stable laminin-binding complexes with integrins α3β1 and α6β1 in the kidney and α3β1 and α6β4 in the skin, has been recently identified in three patients with progressive nephritis, focal GBM irregularities, and splitting of the lamina densa, sensorineural deafness, pretibial epidermolysis bullosa, and α-thalassemia

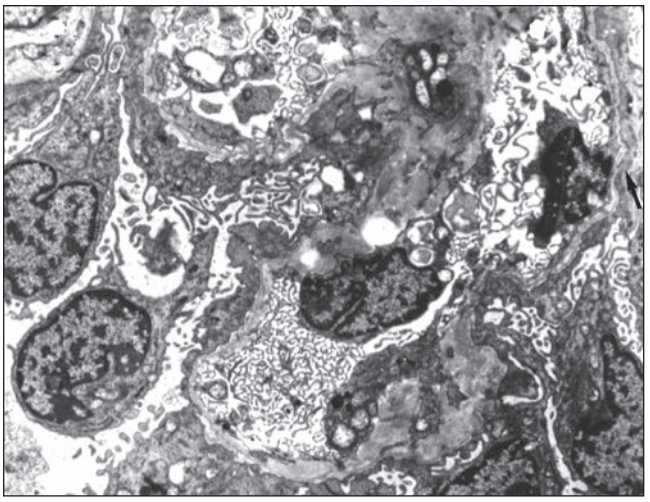


**FIGURE 13.32 Pierson syndrome.** Electron microscopy from a 5-month-old baby with congenital nephrotic syndrome. There is marked disorganization of the GBM with presence of thin segments (*arrow* at far right) and of thick segments with irregular contours. Other changes include diffuse effacement of the foot processes and hypertrophy of endothelial cells with exuberant microvilli formation. (Uranyl acetate-lead citrate, ×5000.). (Courtesy of Dr. Raymonde Bouvier.)

minor (237). Similarly, homozygous mutations in the integrin α3 gene *ITGA3* were recently identified in three unrelated infants with a severe multiorgan disease including congenital nephrotic syndrome, interstitial lung disease, and epidermolysis bullosa (238). Loss of this integrin subunit led to the skin, lung, and kidney basement abnormalities and development of FSGS.

## REFERENCES


1. Alport AC. Hereditary familial congenital haemorrhagic nephritis. *Br Med J* 1927;1:504.
2. Heptinstall RH. Alport's syndrome, nail-patella syndrome, and cyanotic congenital heart disease. In: Heptinstall RH, ed. *Pathology of the Kidney*, 4th ed. Boston, MA: Little, Brown and Company, 1992:2045.
3. Kashtan CE, Gubler MC, Sisson-Ross S, et al. Chronology of renal scarring in males with Alport syndrome. *Pediatr Nephrol* 1998;12:2458.
4. Gubler MC, Levy M, Broyer M, et al. Alport's syndrome: a report of 58 cases and a review of the literature. *Am J Med* 1981;70:493.
5. Hinglais N, Grünfeld JP, Bois E. Characteristic ultrastructural lesion of the glomerular lesion of the glomerular basement membrane in progressive hereditary nephritis (Alport's syndrome). *Lab Invest* 1972;27:473.
6. Yoshikawa N, Ito H, Matsuyama S, et al. Hereditary nephritis in children with and without characteristic glomerular basement membrane alterations. *Clin Nephrol* 1988;30:122.
7. Heidet L, Gubler MC. The renal lesions of Alport syndrome. *J Am Soc Nephrol* 2009;20:1210.
8. Nasr SH, Markowitz GS, Goldstein CS, et al. Hereditary nephritis mimicking immune complex-mediated glomerulonephritis. *Hum Pathol* 2006;37:547.
9. Churg J, Sherman RL. Pathologic characteristics of hereditary nephritis. *Arch Pathol* 1973;95:374.
10. Spear GS, Slusser RJ. Alport's syndrome: emphasizing electron microscopic studies of the glomerulus. *Am J Pathol* 1972;69:213.
11. Rumpelt HJ, Langer KH, Schärer K, et al. Split and extremely thin glomerular basement membranes in hereditary nephropathy (Alport's syndrome). *Virchows Arch A Pathol Anat Histol* 1974;364:225.
12. Mazzucco G, Barsotti P, Muda AO, et al. Ultrastructural and immunohistochemical findings in Alport's syndrome: a study of 108 patients from 97 Italian families with particular emphasis on *COL4A5* gene mutation correlations. *J Am Soc Nephrol* 1998;9:1023.
13. White RHR, Raafat F, Milford DV, et al. The Alport nephropathy: clinicopathological correlations. *Pediatr Nephrol* 2005;20:897.
14. Rumpelt HJ. Hereditary nephropathy (Alport syndrome): correlations of clinical data with glomerular basement membrane alterations. *Clin Nephrol* 1980;13:203.
15. Baumal R, Thorner PS, Valli VEO, et al. Renal disease in carrier female dogs with X-linked hereditary nephritis. *Am J Pathol* 1991;139:751.
16. Gubler MC, Beaufils H, Noël LH, et al. Significance of thin glomerular basement membranes in hematuric children. *Contrib Nephrol* 1990;80:141.
17. Arrondel C, Deschenes G, Le Meur Y, et al. Characterization of a large tandem duplication within the *COL4A5* gene as the founder mutation responsible for the high prevalence of Alport syndrome in French Polynesia. *Kidney Int* 2004;65:2030.
18. Petterson E, Törnroth T, Wieslander J. Abnormally thin glomerular basement membrane and the Goodpasture epitope. *Clin Nephrol* 1990;33:105.
19. Moghal NE, Milford DV, White RH, et al. Coexistence of thin membrane and Alport nephropathies in families with haematuria. *Pediatr Nephrol* 1999;13:778.
20. Hill GS, Jenis EH, Goodloe S. The nonspecificity of the ultrastructural alterations in hereditary nephritis. *Lab Invest* 1974;31:516.
21. Akhtar M, Al-Sabban E. Alport-like glomerular changes in a patient with nephrotic syndrome: report of a case. *Pediatr Nephrol* 2000;14:973.
22. Ito S, Hataya H, Ikeda M, et al. Alport syndrome-like basement membrane changes in Frasier syndrome: an electron microscopy study. *Am J Kidney Dis* 2003;41:1110.
23. Chevalier C, Colon S, Faraj G, et al. Glomérulopathie et hypomélanose de Ito. *Nephrologie* 1997;18:125.
24. Merchant SN, Burgess BJ, Adams JC, et al. Temporal bone histopathology in Alport syndrome. *Laryngoscope* 2004;114:1609.
25. Streeten BW, Robinson MR, Wallace R, et al. Lens capsule abnormalities in Alport's syndrome. *Arch Ophthalmol* 1987;105:1693.
26. Ohkubo S, Takeda H, Higashide T, et al. Immunohistochemical and molecular genetic evidence for type IV collagen alpha 5 chain abnormality in the anterior lenticonus associated with Alport syndrome. *Arch Ophthalmol* 2003;121:846.
27. Savige J, Liu J, DeBuc DC, et al. Retinal basement membrane abnormalities and the retinopathy of Alport syndrome. *Invest Ophthalmol Vis Sci* 2010;51:1621.
28. Kashtan C, Fish AJ, Kleppel M, et al. Nephritogenic antigen determinants in epidermal and renal basement membranes of kindreds with Alport-type familial nephritis. *J Clin Invest* 1986;78:10356.
29. Nakanishi K, Yoshikawa N, Iijima K, et al. Immunohistochemical study of α1-5 chains of type IV collagen in hereditary nephritis. *Kidney Int* 1994;46:1413.
30. Timpl R. Structure and biological activity of basement membrane proteins. *Eur J Biochem* 1989;180:487.
31. Hudson BG. The molecular basis of Goodpasture and Alport syndromes: beacons for the discovery of the collagen IV family. *J Am Soc Nephrol* 2004;15:2514.
32. Soininen R, Huotari M, Hostikka SL, et al. The structural genes for alpha1 and alpha2 chains of human type IV collagen are divergently encoded on opposite DNA strands and have an overlapping promoter region. *J Biol Chem* 1988;263:17217.
33. Leinonen A, Mariyama M, Mochizuki T, et al. Complete primary structure of the human type IV collagen alpha 4(IV) chain: comparison with structure and expression of the other alpha (IV) chains. *J Biol Chem* 1994;269:26172.
34. Boye E, Mollet G, Forestier L, et al. Determination of the genomic structure of the *COL4A4* gene and of novel mutations causing autosomal recessive Alport syndrome. *Am J Hum Genet* 1998;63:1329.
35. Heidet L, Arrondel C, Cohen-Solal L, et al. Structure of the human type IV collagen gene *COL4A3* and mutations in autosomal Alport syndrome. *J Am Soc Nephrol* 2001;12:97.
36. Hostikka SL, Eddy RL, Byers MG, et al. Identification of a distinct type IV collagen α chain with restricted kidney distribution and assignment of its gene to the locus of X-linked Alport syndrome. *Proc Natl Acad Sci U S A* 1990;87:1606.

37. Myers JC, Jones TA, Pohjolainen ER, et al. Molecular cloning of alpha5(IV) collagen and assignment of the gene to the region of the X chromosome containing the Alport syndrome locus. *Am J Hum Genet* 1990;46:1024.
38. Sugimoto M, Oohashi T, Ninomiya Y. The genes *COL4A5* and *COL4A6*, coding for basement membrane collagen chains α5(IV) and α6(IV), are located head-to-head in close proximity on human chromosome Xq22 and *COL4A6* is transcribed from two alternative promoters. *Proc Natl Acad Sci U S A* 1994;91:11679.
39. Zhou J, Ding M, Zhao Z, et al. Complete primary structure of the sixth chain of human basement membrane collagen α(IV): isolation of the cDNAs for α6(IV) and comparison with five other type IV collagen chains. *J Biol Chem* 1994;269:13193.
40. Kalluri R, Shield CF, Todd P, et al. Isoform switching of type IV collagen is developmentally arrested in X-linked Alport syndrome leading to increased susceptibility of renal basement membranes to endoproteolysis. *J Clin Invest* 1997;99:2470.
41. Miner JH, Sanes JR. Collagen IV α2, α4, and α5 chains in rodent basal laminae: sequence, distribution, association with laminins, and developmental switches. *J Cell Biol* 1994;127:879.
42. Yoshioka K, Hino S, Takemura T, et al. Type IV collagen α5 chain: normal distribution and abnormalities in X-linked Alport syndrome revealed by monoclonal antibodies. *Am J Pathol* 1994;144:986.
43. Abrahamson DR, Hudson BG, Stroganova L, et al. Cellular origin of type IV collagen networks in developing glomeruli. *J Am Soc Nephrol* 2009;20:1471.
44. Cheong HI, Kashtan CE, Kim Y, et al. Immunohistologic studies of type IV collagen in anterior lens capsules of patients with Alport syndrome. *Lab Invest* 1994;70:553.
45. Cosgrove D, Samuelson G, Pinnt J. Immunohistochemical localization of basement membrane collagens and associated proteins in the murine cochlea. *Hear Res* 1996;97:54.
46. Harvey SJ, Mount R, Sado Y, et al. The inner ear of dogs with X-linked nephritis provides clues to the pathogenesis of hearing loss in X-linked Alport syndrome. *Am J Pathol* 2001;159:1097.
47. Zehnder AF, Adams JC, Santi PA, et al. Distribution of type IV collagen in the cochlea in Alport syndrome. *Arch Otolaryngol Head Neck Surg* 2005;131:1007.
48. Peissel B, Geng L, Kalluri R, et al. Comparative distribution of the α1(IV), α5(IV), and α6(IV) collagen chains in normal human adult and fetal tissues and in kidneys from X-linked Alport syndrome patients. *J Clin Invest* 1995;96:1948.
49. Borza DB, Bondar O, Ninomiya Y, et al. The NC1 domain of collagen IV encodes a novel network composed of the alpha 1, alpha 2, alpha 5, and alpha 6 chains in smooth muscle basement membranes. *J Biol Chem* 2001;276:28532.
50. Feingold J, Bois E, Chompret A, et al. Genetic heterogeneity of Alport syndrome. *Kidney Int* 1985;27:672.
51. Jais JP, Knebelmann B, Giatras I, et al. X-linked Alport syndrome: natural history in 195 families and genotype-phenotype correlations in males. *J Am Soc Nephrol* 2000;11:649.
52. Jais JP, Knebelmann B, Giatras I, et al. X-linked Alport syndrome: natural history and genotype-phenotype correlations in girls and women belonging to 195 families: a "European Community Alport Syndrome Concerted Action" study. *J Am Soc Nephrol* 2003;14:2603.
53. Crawfurd MD, Toghill PJ. Alport's syndrome of hereditary nephritis and deafness. *Q J Med* 1968;25:562.
54. Rheault NN. Women and Alport syndrome. *Pediatr Nephrol* 2012;27:41.
55. Nielsen CE. Lenticonus anterior and Alport's syndrome. *Acta Ophthalmol* 1977;56:518.
56. Pajari H, Setala K, Heiskari N, et al. Ocular findings in 34 patients with Alport syndrome: correlations of the findings to mutations in COL4A5 gene. *Acta Ophthalmol Scand* 1999;77:214.
57. Perrin D, Junger P, Grünfeld JP, et al. Perimacular changes in Alport's syndrome. *Clin Nephrol* 1980;13:163.
58. Tan R, Colville D, Wang YY, et al. Alport retinopathy results from "severe" COL4A5 mutations and predicts early renal failure. *Clin J Am Soc Nephrol* 2010;5:34.
59. Teekhasaenee C, Nimmanit S, Wutthiphan S, et al. Posterior polymorphous dystrophy and Alport syndrome. *Ophthalmology* 1991;98:1207.
60. Rhys C, Snyers B, Pirson Y. Recurrent corneal erosion associated with Alport's syndrome. *Kidney Int* 1997;52:208.
61. Garcia-Torres R, Orozco L. Alport-Leiomyomatosis syndrome: an update. *Am J Kidney Dis* 1993;22:641.
62. Dahan K, Heidet L, Zhou J, et al. Smooth muscle tumors associated with X-linked Alport syndrome: carrier detection in females. *Kidney Int* 1995;48:1900.
63. Heidet L, Dahan K, Zhou J, et al. Deletions of both α5(IV) and α6(IV) collagen genes in Alport syndrome and in smooth muscle cell proliferation associated with Alport syndrome. *Hum Mol Genet* 1995;4:99.
64. Kashtan CE, Segal Y, Flinter F, et al. Aortic abnormalities in males with Alport syndrome. *Nephrol Dial Transplant* 2010;25:3554.
65. Vitelli F, Piccini M, Caroli F, et al. Identification and characterization of a highly conserved protein absent in the Alport syndrome (A), mental retardation (M), midface hypoplasia (M), and elliptocytosis (E) contiguous gene deletion syndrome (AMME). *Genomics* 1999;55:335.
66. McCoy RC, Johnson KH, Stone WJ, et al. Absence of nephritogenic GBM antigen(s) in some patients with hereditary nephritis. *Kidney Int* 1982;21:642.
67. Olson DL, Anand SK, Landing BH, et al. Diagnosis of hereditary nephritis by failure of glomeruli to bind anti-glomerular basement membrane antibodies. *J Pediatr* 1980;96:697.
68. Jeraj K, Kim Y, Vernier RL, et al. Absence of Goodpasture's antigen in male patients with familial nephritis. *Am J Kidney Dis* 1983;2:626.
69. Kashtan C, Kim Y, Lees GE, et al. Abnormal glomerular basement membrane laminins in murine, canine, and human Alport syndrome: aberrant laminin α2 deposition is species independent. *J Am Soc Nephrol* 2001;12:252.
70. Ding J, Yang J, Liu J, et al. Immunofluorescence study of type IV collagen alpha chains in epidermal basement membrane: application in diagnosis of X-linked Alport syndrome. *Chin Med J (Engl)* 1997;110:584.
71. Patey-Mariaud de Serre N, Garfa M, Bessiéres B, et al. Collagen alpha5 and alpha2(IV) chain coexpression: analysis of skin biopsies of Alport patients. *Kidney Int* 2007;72:512.
72. Nakanishi K, Iijima K, Kuroda N, et al. Comparison of α5(IV) collagen chain expression in skin with disease severity in women with X-linked Alport syndrome. *J Am Soc Nephrol* 1998;9:1433.
73. Massella L, Onetti Muda A, Faraggiana T, et al. Epidermal basement membrane α5(IV) expression in females with Alport syndrome and severity of renal disease. *Kidney Int* 2003;64:1787.
74. Sharp A, Robinson D, Jacobs P. Age- and tissue-specific variation of X-chromosome inactivation ratios in normal women. *Hum Genet* 1994;107:343.
75. Heidet L, Cai Y, Sado Y, et al. Diffuse leiomyomatosis associated with X-linked Alport syndrome: extracellular matrix study using immunohistochemistry and in situ hybridization. *Lab Invest* 1997;76:233.
76. Lees GL, Helman G, Kashtan CE, et al. New form of X-linked dominant hereditary nephritis in dogs. *Am J Vet Res* 1999;60:373.
77. Rheault MN, Kren SM, Thielen BK, et al. Mouse model of X-linked Alport syndrome. *J Am Soc Nephrol* 2004;15:1466.
78. Atkin CL, Hasstedt SJ, Menlove L, et al. Mapping of Alport syndrome to the long arm of the X chromosome. *Am J Hum Genet* 1988;42:249.
79. Barker DF, Hostikka SL, Zhou J, et al. Identification of mutations in the *COL4A5* collagen gene in Alport syndrome. *Science* 1990;247:1224.
80. Gross O, Netzer K-O, Lambrecht R, et al. Meta-analysis of genotype-phenotype correlation in X-linked Alport syndrome: impact on clinical counselling. *Nephrol Dial Transplant* 2002;17:1218.
81. Bekheirnia MR, Reed B, Gregory M, et al. Genotype-phenotype correlation in X-linked Alport syndrome. *J Am Soc Nephrol* 2010;21:876.
82. Crockett D, Pont-Kingdon G, Gedge F, et al. The Alport syndrome COL4A5 variant database. *Hum Mutat* 2010;31:E1652.
83. Barker DF, Pruchno CJ, Jiang X, et al. A mutation causing Alport syndrome with tardive hearing loss is common in the Western United States. *Am J Hum Genet* 1996;58:1157.
84. Martin P, Heiskari N, Zhou J, et al. High mutation detection rate in the *COL4A5* collagen gene in suspected Alport syndrome using PCR and direct DNA sequencing. *J Am Soc Nephrol* 1998;9:2291.
85. Inoue Y, Nishio H, Shirakawa T, et al. Detection of mutations in the *COL4A5* gene in over 90% of male patients with X-linked Alport's syndrome by RT-PCR and direct sequencing. *Am J Kidney Dis* 1999;34:854.
86. Wang F, Wang YF, Ding J, et al. Detection of mutations in the *COL4A5* gene by analyzing cDNA of skin fibroblasts. *Kidney Int* 2005;67:1268.

87. Tazón-Vega B, Ars E, Burset M, et al. Genetic testing for X-linked Alport syndrome by direct sequencing of COL4A5 cDNA from hair root RNA samples. *Am J Kidney Dis* 2007;50:257.
88. Artuso R, Fallerini C, Dosa L, et al. Advances in Alport syndrome diagnosis using next-generation sequencing. *Eur J Hum Genet* 2012;20:50.
89. Mochizuki T, Lemmink HH, Mariyama M, et al. Identification of mutations in the α3(IV) and α4(IV) collagen genes in autosomal recessive Alport syndrome. *Nat Genet* 1994;8:77.
90. Lemmink HH, Mochizuki T, van den Heuvel LP, et al. Mutations in the type IV collagen α3 (*COL4A3*) gene in autosomal recessive Alport syndrome. *Hum Mol Genet* 1994;3:1269.
91. Longo I, Scala E, Mari F, et al. Autosomal recessive Alport syndrome: an in-depth clinical and molecular analysis of five families. *Nephrol Dial Transplant* 2006;21:665.
92. Gubler MC, Knebelmann B, Beziau A, et al. Autosomal recessive Alport syndrome: immunohistochemical study of type IV collagen chain distribution. *Kidney Int* 1995;47:1142.
93. Lemmink HH, Nillesen WN, Mochizuki T, et al. Benign familial hematuria due to mutation of the type IV collagen α4 chain. *J Clin Invest* 1996;98:1114.
94. Lees GE, Helman RG, Kashtan CE, et al. A model of autosomal recessive Alport syndrome in English cocker spaniel dogs. *Kidney Int* 1998;54:706.
95. Miner JH, Sanes JR. Molecular and functional defects in kidneys of mice lacking collagen α3(IV): implications for Alport syndrome. *J Cell Biol* 1996;135:1403.
96. Lu W, Phillips CL, Killen PD, et al. Insertional mutation of the collagen genes *COL4A3* and *COL4A4* in a mouse model of Alport syndrome. *Genomics* 1999;61:113.
97. Van der Loop FT, Heidet L, Timmer ED, et al. Autosomal dominant Alport syndrome caused by a *COL4A3* splice site mutation. *Kidney Int* 2000;58:1870.
98. Ciccarese M, Casu D, Wong FK, et al. Identification of a new mutation in the α4(IV) collagen gene in a family with autosomal dominant Alport syndrome and hypercholesterolaemia. *Nephrol Dial Transplant* 2001;16:2008.
99. Pescucci C, Mari F, Longo I, et al. Autosomal-dominant Alport syndrome: natural history of a disease due to *COL4A3* or *COL4A4* gene. *Kidney Int* 2004;65:1598.
100. Kharrat M, Makni S, Makni K, et al. Autosomal-dominant Alport syndrome: study of a large Tunisian family. *Saudi J Kidney Dis Transpl* 2006;17:320.
101. Marcocci E, Uliana V, Bruttini M, et al. Autosomal dominant Alport syndrome: molecular analysis of the *col4a4* gene and clinical outcome. *Nephrol Dial Transplant* 2009;24:1464.
102. Longo I, Porcedda P, Mari F, et al. COL4A3/COL4A4 mutations: from familial hematuria to autosomal-dominant or recessive Alport syndrome. *Kidney Int* 2002;61:1947.
103. Hood JC, Huxtable CR, Naito I, et al. A novel model of autosomal dominant Alport syndrome in Dalmatian dogs. *Nephrol Dial Transplant* 2002;17:2094.
104. O'Leary CA, He X, Bolton JA, et al. Haplotype sharing excludes orthologous COL4A3, COL4A4 and MYH9 loci I, hereditary nephritis in bull terriers. *Anim Genet* 2009;40:252.
105. Epstein CJ, Sahud MA, Piel CF, et al. Hereditary macrothrombocytopathia, nephritis and deafness. *Am J Med* 1972;52:299.
106. Peterson LC, Venkateswara Rao K, Crosson JT, et al. Fechtner syndrome: a variant of Alport's syndrome with leucocyte inclusions and macrothrombocytopenia. *Blood* 1985;65:397.
107. Moxey-Mims MM, Young G, Silverman A, et al. End-stage renal disease in two pediatric patients with Fechtner syndrome. *Pediatr Nephrol* 1999;13:782.
108. Seri M, Cusano R, Gangarossa S, et al. Mutations in *MYH9* result in the May-Hegglin anomaly, and Fechtner and Sebastian syndromes. The May-Hegglin/Fechtner Syndrome Consortium. *Nat Genet* 2000;26:103.
109. Arrondel C, Vodovar N, Knebelmann B, et al. Expression of the nonmuscle myosin heavy chain IIA in the human kidney and screening for *MYH9* mutations in Epstein and Fechtner syndromes. *J Am Soc Nephrol* 2002;13:65.
110. Zhang Y, Conti MA, Malide D, et al. Mouse models of MYH9-related disease: mutations in nonmuscle myosin II-A. *Blood* 2012;119:238.
111. Kopp JB, Smith MW, Nelson GW, et al. MYH9 is a major-effect risk gene for focal segmental glomerulosclerosis. *Nat Genet* 2008;40:1175.
112. Genovese G, Friedman DJ, Ross MD, et al. Association of trypanolytic ApoL1 variants with kidney disease in African Americans. *Science* 2010; 329:841.
113. Nakanishi K, Yoshikawa N, Iijima K, et al. Expression of type IV collagen α3 and α4 chain mRNA in X-linked Alport syndrome. *J Am Soc Nephrol* 1996;7:938.
114. Heidet L, Cai Y, Guicharnaud L, et al. Glomerular expression of type IV collagen chains in normal and X-linked Alport syndrome kidneys. *Am J Pathol* 2000;156:1901.
115. Heidet L, Borza DB, Jouin M, et al. A human-mouse chimera of the α3.α4.α5(IV) collagen protomer rescues the renal phenotype in Col4a3–/– mice. *Am J Pathol* 2003;163:1633.
116. Harvey SJ, Zheng K, Jefferson B, et al. Transfer of the α5(IV) collagen chain gene to smooth muscle restores in vivo expression of the α6(IV) collagen chain in a canine model of Alport syndrome. *Am J Pathol* 2003;162:873.
117. Heikkila P, Tibell A, Morita T, et al. Adenovirus-mediated transfer of type IV collagen alpha5 cDNA into swine kidney in vivo: deposition of the protein into the glomerular basement membrane. *Gene Ther* 2001;8:882.
118. Prodromidi EI, Poulsom R, Jeffery R, et al. Bone marrow-derived cells contribute to podocyte regeneration and amelioration of renal disease in a mouse model of Alport syndrome. *Stem Cells* 2006;24:2448.
119. Sugimoto H, Mundel TM, Sund M, et al. Bone marrow-derived stem cells repair basement membrane collagen defects and reverse genetic kidney disease. *Proc Natl Acad Sci U S A* 2006;103:7321.
120. Katayama K, Kawano M, Naito I, et al. Irradiation prolongs survival of Alport mice. *J Am Soc Nephrol* 2008;19:1692.
121. Rao VH, Lees GE, Kashtan CE, et al. Increased expression of MMP-2, MMP-9 (type IV collagenases/gelatinases), and MT1-MMP in canine X-linked Alport syndrome (SLAS). *Kidney Int* 2003;63:1736.
122. Rao VH, Meehan DT, Delimont D, et al. Role for macrophage metalloelastase in glomerular basement membrane damage associated with Alport syndrome. *Am J Pathol* 2006;69:32.
123. Andrews KL, Betsuyaku T, Rogers S, et al. Gelatinase B (MMP-9) is nor essential in the normal kidney and does not influence progression of renal disease in a mouse model of Alport syndrome. *Am J Pathol* 2000;157:303.
124. Zeisberg M, Khurana M, Rao VH, et al. Stage-specific action of matrix metalloproteinases influences progressive hereditary kidney disease. *PLoS Med* 2006;4:535.
125. Cosgrove D, Rodgers K, Meehan D, et al. Integrin α1β1 and transforming growth factor-β1 play distinct roles in Alport glomerular pathogenesis and serve as dual targets of metabolic therapy. *Am J Pathol* 2000;157:1649.
126. Zeisberg M, Bottiglio C, Khurana M, et al. Bone morphogenic protein-7 inhibits progression of chronic renal fibrosis associated with two genetic mouse models. *Am J Physiol Renal Physiol* 2003;285:F1060.
127. Nichichuk V, Gross O, Reichel C, et al. Delayed chemokine receptor 1 blockade prolongs survival in collagen 4A3-deficient mice with Alport syndrome. *J Am Soc Nephrol* 2005;16:977.
128. Koepke ML, Weber M, Schulze-Lohoff E, et al. Nephroprotective effect of the HMG-CoA-reductase inhibitor cerivastatin in a mouse model of progressive renal fibrosis in Alport syndrome. *Nephrol Dial Transplant* 2007;22:1062.
129. Chen D, Jefferson B, Harvey SJ, et al. Cyclosporine A slows the progressive renal disease of Alport syndrome (X-linked hereditary nephritis): results from a canine model. *J Am Soc Nephrol* 2003;14:690.
130. Callis L, Vila A, Carrera M, et al. Long-term effects of cyclosporine A in Alport's syndrome. *Kidney Int* 1999;55:1051.
131. Massella L, Muda A, Legato A, et al. Cyclosporine A treatment in patients with Alport syndrome: a single-center experience. *Pediatr Nephrol* 2010;25:1269.
132. Charbit M, Gubler MC, Dechaux M, et al. Cyclosporin therapy in patients with Alport syndrome. *Pediatr Nephrol* 2007;22:57.
133. Grodecki KM, Gains MJ, Baumal R, et al. Treatment of X-linked hereditary nephritis in Samoyed dogs with angiotensin converting enzyme (ACE) inhibitor. *J Comp Pathol* 1997;117:209.
134. Gross O, Beirowski B, Koepke ML, et al. Preemptive ramipril therapy delays renal failure and reduces renal fibrosis in *COL4A3*-knockout mice with Alport syndrome. *Kidney Int* 2003;63:764.

135. Proesmans W, VanDyck M. Enalapril in children with Alport syndrome. *Pediatr Nephrol* 2004;19:271.
136. Gross O, Licht C, Anders HJ, et al. Early angiotensin-converting enzyme inhibition in Alport syndrome delays renal failure and improves life expectancy. *Kidney Int* 2012;5:494.
137. Byrne MC, Budisavljevic MN, Fan Z, et al. Renal transplant in patients with Alport's syndrome. *Am J Kidney Dis* 2002;39:769.
138. Kashtan CE. Renal transplantation in patients with Alport syndrome. *Pediatr Transplant* 2006;10:651.
139. Quérin S, Noël LH, Grünfeld JP, et al. Linear glomerular IgG fixation in renal allografts: incidence and significance in Alport's syndrome. *Clin Nephrol* 1986;25:134.
140. Browne G, Brown PA, Tomson CR, et al. Retransplantation in Alport post-transplant anti-GBM disease. *Kidney Int* 2004;5:675.
141. Pedchenko V, Bondar O, Fogo A, et al. Molecular architecture of the Goodpasture autoantigen in anti-GBM nephritis. *N Engl J Med* 2010;363:343.
142. Brainwood D, Kashtan CE, Gubler MC, et al. Targets of alloantibodies in Alport anti-glomerular basement membrane disease after transplantation. *Kidney Int* 1998;53:762.
143. Kalluri R, Weber M, Netzer KO, et al. *COL4A5* deletion and production of posttransplant anti-α3(IV) collagen antibodies in Alport syndrome. *Kidney Int* 1994;45:721.
144. Ding J, Stitzel J, Berry P, et al. Autosomal recessive Alport syndrome: mutation in the COL4A3 gene in a woman with Alport syndrome and posttransplant antiglomerular basement membrane nephritis. *J Am Soc Nephrol* 1995;5:1714.
145. Kalluri R, Van den Heuvel LP, Smeets HJM, et al. A *COL4A3* gene mutation and posttransplant anti-α3(IV) collagen alloantibodies in Alport syndrome. *Kidney Int* 1995;47:1199.
146. Wang XP, Fogo AB, Colon S, et al. Distinct epitopes for anti-glomerular basement membrane Alport alloantibodies and Goodpasture autoantibodies within the noncollagenous domain of α3(IV) collagen: a Janus-faced antigen. *J Am Soc Nephrol* 2005;16:3563.
147. Kashtan CE, Butkowski R, Kleppel MM, et al. Posttransplant antiglomerular basement membrane nephritis in related males with Alport syndrome. *J Lab Clin Med* 1990;116:508.
148. Rogers PW, Kurtzman NA, Bunn SM, et al. Familial benign essential hematuria. *Arch Intern Med* 1973;131:257.
149. Tina L, Jenis E, Jose P, et al. The glomerular basement membrane in benign familial hematuria. *Clin Nephrol* 1982;17:1.
150. Yoshikawa N, Hashimoto H, Katayama Y, et al. The thin glomerular basement membrane in children with hematuria. *J Pathol* 1984;142:253.
151. Schröder CH, Bontemps CM, Assmann KJM, et al. Renal biopsy and family studies in 65 children with isolated hematuria. *Acta Paediatr Scand* 1990;79:630.
152. Abe S, Amagasaki Y, Konishi K, et al. Thin basement membrane syndrome in adults. *J Clin Pathol* 1987;40:318.
153. Foster K, Markowitz GS, D'Agati VD. Pathology of thin basement membrane nephropathy. *Semin Nephrol* 2005;25:149.
154. Gross O, Netzer KO, Lambrecht R, et al. Novel *COL4A4* splice defect and in-frame deletion in a large consanguine family as a genetic link between benign familial haematuria and autosomal Alport syndrome. *Nephrol Dial Transplant* 2003;18:1122.
155. Ozen S, Ertoy D, Heidet L, et al. Benign familial hematuria associated with a novel *COL4A4* mutation. *Pediatr Nephrol* 2001;16:874.
156. Badenas C, Praga M, Tazon B, et al. Mutations in the *COL4A4* and *COL4A3* genes cause familial benign hematuria. *J Am Soc Nephrol* 2002;13:1248.
157. Buzza M, Dagher H, Wang YY, et al. Mutations in the *COL4A4* gene in thin basement membrane nephropathy. *Kidney Int* 2003;63:447.
158. Wang YY, Rana K, Tonna S, et al. *COL4A3* mutations and their clinical consequences in thin basement membrane disease. *Kidney Int* 2004;65:786.
159. Yamazaki H, Nakagawa Y, Saito A, et al. No linkage to *COL4A3* gene locus in Japanese thin basement membrane disease families. *Nephrology* 1995;1:315.
160. Piccini M, Casari G, Zhou J, et al. Evidence for genetic heterogeneity in benign familial hematuria. *Am J Nephrol* 1999;19:464.
161. Slajpah M, Gorinsek B, Berginc G, et al. Sixteen novel mutations identified in COL4A3, COL4A4, and COL4A5 genes in Slovenian families with Alport syndrome and benign familial hematuria. *Kidney Int* 2007;71:1287.
162. Kaneko K, Tanaka S, Hasui M. A family with X-linked benign familial hematuria. *Pediatr Nephrol* 2010;25:545.
163. Zhang KW, Tonna S, Wang YY, et al. Do mutations in COL4A1 or COL4A2 cause thin basement membrane nephropathy? *Pediatr Nephrol* 2007;22:645.
164. Gould DB, Phalan FC, Breedveld GJ, et al. Mutations in Col4a1 cause perinatal cerebral hemorrhage and porencephaly. *Science* 2005;308:1167.
165. Plaisier E, Gribouval O, Alamowitch S, et al. COL4A1 mutations and hereditary angiopathy, nephropathy, aneurysms, and muscle cramps. *N Engl J Med* 2007;357:2687.
166. Yoneda Y, Haginoya K, Arai H, et al. De novo and inherited mutations in COL4A2, encoding the type IV collagen α2 chain cause porencephaly. *Am J Hum Genet* 2012;90:86.
167. Tryggvason K, Patrakka J. Thin basement membrane nephropathy. *J Am Soc Nephrol* 2006;17:813.
168. Aarons I, Smith PS, Davies RA, et al. Thin membrane nephropathy: a clinicopathological study. *Clin Nephrol* 1989;32:151.
169. Tiebosch ATG, Frederik PM, van Breda Vriesman PJC, et al. Thin-basement-membrane nephropathy in adults with persistent hematuria. *N Engl J Med* 1989;320:14.
170. Dische FE, Anderson VER, Keane SJ, et al. Incidence of thin membrane nephropathy: morphometric investigation of a population sample. *J Clin Pathol* 1990;43:457.
171. Haas M. Thin glomerular basement membrane nephropathy: incidence in 3471 consecutive renal biopsies examined by electron microscopy. *Arch Pathol Lab Med* 2006;30:699.
172. Steffes MW, Barbosa J, Basgen JM, et al. Quantitative glomerular morphology of the normal human kidney. *Lab Invest* 1983;49:82.
173. Vogler C, McAdams AJ, Homan SM. Glomerular basement membrane and lamina densa in infants and children: an ultrastructural evaluation. *Pediatr Nephrol* 1987;7:527.
174. Nasr SH, Markowitz GS, Valeri AM, et al. Thin basement membrane nephropathy cannot be diagnosed reliably in deparaffinized, formalin-fixed tissue. *Nephrol Dial Transplant* 2007;22:1228.
175. Nieuwhof CMG, de Heer F, de Leeuw P, et al. Thin GBM nephropathy: premature glomerular obsolescence is associated with hypertension and late onset renal failure. *Kidney Int* 1997;51:1596.
176. Voskarides K, Damianou L, Neocleous V, et al. COL4A3/COL4A4 mutations producing focal segmental glomerulosclerosis and renal failure in thin basement membrane nephropathy. *J Am Soc Nephrol* 2007;18:3004.
177. Schwartz MM. Focal segmental glomerulosclerosis. In: Jennette JC, Olson JL, Schwartz MM, et al., eds. *Heptinstall's Pathology of the Kidney*, 6th ed. Philadelphia, PA: Lippincott William & Wilkins, 2007:155.
178. Torra R, Tazon-Vega B, Ars E, et al. Collagen type IV (α3–α4) nephropathy: from isolated haematuria to renal failure. *Nephrol Dial Transplant* 2004;19:2429.
179. Frasca GM, Onetti-Muda A, Mari F, et al. Thin glomerular basement membrane disease: clinical significance of a morphological diagnosis: a collaborative study of the Italian Renal Immunopathology Group. *Nephrol Dial Transplant* 2005;20:545.
180. Voskarides K, Arsali M, Athanasiou Y, et al. Evidence that NPHS2-R229Q predisposes to proteinuria and renal failure in familial hematuria. *Pediatr Nephrol* 2012;27:675.
181. Little EM. Congenital absence or delayed development of the patella. *Lancet* 1897;2:781.
182. Meyrier A, Rizzo R, Gubler MC. The nail-patella syndrome: a review. *J Nephrol* 1990;2:133.
183. Bongers EMHF, Gubler MC, Knoers NVAM. Nail-patella syndrome: overview on clinical and molecular findings. *Pediatr Nephrol* 2002; 17:703.
184. Sweeney E, Fryer A, Mountford R, et al. Nail patella syndrome: a review of the phenotype aided by developmental biology. *J Med Genet* 2003;40:153.
185. Del Pozo E, Lapp H. Ultrastructure of the kidney in the nephropathy of the nail-patella syndrome. *Am J Clin Pathol* 1970;54:845.
186. Ben Bassat M, Cohen L, Rosenfield J. The glomerular basement membrane in the nail-patella syndrome. *Arch Pathol* 1971;92:350.
187. Looij BJ Jr, te Slaa RL, Hogewind BL, et al. Genetic counselling in hereditary osteo-onychodysplasia (HOOD, nail-patella syndrome) with nephropathy. *J Med Genet* 1988;25:682.

188. Lemley KV. Kidney disease in nail-patella syndrome. *Pediatr Nephrol* 2009;24:2345.
189. Heidet L, Bongers EMHF, Sich M, et al. In vivo expression of putative *LMX1B* targets in nail-patella syndrome kidneys. *Am J Pathol* 2003; 163:145.
190. Renwick JH, Lawler SD. Genetical linkage between the ABO and nail patella loci. *Ann Hum Genet* 1955;19:312.
191. McIntosh L, Clough MV, Schaffer AA, et al. Fine mapping of the nail-patella syndrome locus at 9q34. *Am J Hum Genet* 1997;60:133.
192. Vollrath D, Jaramillo-Babb VL, Clough MV, et al. Loss-of-function mutations in the LIM-homeodomain gene, *LMX1B*, in nail patella syndrome. *Hum Mol Genet* 1998;7:1091.
193. Chen H, Lun Y, Ovchinnokov D, et al. Limb and kidney defects in *Lmx1b* mutant mice suggest an involvement of *LMX1B* in human nail patella syndrome. *Nat Genet* 1998;19:51.
194. Dreyer SD, Zhou G, Baldini A, et al. Mutations in *LMX1B* cause abnormal skeletal patterning and renal dysplasia in nail patella syndrome. *Nat Genet* 1998;19:47.
195. McIntosh I, Dunston JA, Liu L, et al. Nail patella syndrome revisited: 50 years after linkage. *Ann Hum Genet* 2005;69:349.
196. Lee BH, Cho TJ, Choi HJ, et al. Clinico-genetic study of nail-patella syndrome. *J Korean Med Sci* 2009;24(Suppl):S82.
197. Bongers EMHF, Huysmans FT, Levtchenko E, et al. Genotype-phenotype studies in nail patella syndrome show that *LMX1B* mutation location is involved in the risk of developing nephropathy. *Eur J Hum Genet* 2005;13:1019.
198. Morello R, Zhou G, Dreyer SD, et al. Regulation of glomerular basement membrane collagen expression by *LMX1B* contributes to renal disease in nail patella syndrome. *Nat Genet* 2001;27:205.
199. Suleiman H, Heudobler D, Raschta AS, et al. The podocyte-specific inactivation of Lmx1b, Ldb1 and E2a yields new insight into a transcriptional network in podocytes. *Dev Biol* 2007;304:701.
200. Witzgall R. How are podocytes affected in nail-patella syndrome? *Pediatr Nephrol* 2008;23:1017.
201. Boute N, Gribouval O, Roselli S, et al. *NPHS2*, encoding the glomerular protein podocin, is mutated in autosomal recessive steroid-resistant nephrotic syndrome. *Nat Genet* 2000;24:349.
202. Shih NY, Li J, Karpitskii V, et al. Congenital nephrotic syndrome in mice lacking CD2-associated protein. *Science* 1999;286:312.
203. Rohr C, Prestel J, Heidet L, et al. The LIM-homeodomain transcription factor Lmx1b plays a crucial role in podocytes. *J Clin Invest* 2002;109:1073.
204. Miner JH, Morello R, Andrews KL, et al. Transcriptional induction of slit diaphragm genes by *LMX1B* is required in podocyte differentiation. *J Clin Invest* 2002;109:1065.
205. Salcedo JR. An autosomal recessive disorder with glomerular basement membrane abnormalities similar to those seen in the nail-patella syndrome: report of a kindred. *Am J Med Genet* 1984;19:579.
206. Ikeda K, Yokoyama H, Tomosugi N, et al. Primary glomerular fibrosis: a new nephropathy caused by diffuse intra-glomerular increase in atypical type III collagen fibers. *Clin Nephrol* 1990;33:155.
207. Arakawa M, Yamanaka N. *Collagenofibrotic Glomerulonephropathy*. London: Nishimura & Smith-Gordon, 1991.
208. Imbasciati E, Gherardi G, Morozumi K, et al. Collagen type III glomerulopathy: a new idiopathic glomerular disease. *Am J Nephrol* 1991;11:422.
209. Gubler MC, Dommergues JP, Foulard M, et al. Collagen type III glomerulopathy: a new type of hereditary nephropathy. *Pediatr Nephrol* 1993;7:354.
210. Vogt BE, Wyatt RJ, Burke BA, et al. Inherited factor H deficiency and collagen type III glomerulopathy. *Pediatr Nephrol* 1995;9:11.
211. Tamura H, Matsuda A, Kidoguchi N, et al. A family with two sisters with collagenofibrotic glomerulopathy. *Am J Kidney Dis* 1996;27:588.
212. Morita H, Hasegawa T, Minamoto T, et al. Collagenofibrotic glomerulopathy with a widespread expression of type-V collagen. *Virchows Arch* 2003;442:163.
213. Patro KC, Jha R, Sahay M, et al. Collagenofibrotic glomerulopathy—Case report with review of literature. *Indian J Nephrol* 2011;21:52.
214. Ferreira RD, Custódio FB, Guimarães CS, et al. Collagenofibrotic glomerulopathy: three case reports in Brazil. *Diagn Pathol* 2009;4:33.
215. Duggal R, Nada R, Rayar CS, et al. Collagenofibrotic glomerulopathy. *Clin Kidney J* 2012;5:7–12.
216. Yoshioka K, Takemura T, Tohda M. Glomerular localization of type III collagen in human kidney disease. *Kidney Int* 1989;35:1203.
217. Pierson M, Cordier J, Hervouët F, et al. Une curieuse association malformative congénitale et familiale atteignant l'œil et le rein. *J Genet Hum* 1963;12:184.
218. Zenker M, Tralau T, Lennert T, et al. Congenital nephrosis, mesangial sclerosis, and distinct eye abnormalities with microcoria: an autosomal recessive syndrome. *Am J Med Genet* 2004;130A:138.
219. Zenker M, Aigner T, Wendler O, et al. Human laminin β2 deficiency causes congenital nephrosis with mesangial sclerosis and distinct eye abnormalities (Pierson syndrome). *Hum Mol Genet* 2004;13:2625.
220. Wühl E, Kogan J, Zurowska A, et al. Neurodevelopmental deficits in Pierson (microcoria-congenital nephrosis) syndrome. *Am J Med Genet A* 2007;143:311.
221. Bredrup C, Matejas V, Barrow M, et al. Ophthalmological aspects of Pierson syndrome. *Am J Ophthalmol* 2008;146:602.
222. Maselli RA, Ng JJ, Anderson JA, et al. Mutations in LAMB2 causing a severe form of synaptic congenital myasthenic syndrome. *J Med Genet* 2009;46:203.
223. Matejas V, Hinkes B, Alkandari F, et al. Mutations in the human laminin beta2 (LAMB2) gene and the associated phenotypic spectrum. *Hum Mutat* 2010;3:992.
224. Kagan M, Cohen AH, Matejas V, et al. A milder variant of Pierson syndrome. *Pediatr Nephrol* 2008;23:323.
225. Hasselbacher K, Wiggins RC, Matejas V, et al. Recessive missense mutations in *LAMB2* expand the clinical spectrum pof LAMB2-associated disorders. *Kidney Int* 2006;70:1008.
226. Choi HJ, Lee BH, Kang JH, et al. Variable phenotype of Pierson syndrome. *Pediatr Nephrol* 2008;23:995.
227. Glastre C, Cochat P, Bouvier R, et al. Familial infantile nephrotic syndrome with ocular abnormalities. *Pediatr Nephrol* 1990;4:340.
228. Zhao D, Ding J, Wang F, et al. The first Chinese Pierson syndrome with novel mutations in LAMB2. *Nephrol Dial Transplant* 2010;25:776.
229. Mohney BG, Pulido JS, Lindor NM, et al. A novel mutation of LAMB2 in a multigenerational mennonite family reveals a new phenotypic variant of Pierson syndrome. *Ophthalmology* 2011;118:1137.
230. Lehnhardt A, Lama A, Amann K, et al. Pierson syndrome in an adolescent girl with nephrotic range proteinuria but a normal GFR. *Pediatr Nephrol* 2012;27:865.
231. Noakes PG, Miner JH, Gautam M, et al. The renal glomerulus of mice lacking S-laminin/laminin β2: nephrosis despite molecular compensation by laminin β1. *Nat Genet* 1995;10:400.
232. Noakes PG, Gautam M, Mudd J, et al. Aberrant differentiation of neuromuscular junctions in mice lacking s-laminin/laminin beta2. *Nature* 1995;338:229.
233. Chen YM, Kikkawa Y, Miner JH. A missense LAMB2 mutation causes congenital nephrotic syndrome by impairing laminin secretion. *J Am Soc Nephrol* 2011;22:849.
234. Suh JH, Jarad G, VanDeVoorde RG, et al. Forced expression of laminin beta1 in podocytes prevents nephrotic syndrome in mice lacking laminin beta2, a model for Pierson syndrome. *Proc Natl Acad Sci U S A* 2011;108:15348.
235. Goldberg S, Harvey SJ, Cunningham J, et al. Glomerular filtration is normal in the absence of both agrin and perlecan-heparan sulfate from the glomerular basement membrane. *Nephrol Dial Transplant* 2009;24:2044–2051.
236. Lebel SP, Chen Y, Gingras D, et al. Morphofunctional studies of the glomerular wall in mice lacking entactin-1. *J Histochem Cytochem* 2003;51:1467.
237. Karamatic Crew V, Burton N, Kagan A, et al. CD151, the first member of the tetraspanin (TM4) superfamily detected on erythrocytes, is essential for the correct assembly of human basement membranes in kidney and skin. *Blood* 2004;104:2217.
238. Has C, Spartà G, Kiritsi D, et al. Integrin α3 mutations with kidney, lung and skin disease. *N Engl J Med* 2012;366:1508.

CHAPTER 14

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# Renal Disease in Systemic Lupus Erythematosus, Mixed Connective Tissue Disease, Sjögren Syndrome, and Rheumatoid Arthritis



## SYSTEMIC LUPUS ERYTHEMATOSUS

### Historical Perspective

Systemic lupus erythematosus (SLE) is a chronic inflammatory autoimmune disease affecting multiple organ systems, including the skin, joints, heart, lung, kidneys, central nervous system, and serous membranes. The cutaneous manifestations of SLE were the first to be identified. As early as the 13th century, medical writings by Rogerius (*c.* 1230) and Paracelsus (*c.* 1500) proposed the Latin term *lupus*, meaning wolf, to describe the erythematous ulcerative lesions affecting the skin of the cheeks (1). However, it is Kaposi contention, in his lengthy treatise on lupus vulgaris (2), that even the lesions called *herpes esthiomenos* by Hippocrates in the fourth century BC included characteristic examples of this condition. Cazenave and Schedel are generally credited with the first illustration of discoid lupus erythematosus in 1838 (3), which depicts the bilateral butterfly distribution over the cheeks and bridge of the nose, a distribution evocatively portrayed as resembling bat wings by Jonathan Hutchinson (4) in 1870. It was not until the late 19th and early 20th centuries that the visceral manifestations of SLE were generally appreciated (5–7).

William Osler, in historic publications from 1895 through 1903, is credited with first synthesizing the diverse visceral manifestations of SLE into a loosely defined disease entity that included variable features of "gastrointestinal crises, endocarditis,

pericarditis, acute nephritis, and hemorrhage from the mucous surface." In later writings, he emphasized the arthritis, pulmonary, and central nervous system manifestations while remarking that the arthritis was typically nondeforming. He observed the variable clinical presentation and remitting and relapsing nature of the condition: "Recurrence is a special feature of the disease and attacks may come on month after month or even throughout a long period of years. . . . The attacks may not be characterized by skin manifestations; the visceral symptoms alone may be present, and to the outward view, the patient may have no indications whatever of erythema exudativum" (5).

In 1923, Libman and Sacks (8) described four cases of "a hitherto undescribed form of valvular and mural endocarditis," which we now recognize as the sterile thrombotic valvular manifestations of circulating lupus anticoagulant. The distinctive histologic features of the glomerulonephritis were first reported by Baehr et al. (9), who observed that the proliferative features were accompanied by "wire-loop" alterations of the glomerular capillary walls, which they described as a "peculiar hyaline thickening of the capillary walls which is striking even in sections stained with hematoxylin and eosin."

The hypothesis that SLE constitutes an autoimmune disorder was spurred by three major independent laboratory findings in the early 20th century. First, false-positive serologic test results for syphilis using the Wassermann reaction were obtained for up to 35% of SLE patients who lacked clinical evidence of treponemal infection, sometimes preceding the development of clinical features of SLE. It would be many years before the significance of this finding as a manifestation of antiphospholipid (APL) antibody or lupus anticoagulant syndrome was recognized. Later, Hargraves made the highly important discovery of the LE cell in bone marrow preparations of patients with active SLE. He described two types: a mature neutrophil, which he called the *LE cell*, and a histiocyte, which he called a *tart cell*. Both contained phagocytosed nuclear material in which the chromatin pattern was sometimes still discernible (10). Similar LE bodies consisting of altered hematoxylin-staining nuclear material had previously been observed in the heart valves (11), lymph nodes, spleen, and kidneys of patients with active SLE. The cause of the LE phenomenon, the presence of antinuclear antibody (ANA), would not be identified for another decade. The most important historical event in the evolution of concepts about SLE was the discovery by Coons (12) in 1951 of the immunofluorescence technique employing fluorescein-labeled antibodies and its subsequent application to the sera and tissues of SLE patients for the detection of ANAs by Friou (13) in 1958.

With the explosion of knowledge in the early 1970s about the human leukocyte antigen (HLA) system and the role of T and B cells in immunity, the groundwork was laid for investigations into the genetic, molecular, and cellular determinants of autoimmunity in SLE. Although there have been tremendous advances in our understanding of the basis of autoimmunity and the properties of nephritogenic immunoglobulins, a unifying concept of pathogenesis remains elusive.

## Introduction to Clinical Features

Among the diverse organ systems involved in SLE, it is the renal complications that pose the greatest risk of morbidity and mortality and present the most demanding therapeutic challenge. The renal manifestations of SLE, called *lupus nephritis*, are highly pleomorphic with respect to their clinical and morphologic expressions. Clinically, renal involvement may occur at any time in the course of SLE, ranging from the earliest identifiable clinical onset of disease to years after the initial diagnosis. The onset of renal involvement is most common within the first year and clinically affects up to 50% of patients with SLE. Many studies suggest that a much higher percentage of patients would have morphologic evidence of renal disease if renal biopsies were performed systematically on all SLE patients and if histologic studies were supplemented with more sensitive immunofluorescence and electron microscopy studies.

Clinical features range from asymptomatic urinary findings of microhematuria and mild proteinuria to full-blown nephrotic syndrome or rapidly progressive renal failure. In adults and children (14–16), the renal course is frequently complicated by periods of remission and exacerbation that may occur unpredictably at any time in the course of the disease. Moreover, the nephritis has the ability to transform from one morphologic pattern to another spontaneously or after treatment. Because of this heterogeneity of clinical renal manifestations, the typical presenting clinical features will be described separately for each of the morphologic subtypes of lupus nephritis.

## Introduction to Classification

The pathologic manifestations of lupus nephritis are extremely diverse and may affect any or all renal compartments, including glomeruli, tubules, interstitium, and blood vessels. The complexity of the protean renal manifestations of SLE can be most easily approached using the World Health Organization (WHO) classification as an organizational construct. The original WHO Classification was formulated in 1974 at a convocation of renal pathologists and nephrologists in Buffalo, NY, under the auspices of the WHO (17). The classification that they devised has been the framework most widely used by practicing clinicians and pathologists (18) (Table 14.1). In 1982, this original WHO classification was expanded and refined by the International Study of Kidney Disease in Children (ISKDC) (19), with further modifications in 1995 (20)

**TABLE 14.1 Original (1974) WHO classification of lupus nephritis**

| | |
|---|---|
| **Class I** | Normal glomeruli (by LM, IF, EM) |
| **Class II** | Purely mesangial disease<br>a. Normocellular mesangium by LM but mesangial deposits by IF and/or EM<br>b. Mesangial hypercellularity with mesangial deposits by IF and/or EM |
| **Class III** | Focal segmental proliferative glomerulonephritis (<50%) |
| **Class IV** | Diffuse proliferative glomerulonephritis (≥50%) |
| **Class V** | Membranous glomerulonephritis |

LM, light microscopy; IF, immunofluorescence; EM, electron microscopy.

**TABLE 14.2 Modified (1982) WHO classification of lupus nephritis**

| | |
|---|---|
| **Class I** | a. Normal glomeruli (by LM, IF, EM)<br>b. Normal glomeruli by LM but deposits seen by IF and/or EM |
| **Class II** | Purely mesangial alterations (mesangiopathy)<br>a. Mesangial widening and/or mild hypercellularity (+)<br>b. Moderate hypercellularity (++) |
| **Class III** | Focal segmental glomerulonephritis (associated with mild or moderate mesangial alterations)<br>a. With active necrotizing lesions<br>b. With active and sclerosing lesions<br>c. With sclerosing lesions |
| **Class IV** | Diffuse glomerulonephritis (severe mesangial, endocapillary, or mesangiocapillary proliferation and/or extensive subendothelial deposits). Mesangial deposits are present invariably and subepithelial deposits often and may be numerous<br>a. Without segmental lesions<br>b. With active necrotizing lesions<br>c. With active and sclerosing lesions<br>d. With sclerosing lesions |
| **Class V** | Membranous glomerulonephritis<br>a. Pure membranous glomerulonephritis<br>b. Associated with lesions of category II (a or b)<br>c. Associated with lesions of category III (a, b, or c)[a]<br>d. Associated with lesions of category IV (a, b, c, or d)[a] |
| **Class VI** | Advanced sclerosing glomerulonephritis |

[a]Deleted from the 1995 Modified WHO Classification.

LM, light microscopy; IF, immunofluorescence; EM, electron microscopy.

Modified from Churg J, Sobin LH. *Renal Disease. Classification and Atlas of Glomerular Disease.* Tokyo: Igaku-Shoin, 1982.

(Table 14.2). It defined six major classes and a large number of subclasses, with emphasis on distribution, activity, and chronicity of the lesions. Although much more detailed and precise, this revised classification was not as widely accepted because of its cumbersome reliance on subclasses. A third classification was proposed in 2003 by a large consensus conference of renal pathologists, nephrologists, and rheumatologists that was organized jointly by the International Society of Nephrology (ISN) and the Renal Pathology Society (RPS) (Table 14.3). This new classification, which has been entitled "ISN/RPS WHO classification revisited," retains the simplicity of the original 1974 WHO classification while incorporating some of the refinements introduced by the 1982 revised WHO classification (21,22). The ISN/RPS system provides more detailed and more precise pathologic criteria for each class, which facilitates greater reproducibility among pathologists. This updated consensus classification is widely accepted and has superseded prior classifications.

All three classifications are based entirely on an assessment of the glomerular alterations. They rely heavily on the light microscopic findings, while simultaneously integrating information obtained by fluorescence and electron microscopic studies (Table 14.4). Accurate use of the WHO system is best achieved if the same pathologist studies the biopsy by the three modalities of light microscopy, immunofluorescence microscopy, and electron microscopy, especially because the glomerular sampling of relatively focal lesions may not be equally represented in tissue processed for the three techniques. The first step is to determine the presence or absence of glomerular hypercellularity in the mesangial and endocapillary zones and the extent of glomerular involvement. These findings are then interpreted in the context of the distribution of immune deposits in mesangial, subendothelial, and subepithelial locations as detected by light microscopy, immunofluorescence microscopy, and electron microscopy (Fig. 14.1). Because some lesions are focal, the accuracy of the WHO classification depends on the adequacy of the glomerular sampling. According to one study, at least 20 glomeruli were required for accurate classification of lupus nephritis (23).

Generalized adoption of the WHO system for classification of lupus nephritis in academic and community centers alike has had far-reaching impact. It has served to simplify the interpretation of the renal pathologic findings, which are notoriously pleomorphic, when comparing one biopsy to another and comparing neighboring glomeruli in a biopsy specimen. Use of this classification has facilitated the ease and reliability with which nephrologists and nephropathologists communicate information. Greatly improved standardization and reproducibility of biopsy interpretation between centers has been achieved. Most importantly, use of the WHO classification has provided the standardized nosology necessary for careful clinical pathologic studies, which have yielded valuable information about disease subsets with differing prognostic and therapeutic implications.

## Role of Renal Biopsy

Renal biopsy plays an important role in the management of patients with SLE (24). In some patients, it is instrumental in establishing a diagnosis of SLE. This is especially common early in the disease, before overt extrarenal manifestations of SLE are evident. This scenario applies most frequently to patients with mesangial proliferative or membranous patterns who lack serologic markers of SLE and may present many months or even years before the American College of Rheumatology (ACR) criteria for SLE have been met. More typically, a diagnosis of SLE has already been made prior to renal biopsy, based on presenting clinical features and confirmatory serologic tests. Renal biopsy provides the most accurate window into the kidney, because it provides information about the class, severity, activity, and chronicity of the lupus nephritis that cannot be accurately predicted on the basis of clinical manifestations (25). Based on these findings, important decisions about therapy and prognosis are made.

Indications for renal biopsy vary among centers. Few clinicians would advocate baseline renal biopsy in a newly

**TABLE 14.3 ISN/RPS classification of lupus nephritis (LN) (2004)**

| | |
|---|---|
| **Class I** | **Minimal mesangial LN**<br>Normal glomeruli by LM, but mesangial immune deposits by IF |
| **Class II** | **Mesangial proliferative LN**<br>Purely mesangial hypercellularity of any degree or mesangial matrix expansion by LM, with mesangial immune deposits<br>There may be a few isolated subepithelial or subendothelial deposits visible by IF or EM but not by LM |
| **Class III** | **Focal LN**[a]<br>Active or inactive focal, segmental, and/or global endocapillary and/or extracapillary GN involving <50% of all glomeruli, typically with focal subendothelial immune deposits, with or without mesangial alterations<br>**III (A): purely active lesions: focal proliferative LN**<br>**III (A/C): active and chronic lesions: focal proliferative and sclerosing LN**<br>**III (C): chronic inactive with glomerular scars: focal sclerosing LN** |
| **Class IV** | **Diffuse LN**[a]<br>Active or inactive diffuse, segmental, and/or global endocapillary and/or extracapillary GN involving ≥50% of all glomeruli, typically with diffuse subendothelial immune deposits, with or without mesangial alterations. This class is divided into diffuse segmental (IV-S) when >50% of the involved glomeruli have segmental lesions and diffuse global (IV-G) when >50% of the involved glomeruli have global lesions. *Segmental* is defined as a glomerular lesion that involves less than half of the glomerular tuft<br>**IV-S (A) or IV-G (A): purely active lesions: diffuse segmental or global proliferative LN**<br>**IV-S (A/C) or IV-G (A/C): active and chronic lesions: diffuse segmental or global proliferative and sclerosing LN**<br>**IV-S (C) or IV-G (C): inactive with glomerular scars: diffuse segmental or global sclerosing LN** |
| **Class V** | **Membranous LN**[b]<br>Global or segmental subepithelial immune deposits or their morphologic sequelae by LM and by IF or EM, with or without mesangial alterations |
| **Class VI** | **Advanced sclerosing LN**<br>Ninety percent or more of glomeruli globally sclerosed without residual activity |

Indicate the proportion of glomeruli with fibrinoid necrosis and with cellular crescents.

Indicate and grade (mild, moderate, severe) tubular atrophy, interstitial inflammation, and fibrosis, severity of arteriosclerosis or other vascular lesions.

[a]Indicate the proportion of glomeruli with active and with sclerotic lesions.

[b]May occur in combination with III or IV, in which case both will be diagnosed; may show advanced sclerosis.

LM, light microscopy; IF, immunofluorescence; EM, electron microscopy; GN, glomerulonephritis.

diagnosed patient with SLE who lacks clinically apparent renal disease. However, because some cases of severe lupus nephritis are clinically "silent," this liberal approach has some proponents (26). Most nephrologists and rheumatologists would agree that the new appearance of any significant marker of renal disease such as hematuria, proteinuria, nephrotic syndrome, or renal insufficiency at any time in the course of SLE is ample justification for renal biopsy. The recent ACR guidelines for management of lupus nephritis advocate that renal biopsy should be performed in any patient with clinical

**TABLE 14.4 Integration of LM, IF, and EM findings by the WHO class**

| | Light microscopy | | Immunofluorescence | | Electron microscopy | | |
|---|---|---|---|---|---|---|---|
| **Class** | **MES** | **PCW** | **MES** | **PCW** | **MES** | **SENDO** | **SEPI** |
| I | 0 | 0 | + | 0 | + | 0 | 0 |
| II | + | 0 | + | 0 | + | 0 | 0 |
| III | + | + | ++ | + | ++ | + | +/− |
| IV | ++ | ++ | ++ | ++ | ++ | ++ | +/− |
| V | + | ++ | + | ++ | + | +/− | ++ |

LM, light microscopic [abnormalities]; IF, immunofluorescence [positivity]; EM, electron microscopic [location of deposits]; MES, mesangial; PCW, peripheral capillary wall; SENDO, subendothelial; SEPI, subepithelial.

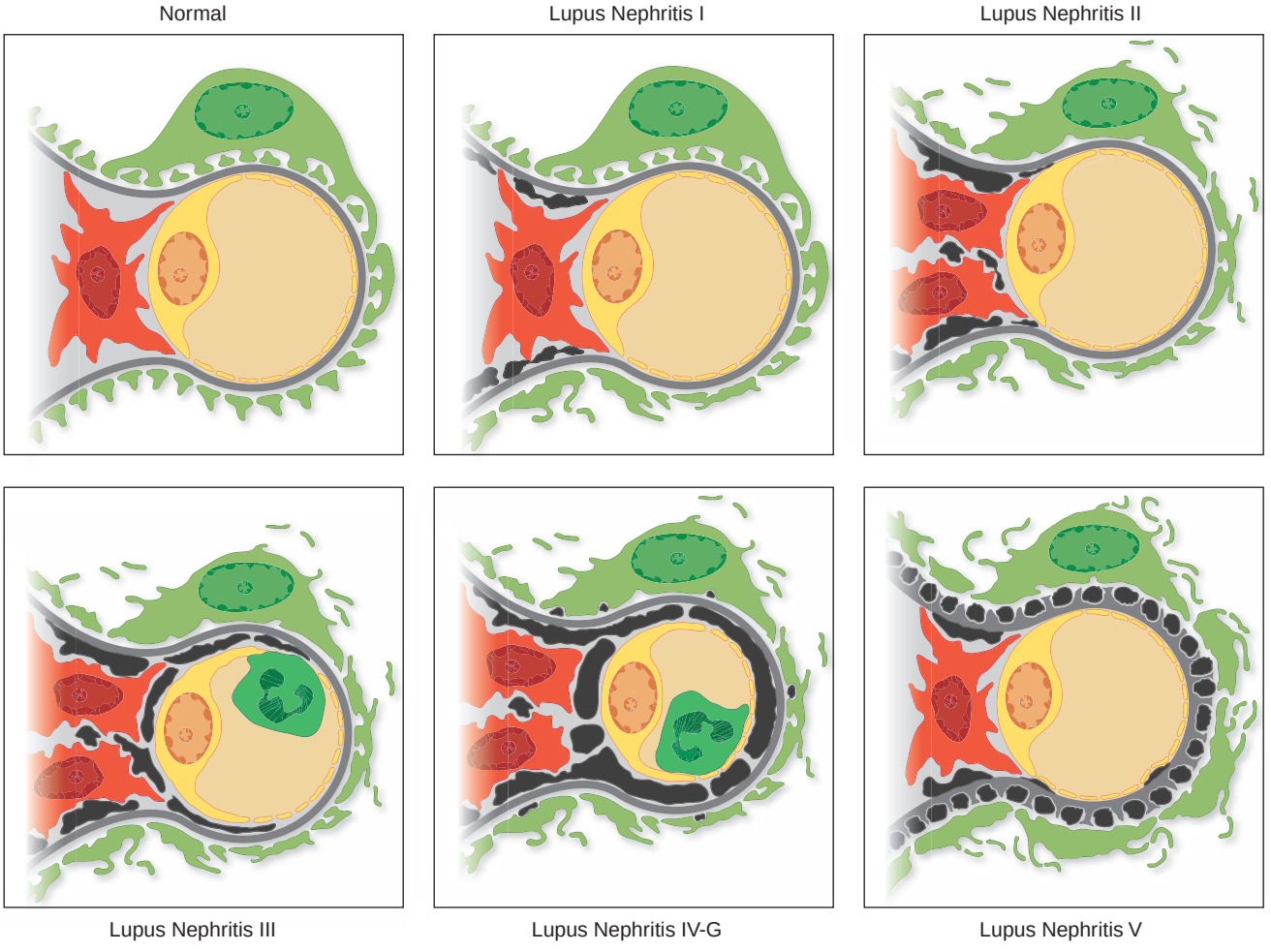



**FIGURE 14.1 Normal capillary with intact podocyte foot processes and no electron-dense deposits.** Class I lupus nephritis with minimal mesangial deposits, focal foot process effacement, and no mesangial hypercellularity. Class II lupus nephritis with substantial mesangial deposits, mesangial hyperplasia, and more extensive foot process effacement. Class III lupus nephritis with scanty subendothelial deposits, mesangial hyperplasia, endocapillary leukocytes, and extensive foot process effacement. Class IV-G lupus nephritis with numerous subendothelial and a few subepithelial deposits, mesangial hyperplasia, endocapillary leukocytes, and extensive foot process effacement. Class V lupus nephritis with numerous subepithelial and a few subendothelial and mesangial deposits and extensive foot process effacement.

evidence of renal involvement defined as one of the following: (a) increasing serum creatinine without compelling alternative causes (such as sepsis, hypovolemia, or medication); (b) confirmed proteinuria of ≥1.0 g per 24 hours or by spot urine protein:creatinine ratio; or (c) combinations of proteinuria ≥0.5 g plus hematuria defined as ≥5 RBCs per hpf or proteinuria ≥0.5 g plus cellular casts (27). Moreover, lupus nephritis is one of the few renal diseases for which "follow-up" renal biopsies are routinely performed in some centers 6 months or more after therapy to gauge the efficacy of treatment and guide further therapeutic management. Repeat renal biopsies are also frequently performed in any patient with a sudden change in renal findings (e.g., new onset of proteinuria, new activity of the urinary sediment, declining glomerular filtration rate [GFR]) that may presage a transformation in the class of lupus nephritis, or reactivation of disease requiring reinstitution or adjustment of therapy.

## Pathologic Findings

### Gross Pathology

Since the advent of the modern therapeutic era, autopsy examination of the kidney from patients who have died of acute SLE has become a rare event. For descriptions of the gross pathology of the acute disease, it is necessary to study the writings of early pathologists such as Klemperer, who described enlarged, swollen kidneys with petechial hemorrhages and focal, shallow, superficial scars (28). In the modern era of immunosuppressive therapy, the appearance of the kidneys at autopsy usually reflects a combination of chronic and acute changes, with a contracted or swollen appearance.

### Light Microscopy

Before considering the individual features that define the various classes of lupus nephritis, it is helpful to discuss the basic types of renal lesions that may be encountered in lupus. These

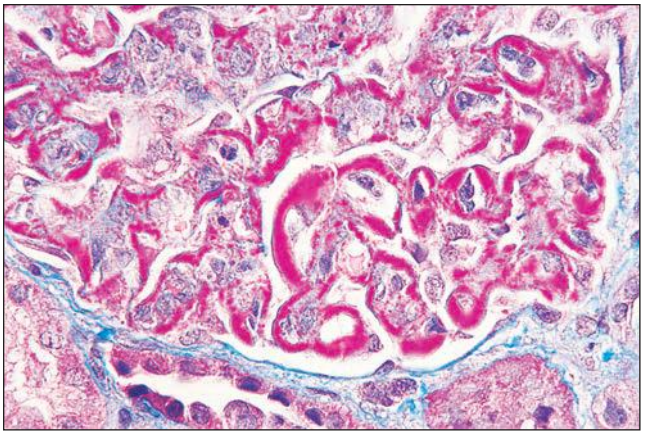


**FIGURE 14.2 Lupus nephritis class IV.** Trichrome stain highlights the presence of global subendothelial fuchsinophilic deposits. (Masson trichrome, ×500.)

general remarks serve as a preface to the more detailed consideration of the WHO classification that follows. Many of these morphologic features pertain to more than one WHO class of lupus nephritis.

### Glomeruli

The major histologic abnormalities of the glomerulus include immune deposits, glomerular proliferation, influx of leukocytes, glomerular necrosis, and scarring. Glomerular proliferation may be mesangial, endocapillary, and extracapillary.

**Immune Deposits** Although glomerular immune deposits are best identified by immunofluorescence and ultrastructural techniques, in lupus nephritis the glomerular immune deposits are also frequently identifiable by light microscopy because of their large size and widespread distribution. As in many other forms of glomerular disease, it is the distribution of deposits in the glomerular tuft that determines the proliferative response, and the glomerular lesions that result are therefore best interpreted in the context of the pattern of immune deposition (29). A number of stains, properly performed, are helpful in demonstrating deposits by light microscopy. Although deposits frequently have a glassy (i.e., hyaline), hypereosinophilic appearance with the hematoxylin and eosin (H&E) stain, they are often difficult to differentiate from the eosinophilic mesangial matrix, glomerular basement membrane (GBM), and cytoplasm of the indigenous glomerular cells without the use of special stains. Particularly helpful are the trichrome stain, which highlights the deposits as red (or fuchsinophilic) against the blue-staining glomerular matrix components (Fig. 14.2) and the Jones methenamine silver or combination methenamine silver-Masson Ponceau stain, which stain the deposits pink or red, respectively, against the black-staining mesangial matrix and GBM.

The mesangium is the most common site for glomerular immune deposits and may be the sole location for renal deposits in some cases. Mesangial deposits in the absence of glomerular capillary wall deposits are the defining feature of class I and class II lupus nephritis. However, mesangial deposits are also found in combination with peripheral glomerular capillary wall deposits in class III, class IV, and class V lupus nephritis. Mesangial deposits and the mesangial proliferation that often accompanies them can be considered the common substratum on which these more complex classes of lupus nephritis are built.

Subendothelial immune deposits are regularly encountered in class III and class IV lupus nephritis. They typically are relatively focal and segmental in class III and more global and diffuse in class IV (especially when accompanied by global endocapillary hypercellularity). When large enough to completely involve the peripheral circumference of the glomerular capillary, they are referred to as classic *wire loops*, which produce a refractile thickening of the glomerular capillary wall in H&E-stained sections, as initially described by Klemperer et al. (28) (Fig. 14.3). Special stains reveal the deposits to be entirely or largely subendothelial, with preservation of an outer peripheral layer of GBM (Fig. 14.4). In florid examples, these massive subendothelial deposits may focally replace the GBM, producing intramembranous deposits best delineated by electron microscopy or extending through the GBM in continuity with overlying subepithelial deposits. In some cases, subendothelial deposits are enclosed by the formation of a subendothelial layer of neomembrane formation, producing a double

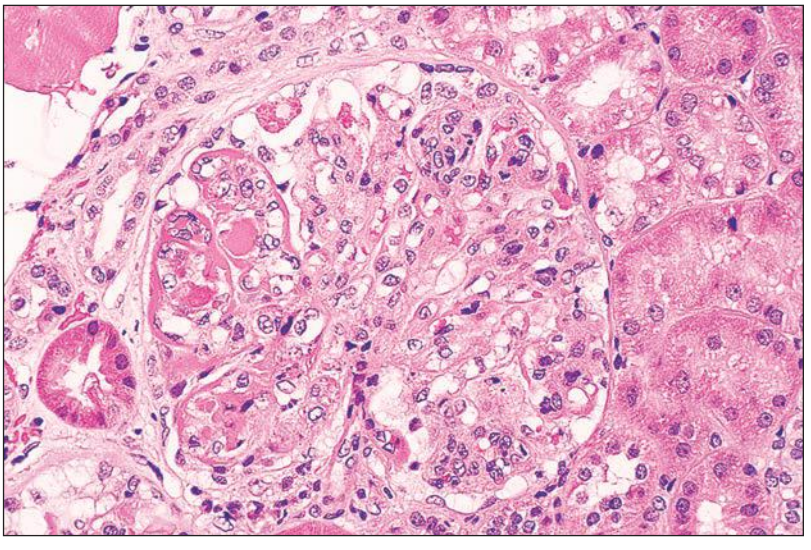


**FIGURE 14.3 Lupus nephritis class IV.** Glomerular capillary walls are segmentally thickened by wire-loop deposits. An intraluminal deposit forms a "hyaline thrombus" in one capillary, and there is global endocapillary proliferation. (H&E; ×320.)

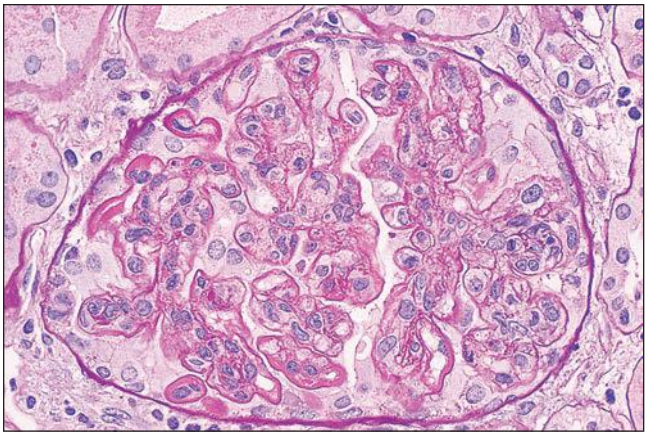


**FIGURE 14.4 Lupus nephritis class IV.** PAS stain highlights the thickening of the glomerular capillary walls by numerous subendothelial deposits. (PAS; ×500.)

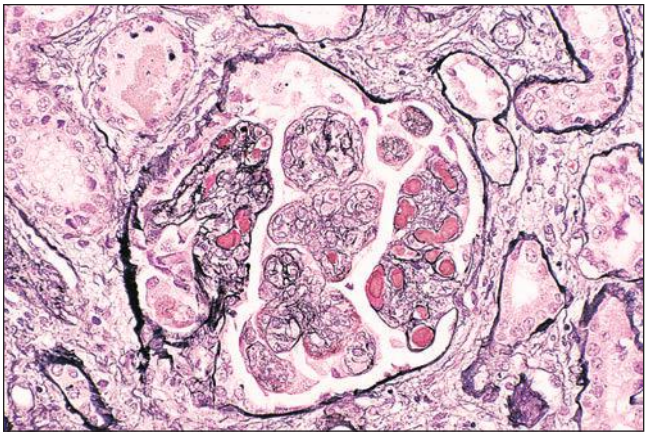


**FIGURE 14.6 Lupus nephritis class IV.** Silver stain is useful to highlight the intraluminal location of the glomerular capillary fibrin thrombi. Some GBMs appear double contoured. (Jones methenamine silver, ×320.)

contour, similar to that observed in membranoproliferative glomerulonephritis. Not surprisingly, mesangial extension or "interposition" frequently accompanies this finding.

Abundant, regularly distributed subepithelial deposits are the defining feature of membranous lupus nephritis (class V), but they may also be seen in association with class III or IV lupus nephritis, as examples of combined class III and V or combined class IV and V lupus nephritis. GBM spikes and a vacuolated texture where cut obliquely can usually be identified with the silver or periodic acid-Schiff (PAS) stain. Scattered subepithelial electron densities may also occur as a sporadic finding in lupus nephritis class III or IV, without the formation of a well-developed membranous pattern. In such cases, the occurrence of these sparse, irregularly distributed, subepithelial deposits is not considered sufficient to justify an additional classification of membranous lupus nephritis class V. The ISN/RPS classification requires subepithelial deposits involving greater than 50% of the tuft of greater than 50% of the glomeruli by light microscopy or immunofluorescence to make a diagnosis of class V plus class III or IV.

Some patients with active class III or IV lupus nephritis have large intracapillary immune deposits forming *hyaline thrombi* (Figs. 14.5 to 14.7). This term is actually a misnomer, because these are not true fibrin thrombi but are instead massive intracapillary immune deposits with the same composition by immunofluorescence as the neighboring subendothelial deposits. Careful serial sectioning usually discloses that these intraluminal deposits without apparent glomerular capillary wall attachment are actually in continuity with large subendothelial deposits in a deeper plane of section. These hyaline thrombi are most common in class IV lupus nephritis, particularly in specimens with extensive wire-loop deposits. We have observed that capillaries with hyaline thrombi or large wire-loop deposits often have less exuberant endocapillary hypercellularity than neighboring capillaries, suggesting possible differences in their ability to incite an inflammatory glomerular response (Fig. 14.8).

Hyaline thrombi must be differentiated from true fibrin thrombi by special stains for fibrin (modified Fraser Lendrum stain and phosphotungstic acid-hematoxylin [PTAH] stain) and

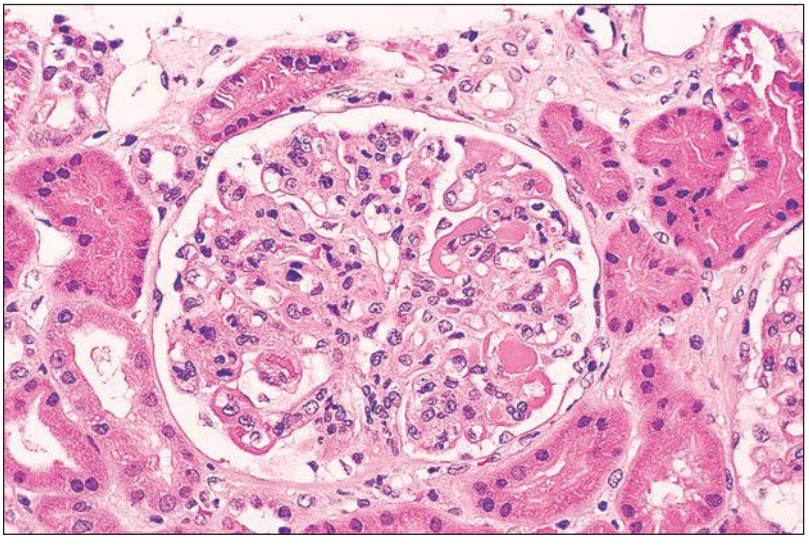


**FIGURE 14.5 Lupus nephritis class IV.** In addition to wire-loop deposits, there are segmental intraluminal deposits forming "hyaline thrombi." (H&E; ×320.)

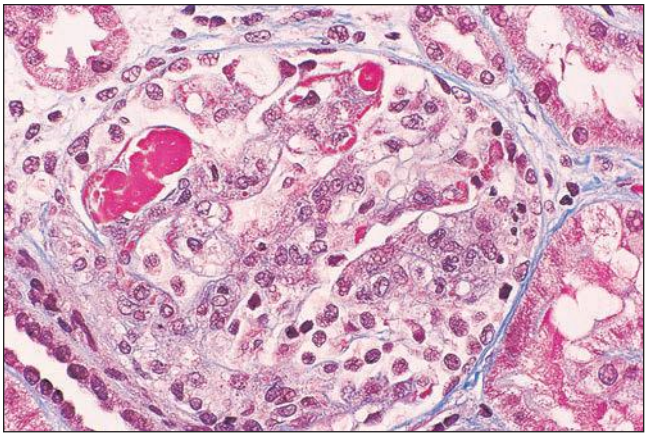


**FIGURE 14.7 Lupus nephritis class IV.** The hyaline thrombi stain red against the blue-staining glomerular capillary walls. (Masson trichrome, ×500.)

by staining for fibrin-related antigens by immunofluorescence. Of these two histochemical stains, PTAH is less reliable, because it sometimes stains immune deposits and fibrin, whereas the Lendrum stain is the more sensitive and specific for fibrin. Of considerable help in differentiating true fibrin thrombi is the appearance of the material in H&E-stained sections. Fibrin often has a darkly eosinophilic fibrillar appearance, whereas hyaline thrombi of the immune deposit type are more lightly eosinophilic, with a homogeneous, glassy, smooth texture.

Kant et al. (30) extensively studied glomerular thrombi in SLE and concluded on the basis of immunofluorescence and clinical data that there are two different mechanisms of thrombosis in lupus nephritis. Thrombosis may complicate any severe active lupus nephritis through complement activation and by triggering the coagulation cascade. Alternatively, glomerular capillary thrombosis (plus arterial and arteriolar thrombosis) may occur as a manifestation of lupus anticoagulant/APL antibody syndrome (discussed in section Thrombotic Microangiopathy). Thrombosis

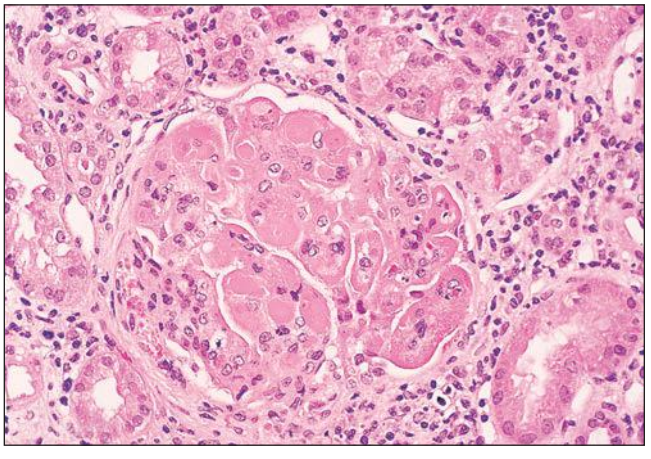


**FIGURE 14.8 Lupus nephritis class IV.** Extensive intraluminal and subendothelial deposits form "hyaline thrombi" and wire loops. Endocapillary proliferation is less conspicuous in the capillaries with voluminous deposits. (H&E; ×320.)

caused by APL antibody may occur as a pure thrombotic microangiopathy or superimposed on virtually any class of lupus nephritis.

**Mesangial and Endocapillary Proliferation** Mesangial hypercellularity and matrix expansion are the first observable response to mesangial deposits. Mesangial hypercellularity and increased mesangial matrix are the only detectable glomerular abnormalities at the light microscopic level in lupus nephritis class II, where they may be diffuse or focal, and segmental or global in the individual glomerulus. Usually, the mesangial immune deposits seen by fluorescence and electron microscopy are more diffuse and regular in distribution than the mesangial proliferative response. There is often a poor correlation between the size and extent of the mesangial immune deposits and the severity of the mesangial hypercellularity. Mesangial hypercellularity is the defining histologic feature of class II lupus nephritis, but it also occurs as an underlying finding in classes III, IV, and V. Like mesangial immune deposits, it is a common base on which the other classes are founded.

Endocapillary hypercellularity can be defined as a proliferation of endothelial cells and mesangial cells together with infiltrating leukocytes (including mononuclear or polymorphonuclear leukocytes) that significantly narrows or occludes the glomerular capillary lumen. Endocapillary hypercellularity is typically focal and segmental in distribution in class III but diffuse and global in class IV disease (Fig. 14.9). Other than neutrophils, the nature of the endocapillary cells is often impossible to determine by light microscopy without the aid of immunohistochemistry using specific markers for monocytes, T lymphocytes, mesangial cells, and endothelial cells. Cells of macrophage/monocyte lineage are particularly numerous in lupus glomerulonephritis and are only exceeded in number in examples of cryoglobulinemic glomerulonephritis (31). They tend to parallel the quantity of immune deposits, complement activation, and proliferative response but do not correlate well with renal function at biopsy or outcome (32,33). On the other hand, the number of macrophages (as well as tubular macrophages) in a second renal biopsy taken 6 months following therapy has been found to correlate well with outcome (34). In computing an activity index, Balow and Austin (35–37) use exudation of more than two neutrophils outside the glomerular capillaries per glomerulus as one of the features of glomerular activity but do not consider lymphocyte or monocyte infiltration. Because neutrophil exudation outside the glomerular capillaries is rare, except in areas of necrosis, this criterion weights even more heavily the importance of necrotizing lesions in the activity index.

**Necrosis** Glomerular necrosis is a feature of class III or IV lupus nephritis and is never observed in pure mesangial proliferative (class II) or membranous (class V) lupus nephritis. It consists of a focus of smudgy fibrinoid obliteration of the glomerular tuft, which is often associated with any or all of the following: deposition of fibrin, GBM rupture or gap formation, and apoptosis of infiltrating neutrophils forming pyknotic or karyorrhectic nuclear debris (Figs. 14.10 and 14.11). The latter change was referred to as "nuclear dust" in the older literature but is now understood to represent a form of apoptosis, which can be confirmed by in situ end labeling of DNA (38). By electron microscopy, the cells undergoing this form of programmed cell death have been identified as infiltrating neutrophils, as well as indigenous glomerular cells (38).

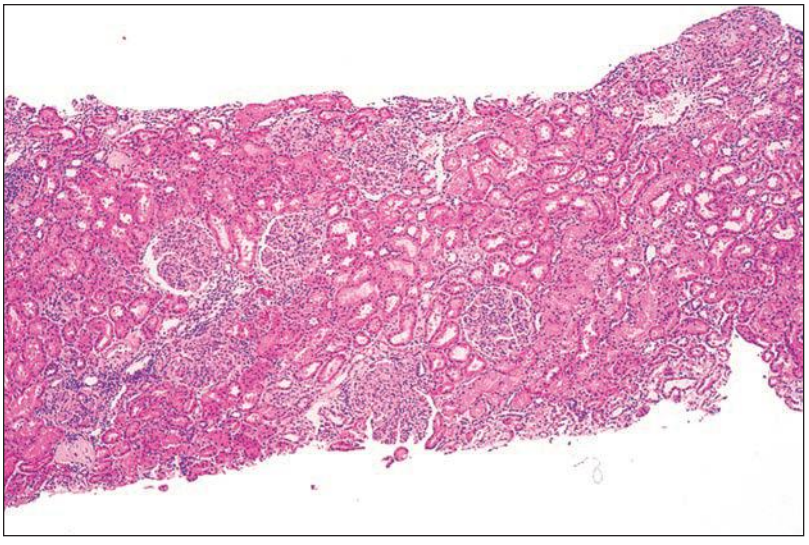


**FIGURE 14.9 Lupus nephritis class IV.** There is relatively uniform diffuse and global endocapillary proliferation. (H&E; ×100.)

Necrotizing lesions are typically segmental in distribution, but more than one glomerular lobule may be affected, particularly in diffuse proliferative lupus nephritis class IV. As in other forms of glomerulonephritis in which necrotizing lesion are common (e.g., pauci-immune crescentic glomerulonephritis), there is evidence of neutrophil infiltration, and early cellular crescents frequently directly overlie the affected lobules. One study found glomerular necrosis to correlate with lower serum complement ($CH_{50}$) levels and more severe proteinuria in a large group of patients with class III and IV lupus nephritis (39). In a large Chinese study, necrotizing lesions strongly correlated with anti-C1q antibodies, as well as anti-dsDNA antibodies (40).

**Hematoxylin Bodies** Hematoxylin bodies are the tissue equivalent of the LE body and consist of naked nuclei whose chromatin has been altered by binding to circulating ANA. They are thought to occur after nuclear exposure to the ambient circulation in the course of individual cell death. Owing to their nuclear origin, they are Feulgen positive. Hematoxylin bodies are most common in necrotizing glomerular lesions. They are considered the only truly pathognomonic lesion in lupus nephritis but are extremely uncommon, affecting approximately 2% of lupus biopsy specimens (41). In H&E-stained sections, hematoxylin bodies consist of rounded, smudgy, lilac staining (amphophilic) structures that are generally smaller than normal nuclei (Fig. 14.12). Hematoxylin bodies may be isolated or clustered. They usually have indistinct borders and appear to merge into the background tissue with surrounding flecks of hematoxyphilic material. Their lilac, hyaline coloration, and smudgy borders allow them to be distinguished from the more darkly basophilic, smaller, and distinctly punctate nuclear fragments observed in foci of karyorrhexis or pyknosis.

**Cellular Crescents** Cellular crescents are a feature of active lupus nephritis that may be encountered frequently in class III or IV lupus nephritis, but never (by definition) in pure class II or class V disease (Fig. 14.13). Crescents may be seen in membranous lupus nephritis in mixed class V and III or class V and IV lesions. Cellular crescents commonly overlie the necrotizing lesions, but they may also occur in glomeruli with nonnecrotizing segmental or global endocapillary hypercellularity. In their activity index formulation, Balow and Austin

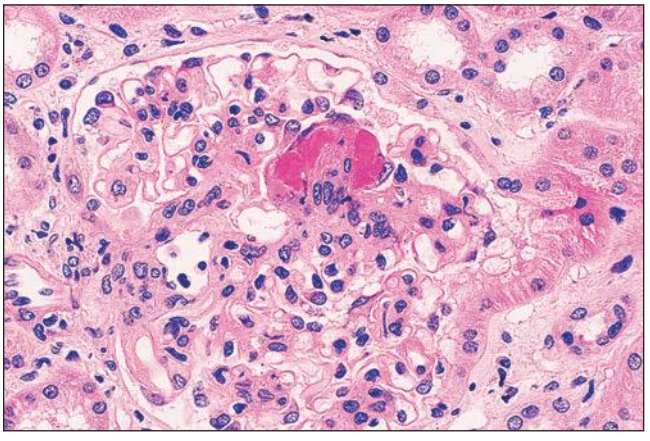


**FIGURE 14.10 Lupus nephritis class III.** There is segmental fibrinoid necrosis with neutrophil infiltration and pyknosis. (H&E; ×500.)

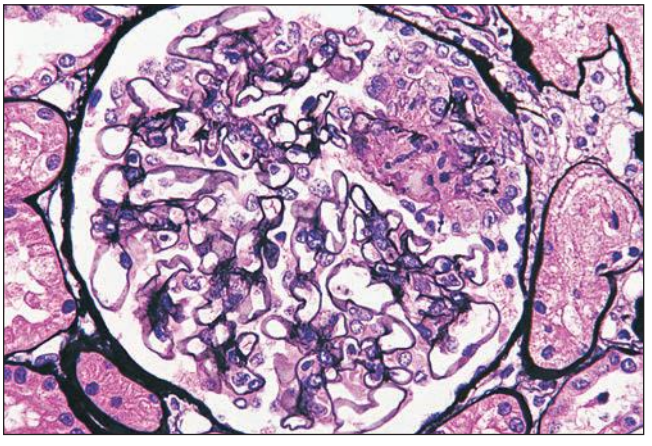


**FIGURE 14.11 Lupus nephritis class III.** The segmental necrotizing lesion displays segmental rupture of the GBM. (Jones methenamine silver, ×500.)

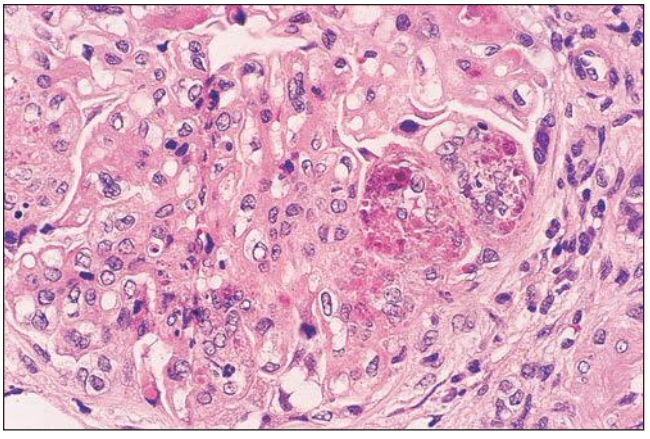


**FIGURE 14.12 Lupus nephritis class IV.** Several glomerular capillaries contain hematoxylin bodies consisting of smudged, coarsely clumped basophilic material. Another lobule contains karyorrhectic nuclear debris. (H&E; ×500.)

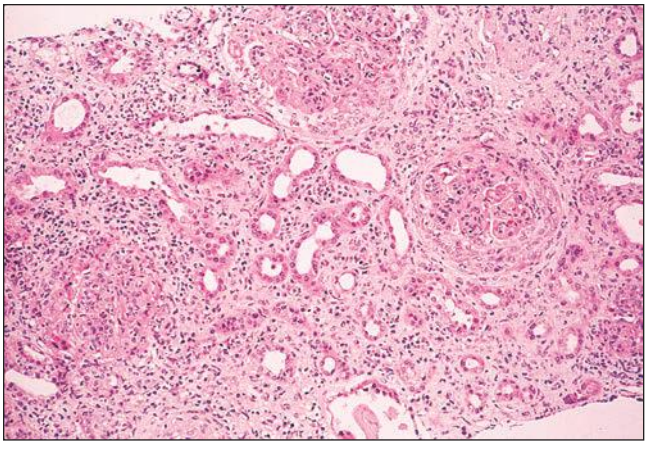


**FIGURE 14.14 Lupus nephritis class IV.** Initial renal biopsy shows diffuse endocapillary proliferation with focal crescents and severe interstitial inflammation. (H&E; ×80.)

(35–37) define cellular crescents as "aggregates comprising two or more layers of proliferating visceral and parietal epithelial cells with infiltrating mononuclear cells lining one fourth or more of the interior circumference of the Bowman capsule." This reasonable definition allows differentiation of crescents from the single layer of reactive hyperplastic visceral epithelial cells commonly encountered in glomeruli with membranous features or undergoing sclerosis. Cellular crescents of inflammatory origin must be differentiated from the epithelial cell proliferation that accompanies the collapsing variant of focal segmental glomerulosclerosis (FSGS) (collapsing glomerulopathy), which has been described in some patients with SLE (see Lupus Podocytopathy).

### Glomerular Scarring

Glomerular obsolescence is a feature of chronic glomerular injury that may result from severe or prolonged glomerular damage in the course of class III, IV, or V lupus nephritis. Although it has not been well studied, it is unlikely that extensive glomerulosclerosis supervenes on pure class II lupus nephritis unless there is transformation into one of the more progressive classes. Focal glomerular sclerosis in a patient with lupus may occur in the course of aging or as a complication of hypertensive arterionephrosclerosis and does not always imply scarring in the course of an immunologically mediated injury. The ISN/RPS classification advocates that such nonspecific glomerulosclerosis be excluded, whenever possible, from the computation of the total percentage of glomeruli affected by scarred lupus nephritis (21,22,42). In class III lupus nephritis, the glomerular scarring is often initially focal and segmental, mirroring the distribution of the proliferative and necrotizing lesions. Serial biopsies can demonstrate an evolution from focal segmental necrotizing glomerulonephritis to focal segmental sclerosing glomerulonephritis, sometimes with associated fibrous crescents or synechiae overlying the sclerotic segments. Similarly, in chronic class IV lupus nephritis, the glomerular scarring is typically more global and diffuse, although segmental sclerosis may affect some glomeruli (Figs. 14.14 and 14.15).

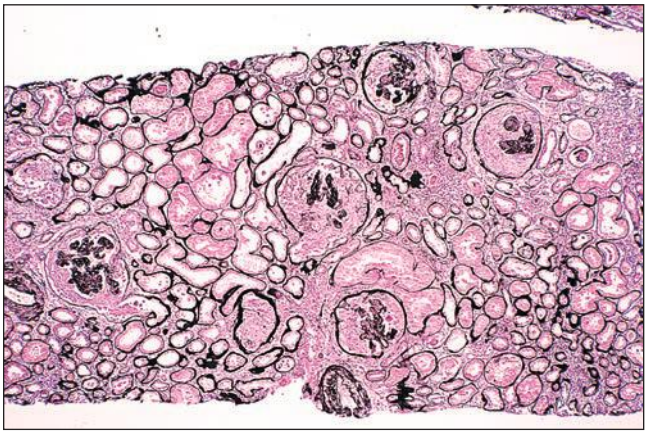


**FIGURE 14.13 Lupus nephritis class IV.** A severe example with extensive cellular crescents. Although the glomerular tufts are compressed, endocapillary proliferation is evident. (Jones methenamine silver; ×80.)

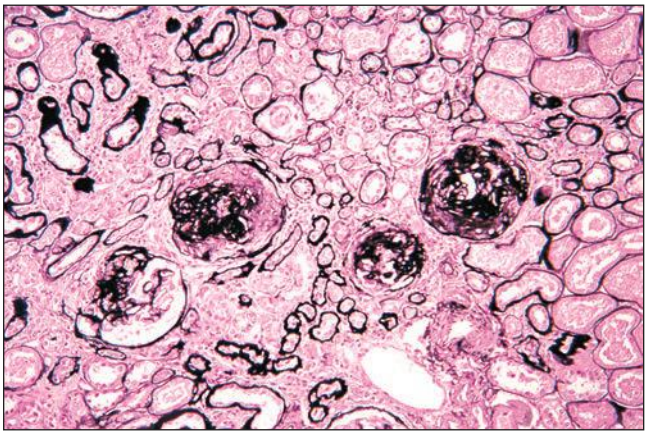


**FIGURE 14.15 Lupus nephritis class IV.** Despite aggressive therapy, repeat renal biopsy 2 years later shows progression to segmental and global glomerulosclerosis with focal fibrous crescents. There is marked reduction in the degree of interstitial inflammation. (Jones methenamine silver, ×80.)

In some patients with class V lupus nephritis, there is progression to chronic renal failure without clear evidence of transformation into a proliferative phase consistent with combined class III or IV disease. This progressive course, which may affect up to 20% of class V patients, can be considered analogous to the situation in primary membranous glomerulonephritis, which progresses to end-stage renal failure in up to 25% of patients within 10 years of initial clinical detection. In a small percentage of lupus patients, an initial renal biopsy performed at the outset of detectable renal disease reveals an advanced picture of diffuse and global glomerular sclerosis affecting 90% or more of glomeruli. Called *lupus nephritis class VI* in the ISN/RPS classification (21,22), this picture is usually the sequela of a previous class IV lupus nephritis. In some patients, the phase of active and potentially treatable glomerulonephritis is clinically occult. At this stage, residual but scanty immune deposits may still be detectable in the sclerotic glomerular tuft by immunofluorescence and electron microscopy.

Because lupus nephritis is a typically remitting and relapsing condition with repeated episodes of reactivation, it is not uncommon to detect sclerosing and active proliferative lesions side by side in renal biopsy specimens, especially in patients with longstanding lupus and a history of active lupus nephritis. When active and chronic scarred lesions are detected in an initial renal biopsy specimen at the apparent outset of disease, the disease onset antedated first clinical recognition.

### Tubules and Interstitium

Tubulointerstitial lesions may be encountered in all classes of lupus nephritis. Even in class I, in which glomeruli appear normal by light microscopy, there may be mild tubulointerstitial disease resulting from arteriosclerosis associated with aging or hypertension that cannot be directly attributed to lupus nephritis. In specimens with only class I or II glomerular changes, the presence of focal active tubulointerstitial lesions typical of class III should raise the possibility of unsampled focal class III glomerular lesions that are not in the planes of section available for examination. Tubulointerstitial disease is most commonly encountered in class IV lupus nephritis but also occurs frequently in class III and to a lesser degree in class V, with lowest frequency found in classes I and II. The types of lesions that affect the tubulointerstitial compartment can be divided into acute and chronic subgroups, and those related to an interstitial inflammatory process or resulting largely from prolonged nephrotic proteinuria.

In patients with nephrotic-range proteinuria, whether in the setting of class III, class IV, or class V lupus nephritis, the tubules may show nonspecific changes common to many conditions with heavy or longstanding glomerular proteinuria. These predominantly affect the proximal tubules and consist of intracytoplasmic lipid resorption droplets that appear as clear vacuoles in H&E-stained sections because of removal of the lipid in the course of tissue processing and protein resorption droplets that appear eosinophilic and strongly PAS positive and usually trichrome red. The latter change has been referred to as "hyaline degeneration" of the proximal tubules, although this is not really a degenerative process but one of active resorption by the proximal tubules of filtered albumin and lipoproteins. In some cases with longstanding nephrotic syndrome, especially in the setting of a membranous lesion, interstitial foam cells may also be found.

Active (or acute) tubulointerstitial lesions most common in class IV and class III lupus nephritis include interstitial inflammation and edema, which may coexist with the more chronic lesions of tubular atrophy and interstitial fibrosis. The interstitial infiltrates consist predominantly of mononuclear leukocytes, including lymphocytes, monocytes, and plasma cells. Neutrophils and eosinophils are seldom identified. There are rare reports of giant cell reaction to deposits involving the tubular basement membranes (43). These leukocytic infiltrates range from sparse to dense and diffuse and are sometimes associated with lymphocytic infiltration of the tubules (i.e., tubulitis) and tubular epithelial degenerative and regenerative changes. Rarely, hematoxylin bodies ingested by neutrophils may be identified in the tubular lumen (44). This active and severe tubulointerstitial damage is most common in severe diffuse proliferative lupus nephritis class IV. Casts of neutrophils, erythrocytes, and shed tubular epithelial cells are readily identified in active class III or IV lupus nephritis. Intratubular oval fat bodies consisting of lipid-laden desquamated epithelial cells are most common in cases with severe nephrotic proteinuria, usually in the setting of class V membranous lupus nephritis or mixed proliferative and membranous glomerulonephritis (i.e., class V and IV or class V and III).

Several investigators have correlated the immunophenotype of the interstitial inflammatory infiltrates with other histopathologic and clinical parameters (31,32,45–47). Most infiltrating mononuclear leukocytes are T lymphocytes, with lesser numbers of monocyte/macrophages, B lymphocytes, and natural killer cells. The relative proportions of CD4 (helper/inducer) and CD8 (suppressor/cytotoxic) T cells vary, with some investigators finding a predominance of $CD8^+$ cells in most patients (31,46) and others a predominance of $CD4^+$ cells (45) or a roughly equal balance (32). Alexopoulos et al. (32) suggested that this disparity may be related in part to treatment, because corticosteroids preferentially reduce $CD8^+$ cells, thereby shifting the CD4:CD8 tissue ratio. Nevertheless, there is general agreement that the percentage of interstitial $CD8^+$ cells is greater than in other forms of glomerular disease such as IgA nephropathy and membranous glomerulonephritis (48,49). One study (46) found the tissue CD4:CD8 ratio to correlate with activity index, whereas others have been unable to demonstrate such a correlation (32). One group identified a correlation between the chronicity index and the extent of interstitial infiltration by T lymphocytes and monocytes (32).

In a recent detailed study, $CD4^+$ T cells were prominent in two thirds of lupus nephritis biopsies and tended to be distributed as broad periglomerular aggregates intermixed with $CD8^+$ T cells (50). By contrast, $CD8^+$ T cells were present in all samples and tended to adhere to the Bowman capsule and infiltrate the tubular epithelium (as CD8 tubulitis). Their differentiation into $CD28^{null}$ memory-effector phenotype suggests participation in adaptive immunity. Study of the T-cell receptor (TCR) β-chain clonotypes revealed oligoclonal T-cell infiltration of the kidney. The clones identified in the kidney represent a small subset of the many clones present in the peripheral circulation, supporting their selection and involvement in adaptive immune responses. Furthermore, when repeat biopsies were performed in individual patients, there was a persistence of the same $CD8^+$ T-cell clones over years, suggesting that they mediate progressive renal injury (50). The intrarenal B-cell population has also been shown to be clonally

expanded and organized into aggregates with focal germinal centers containing follicular dendritic cells (51). The B cells have an immunoglobulin (Ig) repertoire consistent with somatic hypermutation and organ-intrinsic adaptive immune responses. The close proximity of B and T cells suggests that in addition to in situ antibody secretion, resident B cells are contributing to local inflammation by presenting major histocompatibility complex (MHC) class II–restricted antigens to T cells (51). Intrarenal production of anti-dsDNA antibody by resident plasma cells has also been described in the New Zealand Black (NZB)/W murine model of lupus nephritis, indicating that the kidney can be a site of autoantibody formation (52). IL-17–producing double-negative ($CD4^-/CD8^-$) T cells have also been identified in the renal interstitium (53). Several studies using immunostaining for macrophages confirmed the large number of interstitial and intratubular macrophages in diffuse proliferative lupus nephritis and found good correlations between tubular macrophages and serum creatinine, proteinuria, and progression to renal insufficiency (34,54). Urinary biomarkers that reflect the severity and activity of interstitial inflammation in human lupus biopsies include monocyte chemotactic protein 1, hepcidin (produced by monocytes in response to INFα), and liver-type fatty acid–binding protein (produced by the proximal tubule in response to injury) (55).

The strong predominance of T cells among the infiltrating interstitial cells in lupus nephritis suggests a role for cellular immunity in the development of the tubulointerstitial damage. The predominance of CD8 cells in the areas of tubulitis and greater numbers of natural killer cells in biopsies with tubulointerstitial immune deposits raise the question of possible cellular cytotoxicity in response to local autoantigens of renal or systemic origin (32). The presence of follicular structures with germinal centers strongly correlates with tubular basement membrane deposits, suggesting that germinal centers and T- and B-cell aggregates select for cells that locally secrete pathogenic antibodies in the tubulointerstitium (51). The in situ antigens that promote these responses remain unknown but are likely to be important determinants of disease severity. The up-regulation of MHC class II antigen (DR) on the tubular epithelial cells and many of the interstitial leukocytes in human (32,45) and murine lupus nephritis (56,57) also suggests that tubular cells may become activated in lupus and stimulate the influx of activated mononuclear cells. Similar tubular DR expression has been reported in transplant rejection and other renal conditions manifesting tubulitis. Up-regulation of costimulatory molecule CD40 has also been demonstrated in the renal epithelial cells and infiltrating leukocytes of murine and human lupus nephritis (58,59).

Tubulointerstitial immune deposits can be detected by fluorescence and electron microscopy in approximately 50% of patients. They occur more frequently in diffuse proliferative lupus nephritis than in the focal proliferative variant, but they may also occur in some patients with membranous and mesangial proliferative forms. The precise location of these deposits is best elucidated by electron microscopy. They consist of granular electron-dense deposits that involve tubular basement membranes, interstitial capillary basement membranes, and interstitial collagen. They are most frequently associated with the tubular basement membrane, within the tubular basement membrane itself or more commonly abutting the membrane at the interstitial interface (Fig. 14.16). Only rarely are they seen along the epithelial aspect of the tubular basement membrane. These

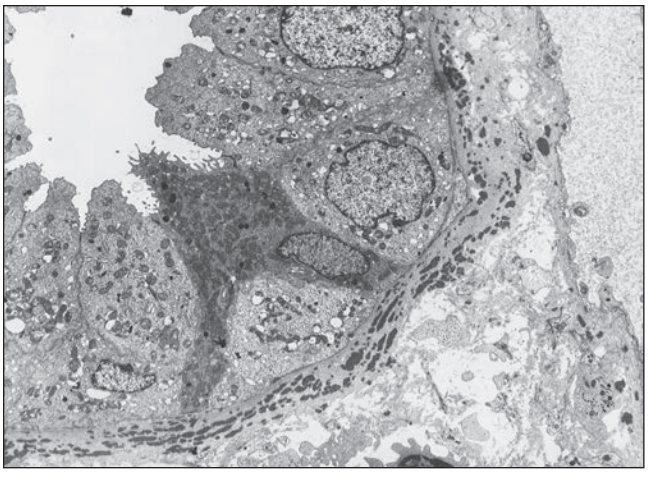


**FIGURE 14.16 Lupus nephritis class IV.** The electron micrograph shows electron-dense deposits within a tubular basement membrane and, to a lesser extent, in the adjacent interstitial collagen. (×2000.)

tubulointerstitial deposits may involve any segment of the nephron and often lack a conspicuous leukocytic response. New layers of tubular basement membrane or interstitial capillary basement membrane may encircle the deposits, producing a lamellated network of basement membrane material (Fig. 14.17).

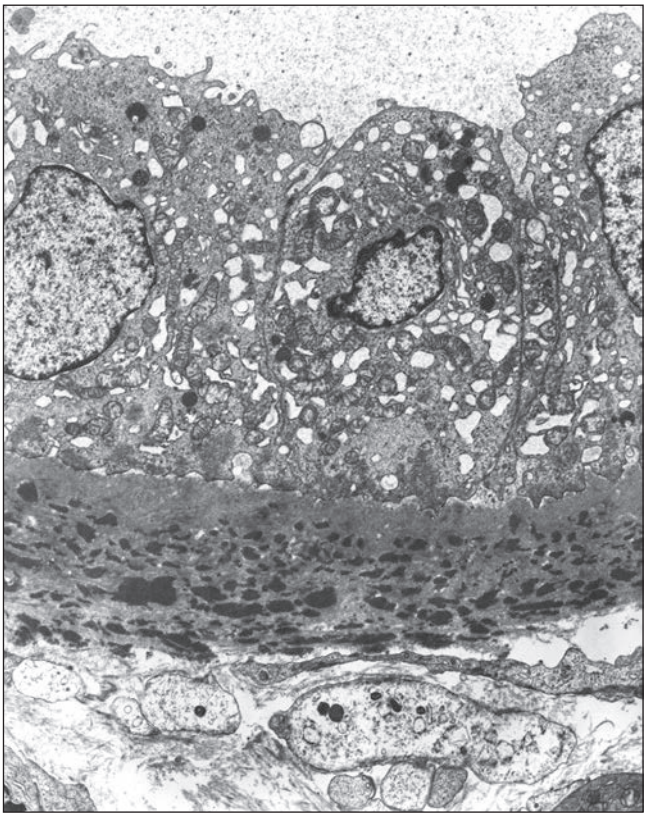


**FIGURE 14.17 Lupus nephritis class IV.** High-power view shows a lamellated network of tubular basement membrane splayed around the tubular basement membrane deposits. (Electron micrograph, ×4000.)

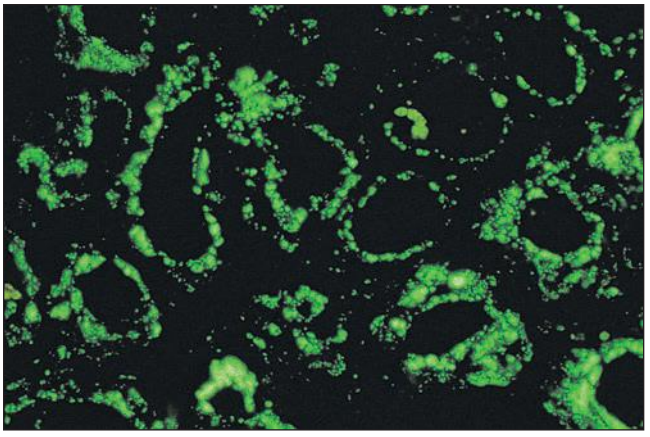


**FIGURE 14.18 Lupus nephritis class IV.** The immunofluorescence micrograph shows abundant granular deposits of IgG within the tubular basement membranes and interstitium. (×200.)

By immunofluorescence, the deposits have a granular to semilinear texture that has a tendency to outline the profiles of tubular basement membranes and interstitial capillaries or to form punctate aggregates in the interstitial collagen (Fig. 14.18). Interstitial capillary immune deposits are particularly specific for lupus nephritis (Fig. 14.19).

The composition of the tubulointerstitial deposits varies. Most have positive staining for IgG, with other immunoglobulins detected less frequently (60,61). In some biopsies, there is positivity for IgG, IgM, and IgA in the tubulointerstitial deposits, and in others, only a single immunoglobulin class may be found. There even may be differences in the composition of IgG subclasses between the tubulointerstitial, vascular, and glomerular compartments of a given biopsy (62). Complement components are associated with the immunoglobulins in most cases and occasionally may be detected in their absence (63), suggesting a role for antibody-independent complement activation.

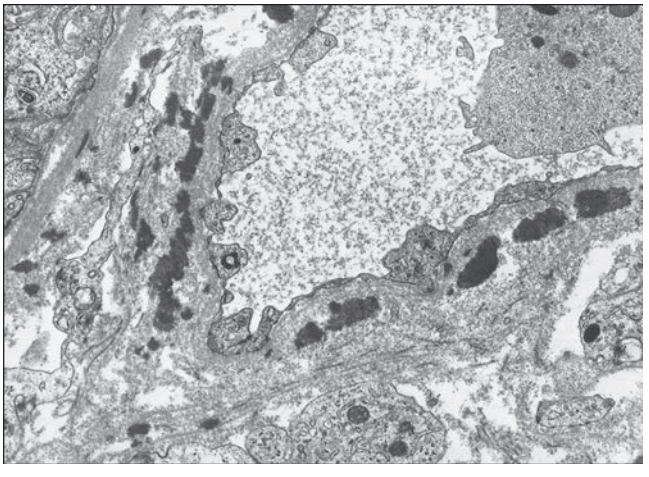


**FIGURE 14.19 Lupus nephritis class IV.** This electron micrographs shows numerous electron-dense deposits within the wall of an interstitial capillary. (×6000.)

In general, the presence of tubulointerstitial deposits correlates well with glomerular lesions, becoming progressively more frequent with increasing glomerular endocapillary hypercellularity (classes III and IV) and being relatively infrequent with membranous (class V) or mesangial proliferative lesions (class II) (47,64). Interstitial inflammation tends to be particularly severe in crescentic forms of lupus nephritis (47). Park et al. (64) and Jeruc et al. (47) made the important observation that there is no correlation between the prevalence of tubulointerstitial deposits and the presence and severity of interstitial inflammation, which may be severe in the absence of detectable deposits. For example, among the 103 patients studied by Park et al. (64), 76 had interstitial infiltrates, but only 32 had tubulointerstitial deposits, and interstitial inflammation without tubulointerstitial deposits was found in all classes of renal lesions. Clearly, mechanisms other than a simple inflammatory response to tubulointerstitial deposits are operant in most cases.

The severity of tubulointerstitial inflammation correlates broadly with glomerular inflammatory lesions (64). It constitutes one of the best morphologic correlates with the degree of renal insufficiency (32,64) and is an accurate prognosticator of subsequent decline in renal function (64). Tubular atrophy, at least in part the result of interstitial inflammation, is one of the strongest predictors of renal failure (36), as it is in many other glomerular diseases. Similarly, increased interstitial volume, which directly parallels the development of tubular atrophy, closely correlates with impaired renal function (65,66). Staining with picro-Sirius red followed by quantitative morphometry and computer imaging has allowed more accurate measurements of interstitial matrix volume, which was a strong predictor of doubling of serum creatinine (67). Morphometric measurement of chronic renal damage using an interactive image analysis system that captures silver-stained images and outlines zones of chronic glomerular and tubulointerstitial injury predicted renal outcome better than WHO class (68). Renal tubular dysfunction has been demonstrated in some patients with lupus nephritis (69,70), and reports include hyperkalemic distal renal tubular acidosis (RTA) with hyporeninemic hypoaldosteronism (71–73).

In most instances of tubulointerstitial deposits, the associated tubular atrophy, interstitial fibrosis, and inflammation accompany severe glomerular lesions. There are rare cases in which severe tubulointerstitial damage occurs in the presence of only trivial glomerular lesions (74–79), sometimes leading to acute renal failure. The presence of abundant tubulointerstitial deposits in most of these patients attests to their pathogenetic relationship to lupus rather than representing a superimposed, unrelated tubulointerstitial process (reviewed in (78)). In some of these cases, the tubulointerstitial damage is at least partially reversible with immunosuppressive therapy (76,78–81). There are rare reports of interstitial nephritis induced by anti–tubular basement membrane deposits, producing a linear fluorescence with antisera to IgG and C1q along the tubular basement membranes and the Bowman capsule (82).

### Vascular Lesions

Vascular lesions are commonly encountered in renal biopsy specimens from patients with SLE and may assume a variety of morphologic forms (83,84), including uncomplicated vascular immune deposits, noninflammatory necrotizing vasculopathy,

**TABLE 14.5 Vascular lesions in lupus nephritis**

| |
|---|
| Arteriosclerosis and arteriolosclerosis |
| Uncomplicated vascular immune deposits |
| Noninflammatory necrotizing vasculopathy (so-called lupus vasculopathy) |
| Thrombotic microangiopathy |
| Associated with HUS/TTP syndrome |
| Associated with APL antibodies |
| Associated with scleroderma/mixed connective tissue disease |
| Necrotizing vasculitis (PAN type) |

HUS, hemolytic uremic syndrome; TTP, thrombotic thrombocytopenic purpura; PAN, polyarteritis nodosa.

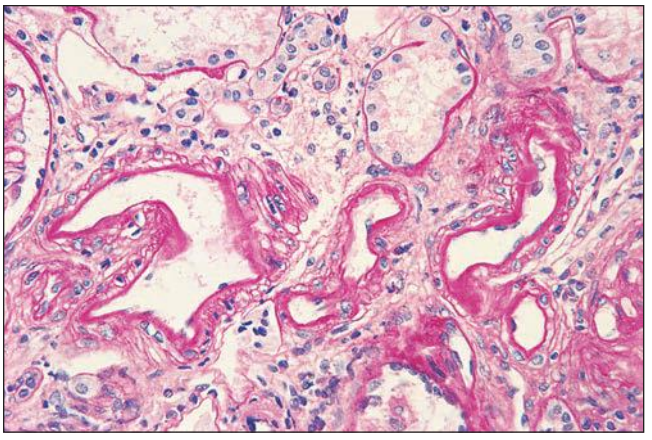


**FIGURE 14.20 Lupus nephritis class IV.** Intimal immune deposits in the walls of interlobular arteries have caused slight thickening of the intimal basement membrane without significant luminal narrowing. (PAS; ×320.)

thrombotic vasculopathy, true inflammatory vasculitis, and nonspecific arteriosclerosis (Table 14.5). Because assessment of vascular lesions is not factored into the ISN/RPS classification of lupus nephritis or formulation of the activity and chronicity index according to the National Institutes of Health (NIH) criteria, renal vascular lesions run the risk of being overlooked. This is especially the case when vascular lesions are focally distributed. To accurately classify renal vascular lesions, vessels must be systematically examined by light, immunofluorescence, and electron microscopy. The morphologic findings must then be analyzed in the context of particular clinical syndromes that may complicate SLE, including APL antibody syndrome (alternatively known as anticardiolipin syndrome), thrombotic thrombocytopenic purpura, renal vein thrombosis (RVT), and accelerated hypertension.

Klemperer reported a high incidence of renal vascular lesions in his autopsy-based study of SLE. The lesions predominantly involved small arteries and arterioles with fibrinoid alteration of the intima and subendothelium (28). Grishman and Venkataseshan (85) observed renal vascular lesions in the autopsy specimens of 8 (33%) of 24 patients who died in the presteroid era and 5 (25%) of 26 who died in the modern era, but with a much lower incidence in 19 (6.9%) of 276 renal biopsy specimens. A greater incidence of renal vascular lesions in autopsy (7 of 20) than biopsy (10 of 200) specimens was also described in an earlier report (86) and may be related to differences in sample size, severity, duration, and treatment of the lupus nephritis. Most studies are in agreement that the presence of renal vascular lesions of the thrombotic, necrotizing, or vasculitic type adversely affects renal outcome.

Attention to the importance of vascular lesions in SLE has been renewed in recent years (83–85,87–89). In a large Italian study of 285 patients with lupus nephritis from 20 nephrology centers, renal vascular lesions were found in 79 cases (27.7%) and included lupus vasculopathy (27 cases), hemolytic uremic syndrome/thrombotic thrombocytopenic purpura (HUS/TTP), or malignant hypertensive lesions (24 cases), vasculitis (8 cases), and arteriosclerosis or arteriolosclerosis (20 cases) (84). Renal vascular lesions were associated with a higher rate of progression to renal failure. The 5- and 10-year renal survival rates were 74.3% and 58.0%, respectively, for patients with renal vascular lesions, compared with 89.6% and 85.9% for those without renal vascular lesions (84). A recent prospective study also found renal vascular lesions to be associated with hypertension and reduced renal function at the time of biopsy, and both variables predicted poor renal outcome in multivariate analyses (87). A large Chinese study of 341 patients with lupus nephritis found renal vascular lesions in 279 (82%), including 253 with uncomplicated vascular immune deposits, 82 with arteriosclerosis, 60 with thrombotic microangiopathy, 13 with noninflammatory necrotizing vasculopathy, and 2 with true arteritis (89). This group found that a statistical model that included vascular lesions was a superior predictor of outcome than traditional NIH activity and chronicity indices (89).

**Uncomplicated Vascular Immune Deposits** The most common renal vascular lesion in SLE is immune complex deposition in the walls of small arteries and arterioles; deposition occurs to a lesser extent in veins (84,85,90,91). The affected vessels usually appear normal by light microscopy. Rarely, thickening of the subendothelial zone or medial intercellular zones by glassy hyaline eosinophilic material is observed by light microscopy, but the vessel lumen usually is not compromised (Fig. 14.20). Diagnosis requires the demonstration of granular deposits of immunoglobulin (IgG, IgM, and IgA in various combinations), often associated with C1q or C3 (Fig. 14.21). Finding IgM or C3 alone is insufficient to diagnose this condition, because these components are frequently nonspecifically trapped in vessels with the ordinary arteriosclerosis or arteriolar hyalinosis that accompanies the more chronic inactive forms of lupus nephritis. By electron microscopy, granular, electron-dense deposits are identified in the intimal and perimyocyte matrix (Fig. 14.22). As in glomerular immune deposits, some of these vascular immune deposits may exhibit an organized fingerprint substructure (83). Such vascular immune deposits may occur in classes II through V of lupus nephritis, but they are most common in the more active proliferative classes (III and IV), especially in association with tubulointerstitial deposits. Uncomplicated vascular immune deposits are usually clinically silent, and they have not been found to confer a higher risk of hypertension or progressive renal disease.

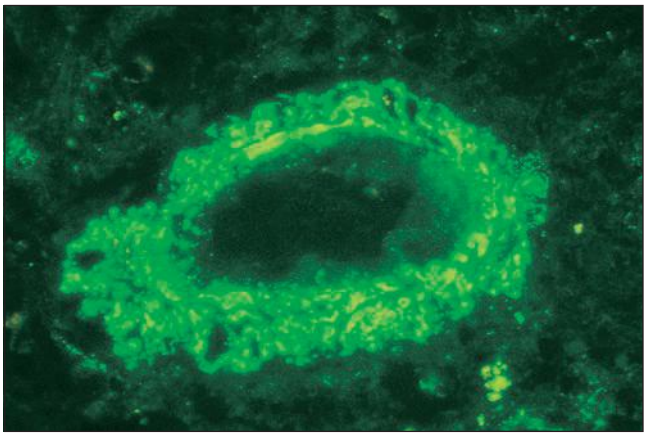


**FIGURE 14.21 Lupus nephritis class IV.** The fluorescence micrograph shows abundant granular staining for IgG within the intima and media of an interlobular artery. (×400.)

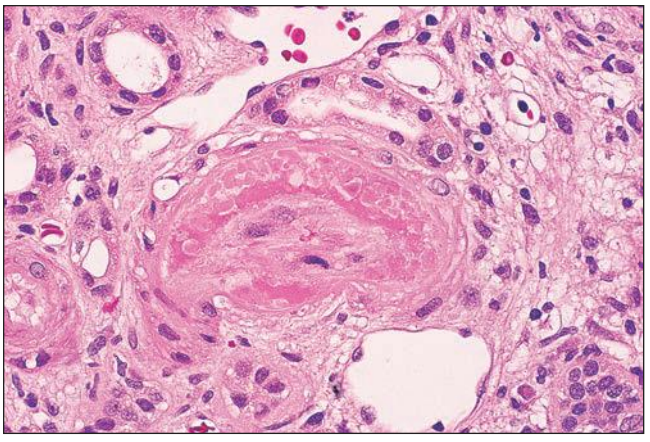


**FIGURE 14.23 Lupus vasculopathy.** The lumen of an arteriole is severely narrowed by intimal deposits of intensely eosinophilic material suggestive of fibrin admixed with more lightly eosinophilic immune deposits. The endothelium is necrotic; there is no inflammation of the vessel wall. (H&E; ×500.)

**Noninflammatory Necrotizing Vasculopathy** Noninflammatory necrotizing vasculopathy (i.e., lupus vasculopathy) is far less common than simple vascular immune deposits (83). It affects predominantly preglomerular arterioles and, to a lesser extent, interlobular arteries, especially in the setting of severe active class IV lupus nephritis. The affected vessels are severely narrowed and sometimes occluded by abundant intimal and luminal deposits of glassy eosinophilic material that may extend into the media (Fig. 14.23). This material is usually fuchsinophilic in trichrome-stained preparations and may demonstrate focal reactivity for fibrin with the Lendrum and PTAH stains (Fig. 14.24). The endothelium is often swollen or denuded, and there is smudgy degeneration and loss of medial myocytes, although without inflammatory infiltration of the vessel wall.

Bhathena coined the term *lupus vasculitis* (92), but we prefer the term *lupus vasculopathy* to depict this lesion because of the absence of vascular infiltration by inflammatory cells (83). Immunofluorescence shows variable staining of the vessel intima and lumen for immunoglobulins (e.g., IgG, IgM, IgA), complement components (C3 and C1q), and fibrin-related antigens (Fig. 14.25). Electron microscopy usually reveals endothelial loss with mural deposits of granular, electron-dense material with the combined appearance of immune deposits and insudated plasma proteins, sometimes associated with fibrillar fibrin. Degenerative changes of the medial myocytes may be seen adjacent to these deposits but without leukocyte infiltration. These morphologic features strongly suggest that the combined processes of vascular immune deposition and intravascular coagulation contribute to their morphogenesis. Severe hypertension is not invariably associated with these lesions and is unlikely to be the primary etiologic factor. However, severe hypertension is common and probably exacerbates the vascular changes (85,93). In most series, lupus vasculopathy carries an ominous prognosis, with frequent, severe renal insufficiency, active urinary sediment, active lupus serologies, hypertension, and rapid progression to renal failure.

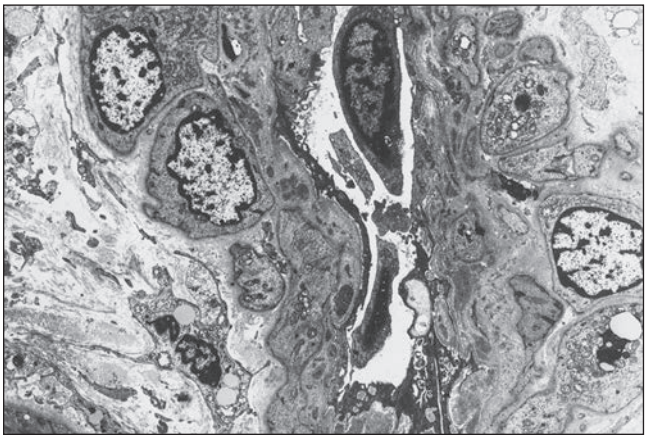


**FIGURE 14.22 Lupus nephritis class IV.** The electron micrograph shows small, granular, electron-dense deposits within the subendothelial basement membrane of an interlobular artery. (×2875.)

**Thrombotic Microangiopathy** Thrombotic microangiopathy may be difficult to differentiate from the necrotizing, noninflammatory lupus vasculopathy described in the previous section (83). It may occur in association with distinct clinical syndromes such as HUS/TTP, lupus anticoagulant/APL syndrome, overlap with scleroderma, or malignant hypertension. However, in a significant number of patients, thrombotic lesions are found in renal biopsies of patients with SLE who lack a recognizable systemic thrombotic process. Thrombotic vascular lesions most commonly affect preglomerular arterioles and interlobular arteries. Unlike lupus vasculopathy, they are also frequently associated with signs of glomerular thrombosis, including glomerular fibrin thrombi, mesangiolysis, double contours of the GBM enclosing subendothelial electron lucent flocculent material, and ischemic glomerulosclerosis

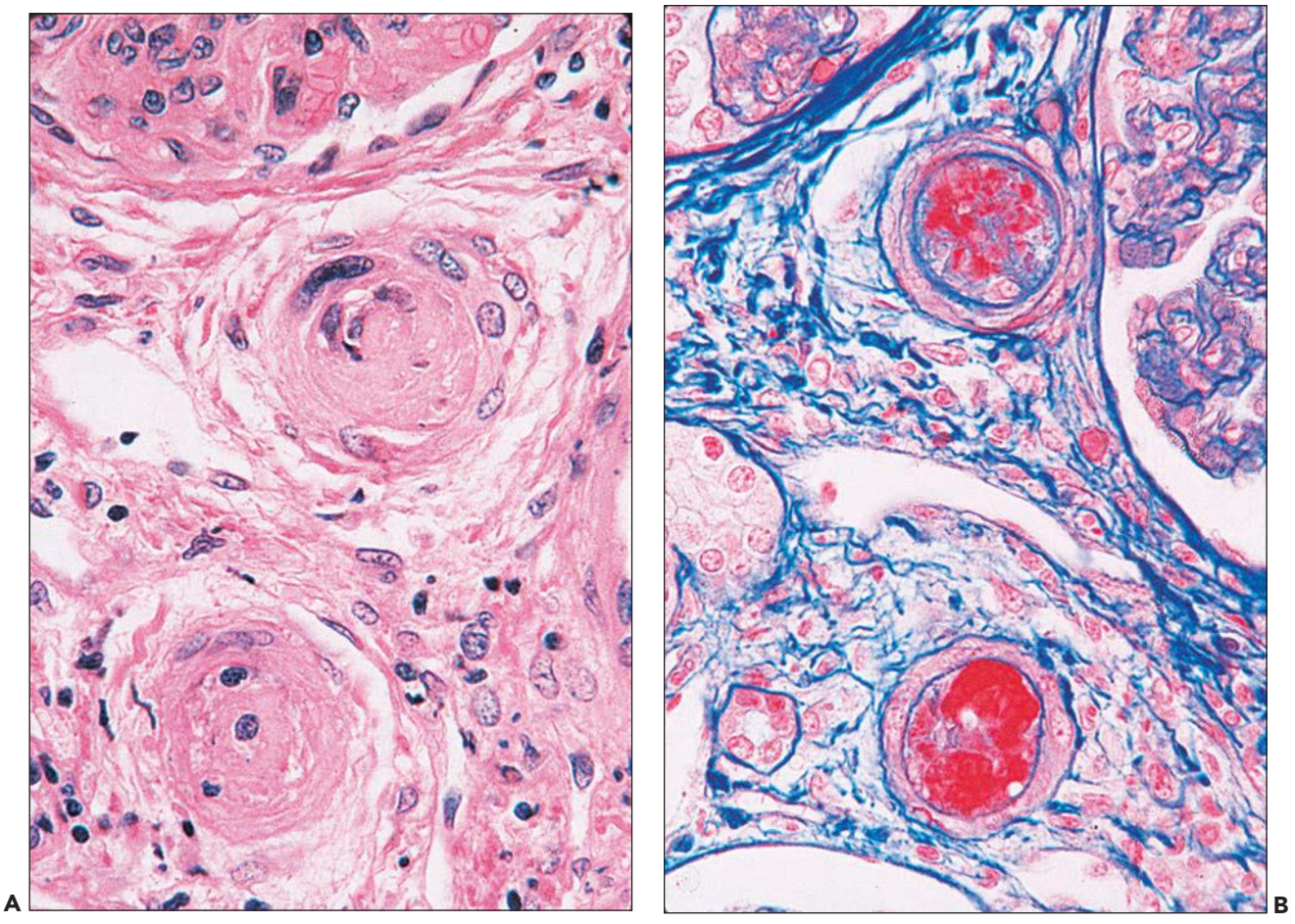



**FIGURE 14.24 Lupus vasculopathy.** A double panel shows occlusion of preglomerular arterioles by eosinophilic deposits **(A)** that stain positive with Lendrum stain for fibrin (**B**). (×500.)

(Fig. 14.26). By light microscopy, the affected vessels are occasionally narrowed or occluded by intraluminal fibrin and platelet thrombi, which may be associated with endothelial swelling and denudation (Fig. 14.27). The products of coagulation may penetrate the intima and sometimes contain fragmented erythrocytes. There is no overt leukocyte infiltration of the media, although a rare neutrophil or lymphocyte may be entrapped in the luminal thrombosis. In the interlobular arteries, the intima may have a more mucoid, edematous appearance, with "onion skin" myointimal proliferation. By fluorescence microscopy, the affected vessels usually reveal intense, dominant staining for fibrin-related antigens, with variable positivity for IgM and C3. In contrast to lupus vasculopathy, IgG is usually absent from these lesions. Recently, C4d has been identified as a potential marker of glomerular thrombotic microangiopathy in lupus nephritis, irrespective of whether there was an associated APL antibody (94). The incidence of thrombotic lesions resembling HUS/TTP was 8% of lupus nephritis patients according to a collaborative Italian study (84). Their presence was associated with reduced (66.3%) 5-year renal survival compared with 89.6% for lupus nephritis patients without renal vascular lesions. They may be superimposed on virtually any class of lupus nephritis including classes II (95), III (95), and IV (96).

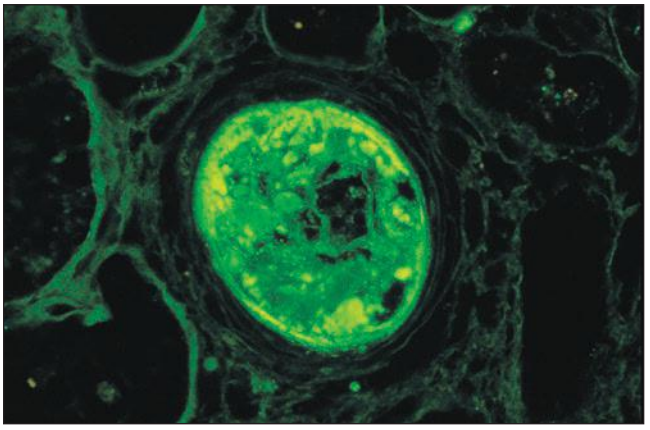


**FIGURE 14.25 Lupus vasculopathy.** An interlobular artery shows staining for IgA within its expanded intima. Similar deposits were seen for IgG, IgM, C3, C1q, and fibrinogen. The vessel lumen is nearly occluded. (×600.)

**Syndrome of Hemolytic Uremic Syndrome or Thrombotic Thrombocytopenic Purpura** Some patients with biopsy features of thrombotic microangiopathy have a clinical syndrome of HUS or TTP, which may precede, be contemporaneous with, or follow clinical onset of SLE (83,97). Symptoms related to TTP include fever, malaise, petechial and purpuric

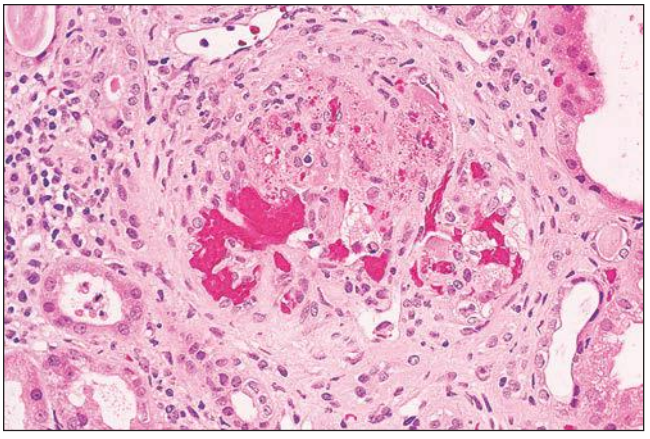


**FIGURE 14.26 Thrombotic thrombocytopenic purpura–like syndromein SLE.** This glomerulus displays features of acute thrombotic microangiopathy, including marked glomerular capillary congestion, endothelial swelling and necrosis, and glomerular capillary thrombosis with entrapment of fragmented erythrocytes (i.e., schistocytes). (H&E; ×320.)

skin lesions, abdominal pain and bleeding, and neurologic symptoms, including reduced consciousness, seizures, transient pareses, and coma (95,98,99). Most well-documented cases have evidence of thrombocytopenia and microangiopathic hemolytic anemia. The results of other coagulation studies (e.g., prothrombin time, partial thromboplastin time, fibrin degradation products) have been normal, and the lupus anticoagulant is absent in most patients (83,95). Some cases have been linked to autoantibody to the von Willebrand factor cleaving protease ADAMTS-13 (100,101). An association with decreased serum complement factor H levels in some individuals suggests a role for dysregulation of the alternative complement pathway (101,102). Renal manifestations of the TTP-like process range from asymptomatic urinary abnormalities to mild reduction in renal function to fulminant oligoanuric renal failure requiring dialysis. Hypertension has been

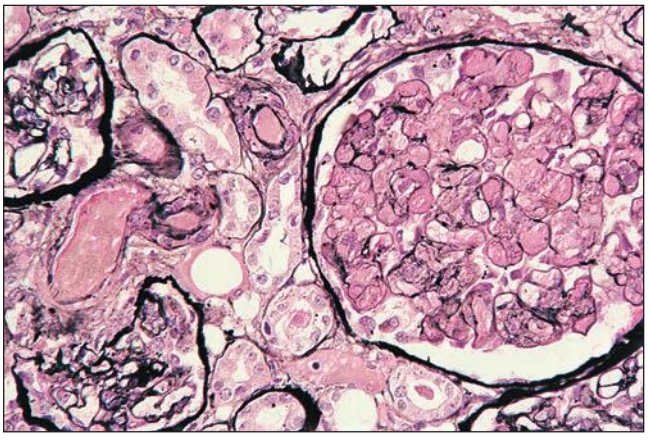


**FIGURE 14.27 Thrombotic thrombocytopenic purpura–like syndrome in SLE.** The preglomerular arteriole and many glomerular capillaries are occluded by fibrin thrombi. The arteriolar and glomerular endothelium appears denuded. (Jones methenamine silver, ×320.)

absent in most cases (95,97,103) but present in others (84). The TTP has responded to the same therapeutic strategies used in idiopathic TTP. In the modern era, plasma exchange and plasma infusion have achieved greatly improved survival compared with older forms of therapy, including steroids, antiplatelet agents, and splenectomy.

**Lupus Anticoagulant or Antiphospholipid Syndrome**

Although Conley and Hartmann first described a lupus inhibitor in 1951, it was only in the 1980s that the lupus anticoagulant or APL syndrome was fully recognized as a major complication of SLE and related collagen-vascular diseases. Patients with this condition may have SLE, a lupus-like condition that fails to fulfill ACR criteria for SLE (i.e., usually low-titer ANA with negative results for anti-DNA antibody), or primary APL syndrome (104–108). Slow recognition of the nature and clinical features of the APL syndrome is related in part to the diverse organ system involvement, which overlaps between specialties, including hematology, rheumatology, neurology, nephrology, and obstetrics (109,110). The kidney is commonly affected in this condition, with overt renal manifestations in approximately 25% of patients (109). APL antibodies have been detected in 25% to 50% of SLE patients, although a smaller percentage develops any clinical signs or symptoms of thrombosis (111,112). In an analysis of 29 published series comprising greater than 1000 patients with SLE, there was a 34% incidence of lupus anticoagulant and 44% incidence of anticardiolipin antibodies (113).

The clinical features of APL syndrome are diverse and include superficial and deep venous thromboses, spontaneous abortions (from placental thrombosis), pulmonary hypertension, cerebral infarcts and transient ischemic attacks, Budd-Chiari syndrome, livedo reticularis, cardiac valvular disease, adrenal hemorrhage, thrombocytopenia, and vague constitutional symptoms (114–117). Renal manifestations range from microthrombosis of glomerular capillaries and arterioles to thrombosis of intraparenchymal arteries to thrombosis of the main renal artery and vein, with secondary renovascular hypertension, renal cortical necrosis, and infarction (63,106,118–126) (Figs. 14.28 and 14.29). Thrombi may be in various stages of organization and recanalization. Some cases are associated with ischemic subcapsular cortical scars (127). These features may produce a spectrum of renal clinical manifestations, including asymptomatic hematuria and mild proteinuria, hypertension (ranging from mild to malignant range), mild to severe renal insufficiency, nephrotic-range proteinuria, and rapidly progressive renal failure (126,128–133). Some patients with SLE develop catastrophic antiphospholipid syndrome (CAPS), also known as Asherson syndrome, which frequently involves the kidney and is life threatening (134). CAPS is defined as follows: (a) clinical evidence of involvement of at least three organ systems over a period of less than 1 week, (b) histologic confirmation of thrombosis in at least one organ system, and (c) documented APL antibody, which is usually present at high titer.

In 1981, Kant et al. (30) first called attention to the strong association between the existence of a circulating lupus anticoagulant and the occurrence of glomerular capillary thrombosis, which often could not be explained by the activity of the glomerulonephritis. In 1988, Kincaid-Smith described

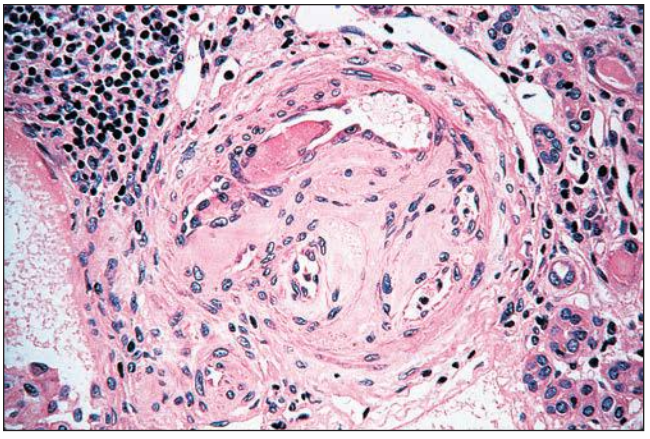


**FIGURE 14.28 Lupus anticoagulant syndrome in SLE.** An interlobular arteries contains fresh and recanalized thrombus. (H&E, ×400.)

a syndrome of pregnancy-associated thrombotic microangiopathy in 12 young women with APL syndrome, 8 of whom had no evidence of SLE and 4 of whom had SLE, including 2 with proliferative glomerulonephritis (135). D'Agati et al. (129) stressed that the occurrence of thrombotic microangiopathy in APL syndrome could not be accounted for by secondary intravascular coagulation caused by active proliferative and necrotizing lupus nephritis.

In SLE, thrombotic microangiopathy related to APL syndrome may occur in a variety of classes of lupus nephritis. In a study of 26 patients with APL antibodies and renal disease but failing to fulfill ACR criteria for SLE, there was a particular predominance of membranous lupus-like nephritis with or without features of associated thrombotic microangiopathy on renal biopsy (136). In these 26 cases, renal biopsy disclosed pure thrombotic microangiopathy in 4 cases, thrombotic microangiopathy combined with lupus-like nephritis in 11 cases, and lupus-like nephritis alone in 4. The lupus-like nephritis consisted of membranous glomerulonephritis in 11, mesangial proliferative in 3, and focal proliferative in 1 (136). In a study by Daugas et al. (127), renal lesions of APL syndrome were detected in 32% of lupus patients with renal biopsies, superimposed on or independently of lupus nephritis. The renal biopsy findings of APL nephropathy were statistically associated with lupus anticoagulant, but not with anticardiolipin antibodies, and were independent risk factors for the development of hypertension, elevated serum creatinine, and increased interstitial fibrosis (127). Moroni et al. (137) prospectively followed 111 patients with lupus nephritis for mean of 173 months and found an overall prevalence of APL antibody in 26% of patients; the presence of an APL antibody was associated with worse renal survival. In a study of 151 lupus patients with or without APL antibody, renal biopsy features of thrombotic microangiopathy were identified in 40% of lupus patients with APL antibody compared to only 4% of lupus patients without APL antibody (138). There was a higher frequency of hypertension and renal insufficiency in those with nephropathy related to APL antibody. Interestingly, some patients with apparently primary APL syndrome evolve into SLE over time, suggesting these conditions are related (139).

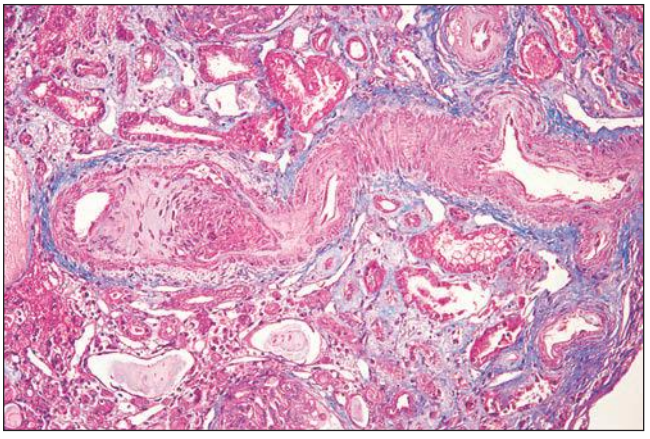


**FIGURE 14.29 Lupus anticoagulant syndrome in SLE.** An interlobular artery is narrowed by organizing thrombus. There is adjacent tubular atrophy and interstitial fibrosis. (Masson trichrome; ×200.)

The APL antibody syndrome is caused by antibodies to a family of naturally occurring phospholipids (including cardiolipin, phosphatidylcholine, phosphatidylserine, phosphatidylinositol, and others) and/or to the β2-glycoprotein 1 (β2GP-1) to which they bind (140). Laboratory diagnosis is made by demonstration of a positive lupus anticoagulant or demonstration of APL or β2GP-1 antibodies by enzyme-linked immunosorbent assay (ELISA).

The lupus anticoagulant test depends on the prolongation of clotting tests such as the activated partial thromboplastin time, kaolin clotting time, or dilute Russell viper venom time owing to interference with the phospholipid component of the prothrombin activator complex, consisting of factors Xa, V, $Ca^{2+}$, and phospholipid. LAC activity is defined as an unexplained prolongation of the activated partial thromboplastin time that is not reversed when the patient's plasma is diluted 1:1 with normal platelet-free plasma (a procedure that would be expected to reverse clotting caused by factor deficiencies) (141). This occurs because the patient's plasma contains an inhibitor. Although the presence of a lupus anticoagulant prolongs phospholipid-dependent coagulation tests in vitro, it rarely causes bleeding problems. Thus, in so far as the "lupus anticoagulant" promotes coagulation in vivo, it is something of a misnomer.

ELISA for APL antibodies uses a phospholipid matrix of hexagonal-phase phospholipids (142), which more closely resemble the structure of endothelial membrane-associated phospholipids, or a platform of β2GP-1 itself. Some ELISAs are able to distinguish APL antibody reactive to phospholipids alone from antibody to phospholipids that requires the presence of β2GP-1 and from antibody to β2GP-1 that is independent of the presence of phospholipids. ELISA for APL antibodies is reported as specific titers and an immunoglobulin class (IgG, IgM, or IgA), both of which may have prognostic importance. In SLE patients, high-titer IgG APL antibodies correlate best with thrombotic episodes and obstetrical complications, although even moderate titers of IgG APL antibody may give rise to severe thrombotic events. In general, the titers of APL antibodies do not correlate with anti-DNA antibody titers or complement levels. IgM APL antibodies are less

closely linked to APL syndrome and may be a manifestation of drug-induced APL antibody, particularly from procainamide. IgA APL antibody has been linked to a higher incidence of thrombocytopenia in some reports. Many patients have APL antibodies of more than one class. False-positive Venereal Disease Research Laboratory (VDRL) test results have been obtained from APL antibodies reactive with the cardiolipin and phosphatidylcholine component of beef heart used in this assay.

The pathogenesis of thrombosis in patients with APL syndrome is uncertain. In many patients, the binding of anticardiolipin antibody to phospholipid appears to depend on a plasma cofactor, β2GP-1, which has a high affinity for anionic phospholipids (143–145). The means by which this interaction promotes coagulation may involve direct endothelial damage or platelet activation. There is also evidence that these antibodies bind to lipid surfaces that have been altered by oxidants, thereby exposing novel epitopes that are themselves immunogenic (146). Altered endothelial cell functions may result, promoting thrombosis or decreased fibrinolysis through reduced production of free protein S, decreased activation of protein C by thrombomodulin, reduced prostacyclin, and reduced prothrombinase activity.

Optimal therapy for SLE patients with APL antibodies, with and without thrombotic events, has not been clearly defined. Most clinicians advocate high-dose anticoagulation with heparin followed by long-term warfarin for patients with APL antibodies who have suffered thrombotic complications to prevent recurrent thromboses. More controversial are the large numbers of lupus patients with APL antibody who have not manifested clinical features of APL syndrome. Options include no specific therapy or aspirin alone; a role for prophylactic anticoagulation has not been defined (147). Although steroid or cytotoxic therapy may alter APL antibody titers, immunosuppressive therapy does not reduce the risk of thrombosis (140).

**Renal Vasculitis** True inflammatory vasculitis in which there is leukocyte infiltration of vessel walls, often accompanied by necrosis, is the least common vascular lesion encountered in renal biopsies from lupus patients (83). This form of renal vasculitis, which is histologically indistinguishable from that seen in polyarteritis nodosa or microscopic polyangiitis, is so rare in lupus nephritis that some have questioned whether it may not represent an overlap with some other forms of vasculitis. Some of these patients have clinical evidence of systemic vasculitis, but others appear to have renal-limited vasculitis. We have seen an example of necrotizing inflammatory vasculitis in the kidney and probable cerebral vasculitis with associated seropositivity for antineutrophil cytoplasmic antibody (ANCA). This form of renal vasculitis has not been studied systematically, owing to the rarity of the lesion and the fact that most of the reported patients antedate the availability of serologic testing for ANCA. This form of renal vasculitis was observed in 1 (1.8%) of 56 patients reported by Appel et al., 1 (0.3%) of 326 reported by Grishman et al., 8 (2.8%) of 285 reported by Banfi et al., and 2 of 279 (0.7%) reported by Wu et al. (18,84,85,89). Morphologically, these lesions are the only form of lupus-associated vascular disease in which there is true inflammatory infiltration of the intima and media by neutrophils and mononuclear leukocytes, often accompanied by fibrinoid necrosis and rupture of elastic lamellae (Fig. 14.30).

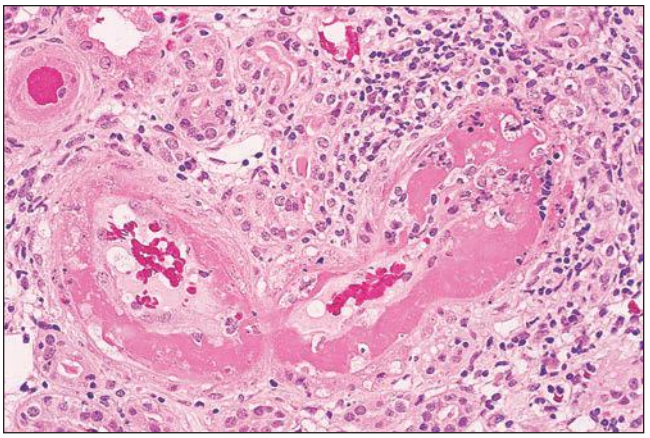


**FIGURE 14.30 Acute necrotizing vasculitis in SLE.** This patient with class IV lupus nephritis and multisystem vasculitis had necrotizing, inflammatory vasculitis of several small interlobular arteries. There is transmural infiltration by neutrophils and lymphocytes, with abundant intimal fibrin and necrosis of the media. (H&E; ×320.)

Immunofluorescence discloses strong staining for fibrin-related antigens, with weak and more variable staining for immunoglobulin and complement, probably representing nonspecific trapping of plasma proteins in areas of necrosis. Electron microscopic descriptions of these lesions are lacking, and it is unknown whether affected vessels contain electron-dense immune deposits.

This lesion may occur in the setting of any class of active or inactive lupus nephritis. Because of its extremely poor prognosis and need for aggressive immunosuppressive therapy, the occurrence of true inflammatory vasculitis in a renal biopsy specimen should be promptly reported.

## Immunofluorescence Microscopy

Lupus nephritis is one of the few diseases of the kidney in which immune deposits can be detected in all renal compartments, including the glomeruli, tubules, interstitium, and blood vessels. The immunofluorescence staining pattern is often extremely helpful in confirming a diagnosis of lupus nephritis when the diagnosis of SLE may be in doubt at the time of biopsy.

Although individual patterns of immunofluorescence staining are highly variable and are discussed in more detail in the section on the various ISN/RPS classes, some general remarks are applicable to all classes. Data from four different series giving the frequency of positivity of different antisera are listed in Table 14.6 (18,148–150). IgG is found almost universally. There are codeposits of IgM and IgA in most specimens. The corresponding presence of both light chains indicates the polyclonal nature of the immunoglobulins. Some investigators have identified a more intense staining of the IgG and IgA deposits with antisera to $\lambda$ than $\kappa$ (151), although this $\lambda$ dominance is less pronounced than that observed in IgA nephropathy (152). Others have observed a $\kappa$ predominance or equal intensity of light chain staining in lupus nephritis (152). C3 is the most frequent complement component, followed by C1q, which usually stains very intensely, sometimes in the absence of C3 and C4 (152). C4 stains least frequently and often most faintly. The presence of early complement components C1q

**TABLE 14.6 Incidence of positive glomerular immunofluorescence for different antisera from four series**

| Antiserum | Appel et al. (18) | Cameron et al. (741) | Hill et al (149) | Sinniah and Feng (150) | Total | Percentage positive |
|---|---|---|---|---|---|---|
| IgG | 24/27 | 38/41 | 56/64 | 32/32 | 150/164 | 91.5 |
| IgM | 26/29 | 29/41 | 41/64 | 16/32 | 112/166 | 67.5 |
| IgA | 13/28 | 23/41 | 41/64 | 21/32 | 98/165 | 59.4 |
| C1q | 24/31 | 21/32 | 30/40 | — | 75/103 | 72.8 |
| C4 | — | 18/31 | 20/53 | — | 38/85 | 44.7 |
| C3 | 26/29 | 32/41 | 47/63 | 29/32 | 134/165 | 81.2 |
| Fibrin(ogen) | 6/13 | 12/40 | 17/62 | 29/32 | 64/147 | 43.5 |

and C4 attests to the activation of complement by the classic pathway (153). The staining pattern is called "full house" (meaning 3 of a kind and 2 of a kind in poker parlance) when deposits containing all three immunoglobulin classes (IgG, IgM, and IgA) and both complement components (C3 and C1q) are present.

Properdin can also be identified in the glomerular deposits (154), suggesting that complement is also activated through the alternative pathway. Membrane attack complex (C5 through C9) has also been identified in the glomerular deposits (155). Fibrin-related antigens are commonly intensely positive in areas corresponding to necrotizing lesions and in the urinary space in association with active crescents (Fig. 14.31). Weaker positivity for fibrin may also persist in older fibrous crescents. In contrast to the intense and discretely localized positivity for fibrin in necrotizing glomerular lesions, it is not uncommon to observe a more generalized and weaker positivity for fibrin outlining the glomerular tuft in cases of diffuse proliferative glomerulonephritis without necrotizing features by light microscopy. It is likely that fibrin is deposited in the course of activation of the coagulation cascade through glomerular capillary immune deposition, leukocyte infiltration, and endothelial activation. Because antibodies to fibrin-related antigens do not differentiate fibrin I, fibrin II (cross-linked fibrin), fibrinogen, and fibrin degradation products, more specific antisera can be used to differentiate between these coagulation products (156,157). These studies show that fibrinogen may be detected in glomeruli without histologically identifiable thrombi and that the presence of cross-linked fibrin correlates best with active necrotizing lesions (157). C4d is a newly identified biomarker associated with glomerular thrombosis in lupus nephritis (94) but can also colocalize with immune deposits.

Another commonly observed phenomenon in immunofluorescence studies is the detection of tissue ANA in renal biopsies (158). Although ANA is detectable by indirect immunofluorescence using tumor cell lines as substrate in up to 98% of lupus patients, for unknown reasons only a fraction of patients with lupus exhibit ANA reactivity in their own renal biopsies. Reactivity consists of positive staining of the renal epithelial nuclei with antisera to IgG (in a homogeneous, speckled, or rim pattern similar to that observed in serum ANA testing) (Fig. 14.32). This phenomenon has long been considered an artifact of the cryostat sectioning allowing ambient ANA in the patient's serum to bind to the nuclei exposed in the course of sectioning, rather than representing an in vivo phenomenon. ANA reactivity is revealed by adding fluoresceinated

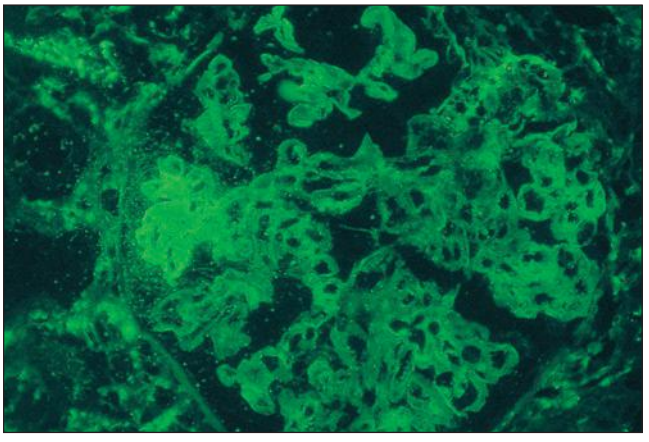


**FIGURE 14.31 Lupus nephritis class III.** There is strong staining for fibrin/fibrinogen in the distribution of a segmental necrotizing lesion. (Immunofluorescence micrograph, ×500.)

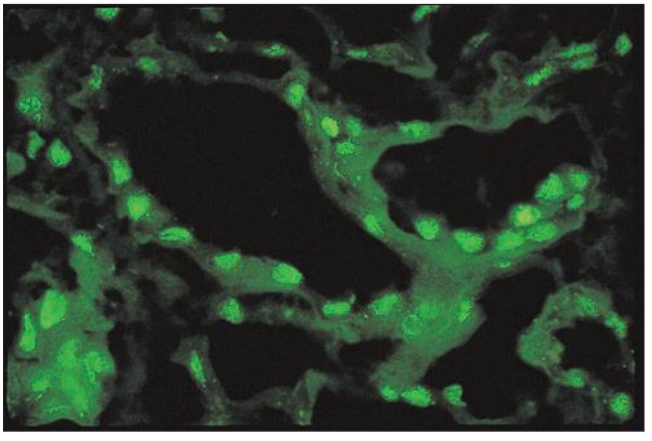


**FIGURE 14.32 Lupus nephritis class IV.** The immunofluorescence micrograph shows tubular ANA reactivity in this cryostat section stained for IgG. This finding has been referred to as "tissue ANA." (×600.)

rabbit antihuman IgG in a standard fashion. However, experimental data on murine lupus suggest that ANA may penetrate and bind to the nuclei of intact cells and therefore may occur in vivo (159,160). In cases with strong tissue ANA (particularly speckled type), the diffuse nuclear staining for IgG may interfere with detection of true immune deposits in glomeruli and the tubulointerstitium, requiring careful integration with the results of immunofluorescence staining for the other immune reactants.

## Electron Microscopy

The electron microscopic appearances of the kidneys from SLE patients are exceedingly diverse. Deposits in the glomeruli may range from sparse and small to abundant and large. The mildest glomerular alteration consists of small, discrete, mesangial electron-dense deposits (in lupus nephritis class I or II). In specimens with severe glomerular immune deposition, electron-dense deposits consist of copious mesangial deposits and involve subendothelial, intramembranous, and subepithelial locations, even to the point of obliterating the glomerular capillary lumina in the more dramatic examples of diffuse proliferative glomerulonephritis (Fig. 14.33). Many researchers (18,161,162) have emphasized that voluminous deposits in all these locations simultaneously are seldom found in conditions other than lupus nephritis, with the exception of some forms of primary and secondary membranoproliferative glomerulonephritis type 3.

Of the types of deposits found in lupus nephritis, the subendothelial deposits correlate best with clinically active disease. These subendothelial deposits distributed in a diffuse and global pattern are the ultrastructural hallmarks of the most ominous pattern of lupus nephritis class IV, whereas they are more focal or segmental in class III. The various patterns of immune deposit formation in the glomerulus will be described later in the section on WHO classification of glomerular lesions.

In lupus nephritis, electron-dense deposits are predominantly finely granular in texture, but in a few cases the deposits may be partially organized, exhibiting a fingerprint, microtubular, or lattice-like substructure (Figs. 14.34 and 14.35). This

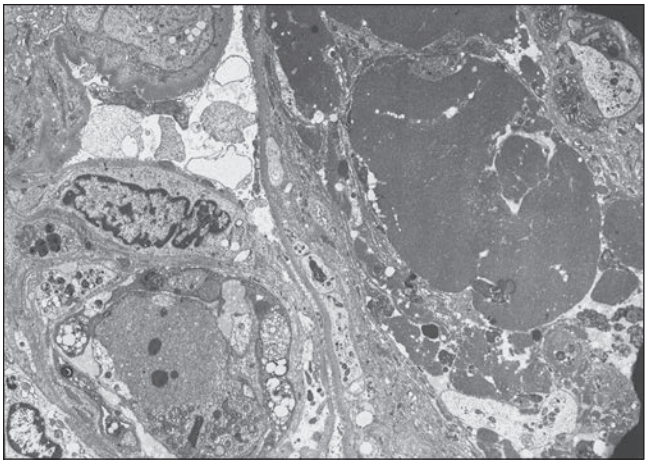


**FIGURE 14.33 Lupus nephritis class IV.** Electron micrograph shows luminal obliteration by a massive glomerular capillary electron-dense deposit corresponding to a "hyaline thrombus." (×2300.)

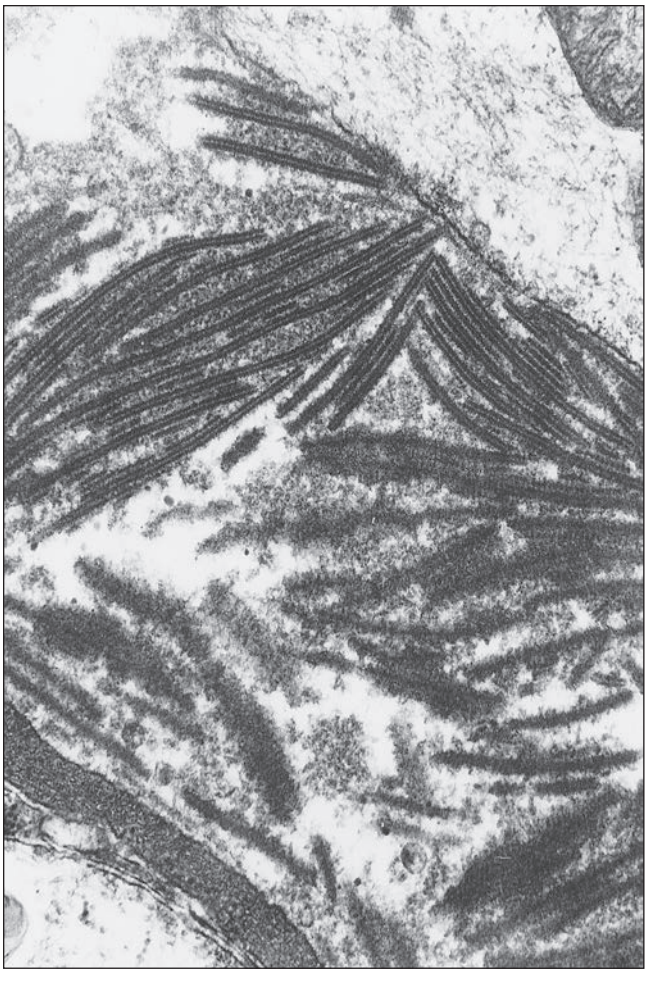


**FIGURE 14.34 Lupus nephritis class IV.** Electron micrograph shows an organized mesangial electron-dense deposit with tubulofibrillar substructure resembling that seen in cryoglobulinemia. (×80,000.)

substructure usually does not affect all deposits completely or uniformly but is identified focally in just a portion of an otherwise granular deposit, especially in the larger intraluminal or subendothelial deposits. Fingerprint substructure, first described by Grishman et al. (163), consists of curved arrays of from two to six parallel dark bands of various lengths alternating with light bands arranged around a center, producing a configuration that mimics a fingerprint (Figs. 14.36 and 14.37). The diameter of the bands varies from 10 to 15 nm, and the distance from the center of one band to the center of the next gives a periodicity of 22 to 29 nm. These bands are usually curved, but some may be straight and seemingly tubular. Cross-striations are sometimes discernible between the bands (Fig. 14.37). These structures somewhat resemble the annular-tubular deposits regularly identified in mixed cryoglobulinemia (see Chapters 22 and 23), suggesting that they represent cryoglobulins. Type 3 cryoglobulins containing two polyclonal immunoglobulins of various classes are common in SLE.

Although this hypothesis has not been studied systematically in a large number of patients, cryoglobulinemia was documented in three of five lupus patients with glomerular fingerprint deposits (164). Several cases of lupus nephritis

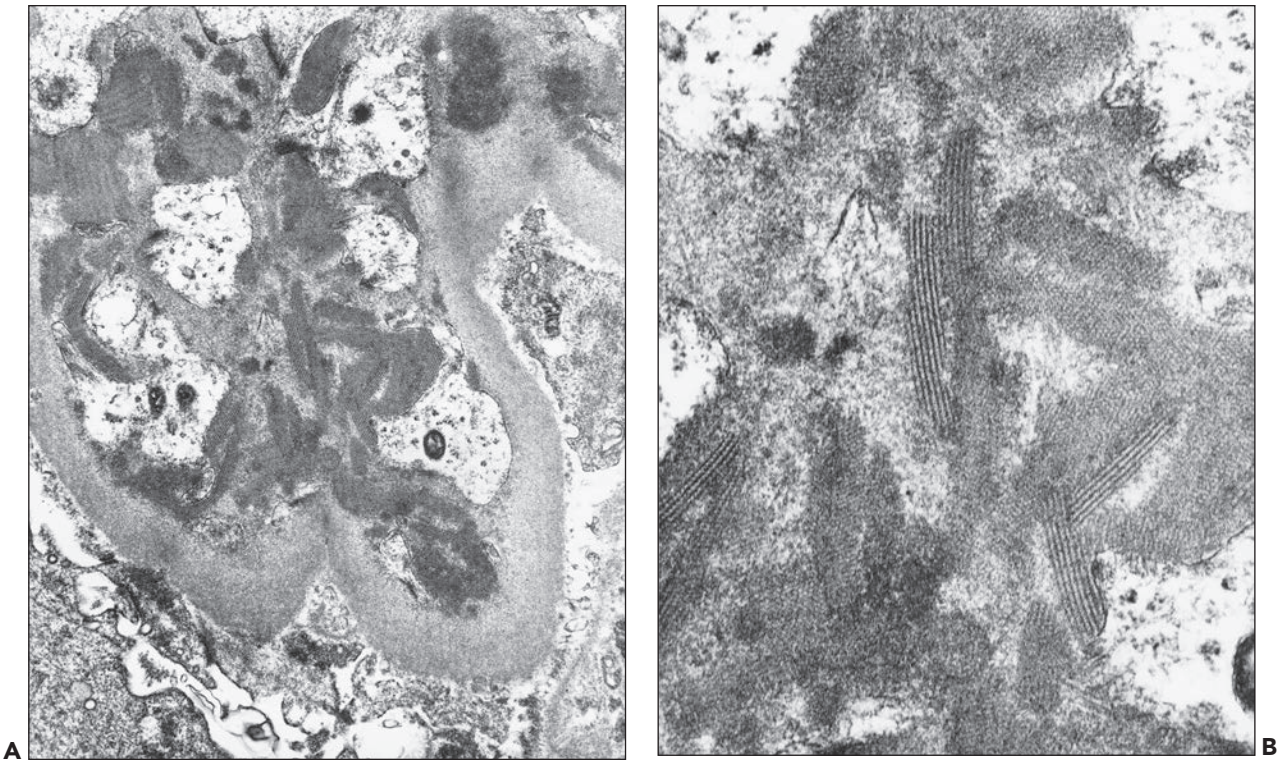



**FIGURE 14.35 Lupus nephritis class IV.** A large subendothelial deposit displays an organized substructure composed of parallel linear arrays resembling those seen in some forms of cryoglobulinemia. (Electron micrographs; **A**, ×10,000; **B**, ×30,000.)

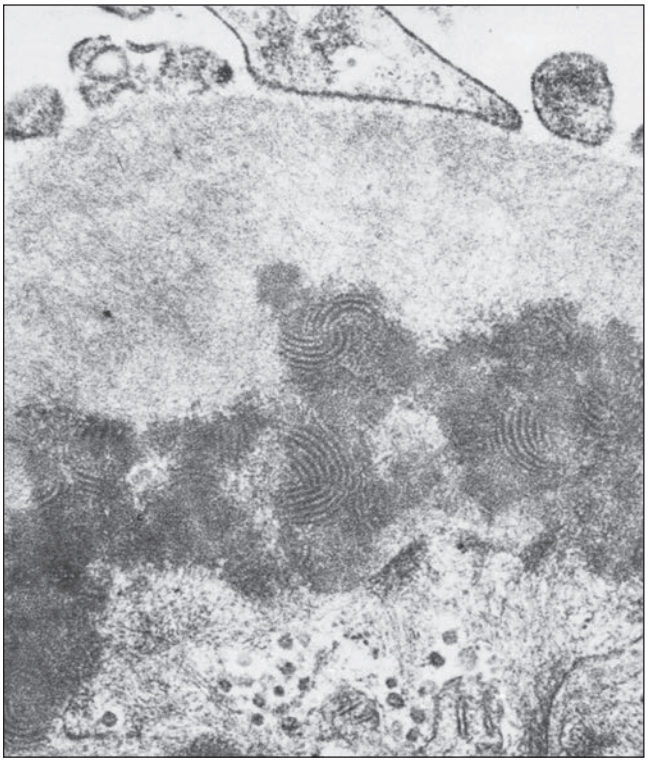


**FIGURE 14.36** A subendothelial electron-dense deposit displays a fingerprint-whorled, lamellated substructure. (×40,000.) (From Churg J, Grishman E. Ultrastructure of immune deposits in renal glomeruli. *Ann Intern Med* 1972;76:479.)

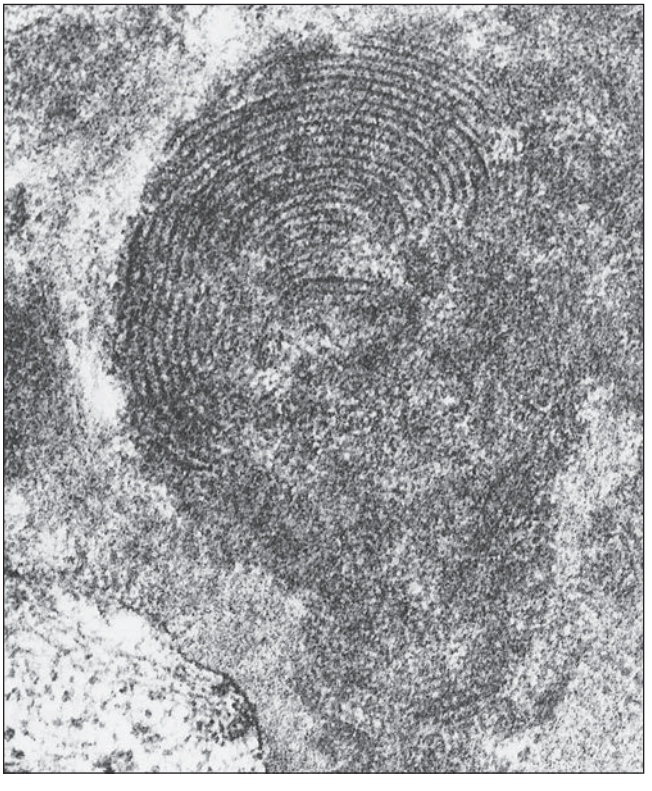


**FIGURE 14.37 Electron micrograph of a subendothelial deposit with a fingerprint substructure shows it to be composed of curvilinear, concentric, membranous structures with fine cross-hatching.** The remainder of the deposit has a granular texture. (×100,000.)

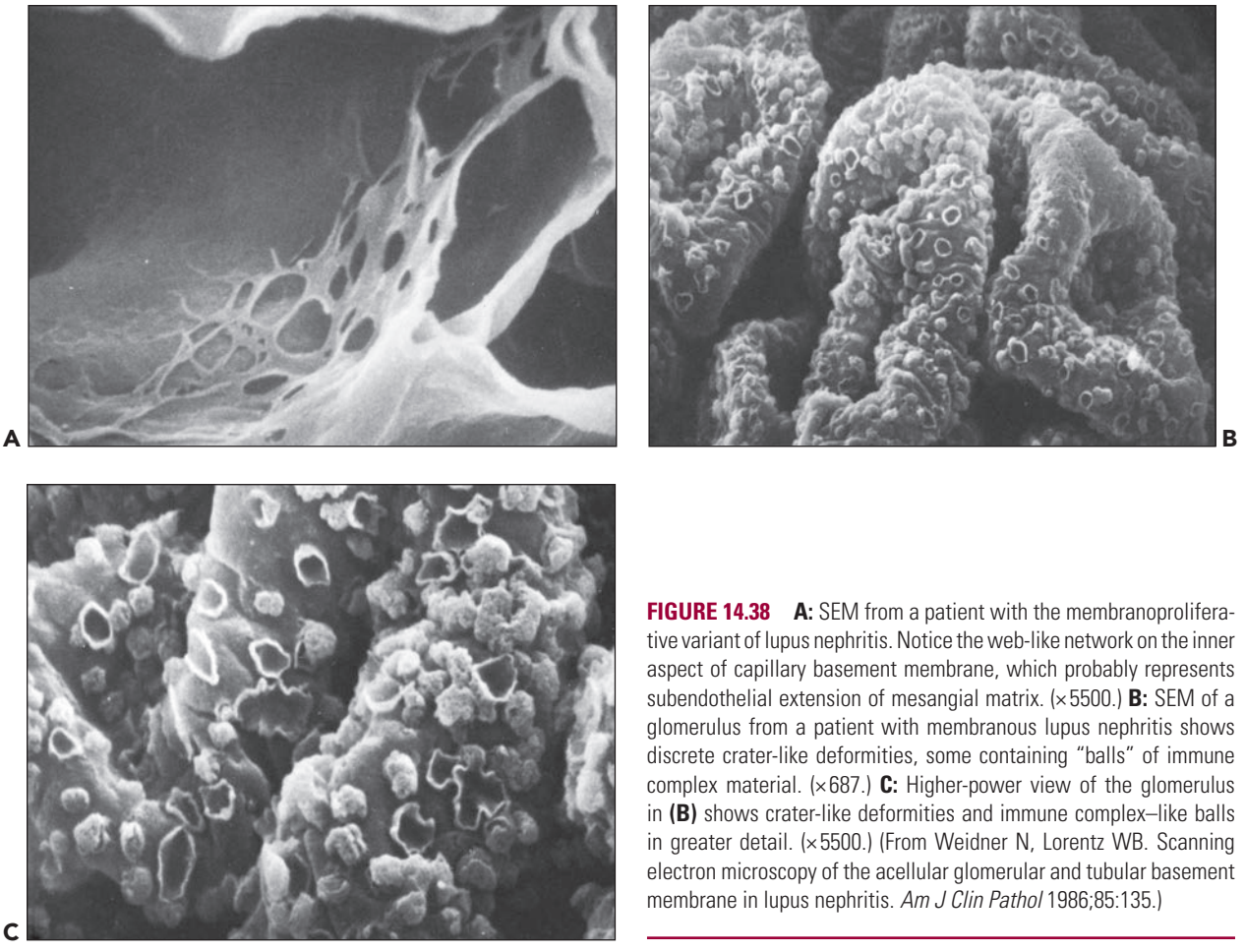



**FIGURE 14.38** **A:** SEM from a patient with the membranoproliferative variant of lupus nephritis. Notice the web-like network on the inner aspect of capillary basement membrane, which probably represents subendothelial extension of mesangial matrix. (×5500.) **B:** SEM of a glomerulus from a patient with membranous lupus nephritis shows discrete crater-like deformities, some containing "balls" of immune complex material. (×687.) **C:** Higher-power view of the glomerulus in **(B)** shows crater-like deformities and immune complex–like balls in greater detail. (×5500.) (From Weidner N, Lorentz WB. Scanning electron microscopy of the acellular glomerular and tubular basement membrane in lupus nephritis. *Am J Clin Pathol* 1986;85:135.)

with a mixed IgG-IgA-IgM cryoglobulin exhibited the same fingerprint substructures on ultrastructural study of the cryoprecipitate and the glomerular deposits (164,165). Moreover, fingerprint substructures similar to those in SLE have also been described in some cases of monoclonal IgG cryoglobulinemia (166). It appears that the fingerprint deposits are not specific for SLE but reflect the composition of the associated immune deposits.

Schwartz et al. (167) pointed out that the subepithelial deposits seen in diffuse proliferative lupus nephritis are often different from those in membranous lupus nephritis. In the membranous form, the subepithelial deposits tend to be more uniform in size, consistency, and distribution. In contrast, they are often irregularly distributed and heterogeneous in size in the diffuse proliferative form, in which one loop may have many small subepithelial deposits and an adjacent loop may have none at all. Moreover, in lupus nephritis, it is common for some subepithelial deposits to traverse the full thickness of the GBM in continuity with underlying subendothelial deposits, a phenomenon not observed in primary membranous glomerulonephritis. Fingerprint substructure may also be identified in the subepithelial deposits of lupus nephritis but not in primary membranous glomerulonephritis.

The use of scanning electron microscopy has illustrated the diversity and complexity of the GBM alterations in lupus nephritis. Using a technique to extract the cells and deposits from the GBMs, Weidner and Lorentz (168) examined the naked GBM in several classes of lupus nephritis. In focal and diffuse proliferative lupus nephritis they found small craters on the GBM, sometimes in clusters corresponding to the sites of small subepithelial and incorporated intramembranous deposits. In some cases with membranoproliferative features, extensive web-like arrays of basement membrane were found on the inner aspect of the capillary walls, corresponding to sites of GBM remodeling and subendothelial extension of mesangium into the peripheral capillaries (Fig. 14.38). The most dramatic alterations of GBM texture were identified in the forms of membranous lupus nephritis. Depending on the stage of development, the membranous alterations produced fields of crater-like pitting of the GBM or nodular plaques consisting of incorporated deposits (see Fig. 14.38). More advanced cases exhibited a complex moth-eaten appearance corresponding to intramembranous deposits with extensive remodeling of the GBM.

The ultrastructural morphogenesis of hematoxylin bodies was elegantly described by Cohen and Zamboni (169) (Fig. 14.39). They consist of two major components of probable nuclear and cytoplasmic origin. The first, which probably is nuclear, occupies a central position and ranges from irregular aggregates of marginated and coarsely clumped chromatin to more spheroid masses of moderately electron-dense, finely granular, or amorphous material. These masses are sometimes lobulated, suggesting a possible neutrophilic origin. This central

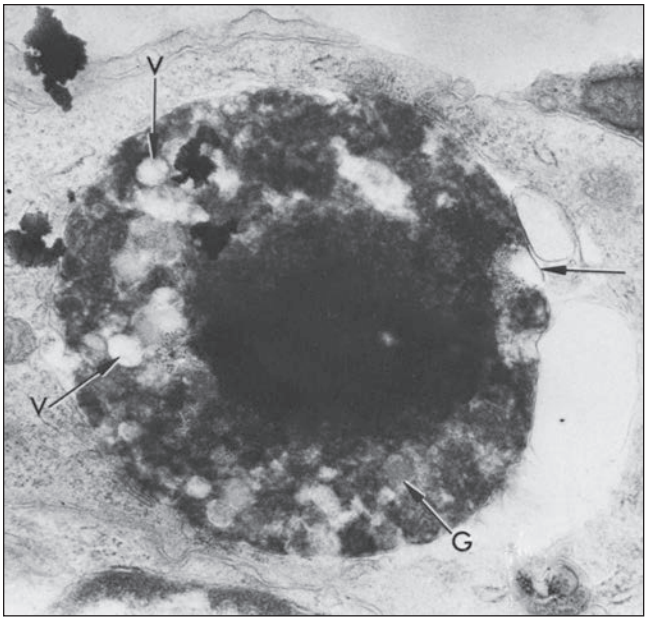



**FIGURE 14.39 Lupus nephritis class IV.** Electron micrograph of a hematoxylin body with a densely rounded central core of altered nuclear material and surrounding cytoplasmic elements consisting of degenerating organelles, all contained within a discontinuous membrane (*arrows*). V, vacuoles; G, granules. (×21,402.) (From Cohen AH, Zamboni L. Ultrastructural appearance and morphogenesis of renal glomerular hematoxylin bodies. *Am J Pathol* 1977;89:105.)

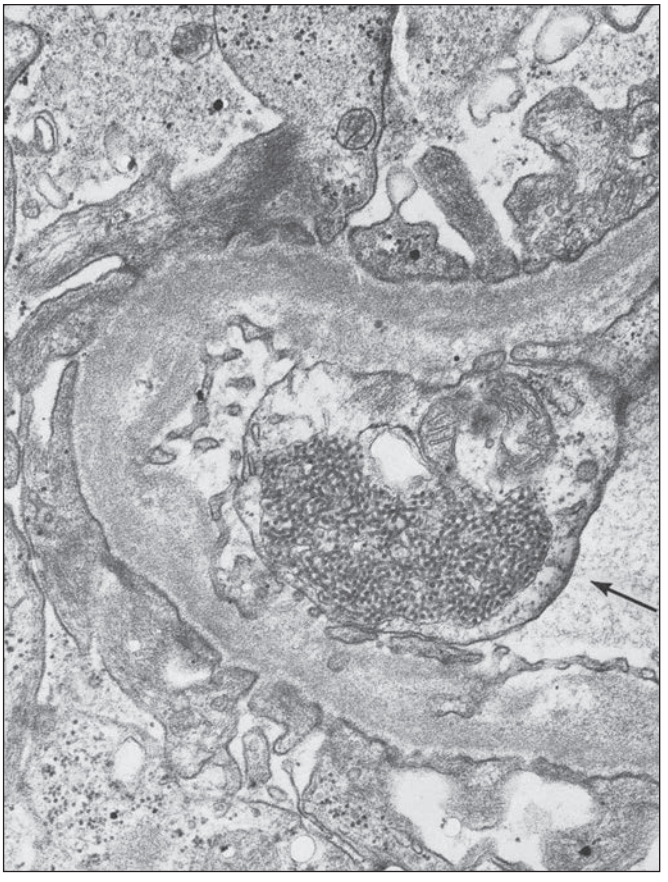


**FIGURE 14.40** The electron micrograph shows TRIs within the endothelial cell (*arrow*) of a glomerular capillary and scattered, subepithelial, electron-dense deposits. (×14,000.) (Courtesy of Dr. Antonovych, Armed Forces Institute of Pathology, Washington, DC.)

component is partly or completely enveloped by membranes. The second component, presumed to be cytoplasmic in origin, consists of aggregates of vesicles, vacuoles, glycogen granules, and other spheroidal or rod-shaped granules, some of which exhibit swollen cristae identifiable as degenerating mitochondria. In some cases, the characteristics of the granules are identical to those of specific granules of neutrophils. These cytoplasmic components are surrounded by a continuous or fragmented membrane, which probably represents the original plasma membrane. These structures are enclosed within large phagocytic vacuoles of cells that appear to be mesangial or monocytic in origin. Adjacent mesangial deposits are often identified surrounding the phagocytic mesangial cell. The morphologic appearance of these structures is similar to the description of the LE cell by Maldonado et al. (170). Grishman and Churg (171) published electron microscopic studies of hematoxylin bodies that occurred in arterial walls in the absence of inflammation.

Another common and characteristic ultrastructural finding in lupus nephritis is intracellular tubuloreticular inclusions (TRIs) (172) (Fig. 14.40). These structures are most often identified in glomerular endothelial cells, in which they may reach large size and commonly number several per glomerulus. They are also readily detected in the endothelium of interstitial capillaries and arteries of the kidney. Rarely can they be identified in glomerular epithelial or mesangial cells. TRI also occur in affected and normal skin in chronic discoid and systemic lupus, in synovium, and in lymphocytes (173–175). These structures were first reported in the kidney biopsy of patients with SLE by Gyorkey et al. (176) in 1969 and consist of 23-nm interanastamosing tubular structures measuring 8 nm in their inner diameter and up to 100 nm long. They are always located in dilated cisternae of endoplasmic reticulum. When first reported, their resemblance to myxovirus particles suggested that they might represent viral particles (176,177). However, Schaff et al. (178) demonstrated that the inclusions were not digestible by ribonuclease or deoxyribonuclease and had chemical properties suggestive of phospholipid and acid glycoproteins, a composition not consistent with actual virions. These structures are inducible in normal lymphocytes on exposure to interferon-$\alpha$ in vitro, earning them the eponym *interferon footprints* (179).

The precise cellular function of these structures remains unknown. Although they are identifiable in most cases of lupus nephritis, TRI are not specific for SLE (180). They are common in patients with human immunodeficiency virus (HIV) infection and to a lesser extent other collagen-vascular diseases (181). They may even be identified in a few healthy individuals. In some patients with apparent primary membranous glomerulonephritis, the finding of TRI may presage development of overt SLE months to years later (182–184).

In cases with significant glomerular proteinuria (most common in class V but also observable in class IV and class III nephritis), the podocytes display a variety of cytoplasmic

alterations that are common to many other glomerular diseases manifesting altered glomerular permeability. These ultrastructural alterations include variable foot process effacement, condensation of cytoskeletal microfilaments, microvillous transformation, and cellular hypertrophy with increased organelles, including endoplasmic reticulum, mitochondria, and membrane bound vesicles, some of which contain electron-dense material suggestive of resorbed proteins.

## Classification of Lupus Nephritis

### Historical Perspective

Because of the wide variety of renal manifestations of SLE, several classifications of lupus nephritis have been proposed. The first to gain wide acceptance were those of Pollak et al. (185) in Chicago and the New York group of Baldwin et al. (186). These early classifications were based entirely on the light microscopic appearance of the glomeruli and essentially recognized three major categories: focal proliferative lupus nephritis (i.e., lupus glomerulitis), diffuse proliferative lupus nephritis (i.e., active lupus glomerulonephritis), and membranous glomerulonephritis. These seminal classification systems of the 1960s failed to acknowledge the existence of milder mesangial forms of the disease because the existence of the mesangial cell was not yet fully appreciated. It was not until 1977 that Baldwin et al. (93) added a fourth category of mesangial lupus nephritis. The subclassification of mesangial lupus nephritis into forms with mesangial hypercellularity and mesangial deposits (IIb) compared with those with mesangial deposits but lacking mesangial hypercellularity (IIa) was solidified by Appel et al. (18) in their 1978 study of the natural history and biopsy features of a large population of patients with lupus nephritis.

The original WHO classification, which arose from a meeting of renal pathologists and nephrologists in Buffalo in 1974, was first published in preliminary form in 1975 by McCluskey (17), with further refinements in 1978 (18) (see Table 14.1). It was further modified by the Pathology Advisory Group of the ISKDC during a meeting in Paris in 1980 to define a number of subclasses (see Table 14.2). This modified classification, published in 1982, was the first to receive the formal imprimatur of the WHO (19). The use of numerous subcategories and the handling of mixed classes made this classification cumbersome to use. Most controversial was the subdivision of the membranous category into designations Va through Vd. Many renal pathologists preferred to treat lesions in class Vc as mixed classes III and V lupus nephritis and those in class Vd as mixed classes IV and V lupus nephritis. This bias is justified by the ample clinical evidence that patients in class Vc and Vd do not behave as membranous disease but have a poor prognosis even worse than that of uncomplicated diffuse proliferative lupus nephritis (187). Accordingly, subgroups Vc and Vd were deleted from the 1995 modified classification (20).

The ISN/RPS 2003 WHO classification revisited was formulated by a group of 23 individuals including renal pathologists, nephrologists, and rheumatologists at Columbia University in May 2002 (see Fig. 14.1 and Table 14.3) (21,22). The meeting was spurred by a universally perceived need to reexamine the existing classifications, eliminate ambiguities, clarify and standardize definitions, and facilitate uniformity in reporting. The 2003 classification has the advantage of defining very precisely the distinctions between each class and the threshold for the diagnosis of each. Features of activity and chronicity are clearly delineated. In many ways, it is a return to the simplicity of the original WHO classification, while incorporating modern refinements in our understanding of activity and chronicity. Inconsistencies, such as the designation of a normal biopsy as a form of class I lupus nephritis, were eliminated. Mesangial lesions are clearly separated into class I and class II depending on the absence or presence of histologically identifiable mesangial expansion. Classes III and IV are separated unequivocably based on the percentage of glomeruli affected by active and chronic lesions. The classification requires that sclerotic glomeruli representing scarred lesions of lupus nephritis be factored into the total number of glomeruli affected. Probably the most controversial aspect is the introduction of a subdivision of class IV based on whether the lesions are predominantly segmental or global, which was designed to test the significance of this differentiation. The threshold for the diagnosis of membranous class V as an isolated or additional class (superimposed on a proliferative class) is clearly defined. And finally, the threshold for a diagnosis of class VI is delineated with the qualification that there should be no residual activity. The classification requires that the diagnostic line include entries for the attendant tubulointerstitial and vascular lesions.

Table 14.7 details the incidence of the various classes of lupus nephritis in renal biopsy specimens using the ISN/RPS classification in several large series, comprising 1341 subjects in total. The average frequencies were class I, 1.3%; class II, 9.3%; class III, 20.6%; class IV, 46.6%; class V, 20.3%; and class VI, 1.6%. Although there is some variability between series, the diffuse proliferative category is the most frequent, accounting for 37% to 66% of patients who have had biopsies. Membranous lesions are less common, averaging 20.3% (range, 8% to 29%).

**TABLE 14.7 Frequencies of histologic patterns of lupus nephritis (ISN/RPS classification)**

| | | Histologic type (%) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Study | Number of patients | Class I | Class II | Class III | Class IV | Class IV-S | Class IV-G | Class V | Class VI |
| Markowitz (234) | 640 | <1% | 7.8% | 23.7% | 53.1% | — | — | 15% | <1% |
| Kojo (221) | 99 | 3% | 13% | 9% | 66% | 20% | 46% | 8% | 1% |
| Yokoyama (215) | 60 | 15% | 17% | 13% | 38% | 10% | 28% | 17% | — |
| Seshan (193) | 541 | 1% | 10% | 20% | 37% | 16% | 21% | 29% | 3% |

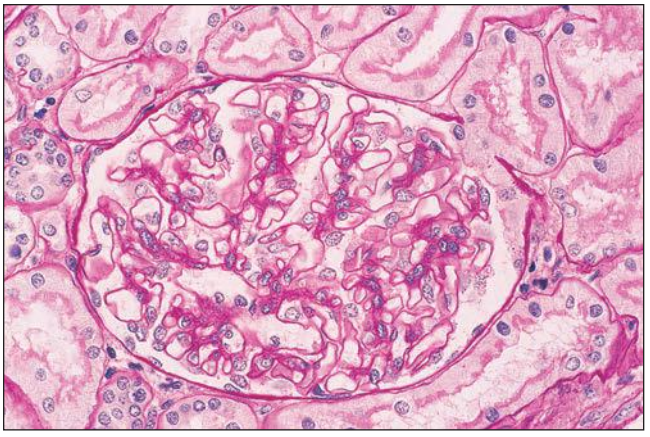


**FIGURE 14.41 Lupus nephritis class I.** The glomerulus is normal in cellularity, and the GBMs are unremarkable. (PAS; ×500.)

## Pathologic Findings by ISN/RPS Classification With Clinical Correlates

### Class I: Minimal Mesangial Lupus Nephritis

According to the ISN/RPS classification, class I denotes normal glomeruli by light microscopy with mesangial immune deposits detected by immunofluorescence and/or electron microscopy. This is the mildest glomerular lesion in lupus nephritis. In the original WHO classification, class I had been defined as a normal renal biopsy from a patient who fulfills ACR criteria for SLE. There are few examples of class I lupus nephritis so defined because these patients generally have no clinical renal abnormalities and are not referred to a nephrologist for biopsy. Because normal biopsies in SLE are so rare and to classify them as a form of "lupus nephritis" is a contradiction in terms, this normal category was eliminated from the ISN/RPS classification and replaced with the original WHO class IIa.

There are no glomerular histologic abnormalities, and the glomerular tuft is normocellular (Fig. 14.41). By immunofluorescence, there are immune deposits limited to the mesangium (Fig. 14.42). The mesangial deposits tend to be small and vary from segmental to global in distribution. By electron microscopy, small electron-dense deposits are present in the mesangium (Fig. 14.43). No electron-dense deposits are identified involving the peripheral glomerular capillary walls.

Patients with class I lupus nephritis usually have minimal urinary findings of microhematuria or mild subnephrotic proteinuria. Renal function is normal. Despite the very mild clinical renal abnormalities, the systemic manifestations of lupus and lupus serologies may be active.

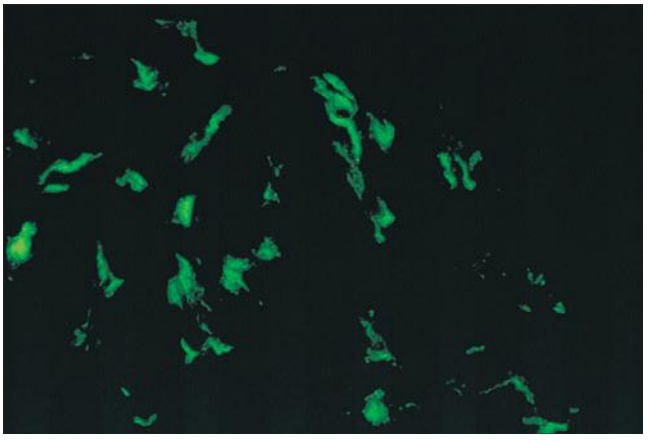


**FIGURE 14.42 Lupus nephritis class I.** Immunofluorescence microscopy shows delicate mesangial positivity for immunoglobulin G, although no abnormalities were seen by light microscopy. (×600.)

### Class II: Mesangial Proliferative Lupus Nephritis

According to the ISN/RPS schema, class II is defined as purely mesangial hypercellularity of any degree and/or mesangial matrix expansion by LM with mesangial immune deposits. There may be rare isolated minute subepithelial or subendothelial deposits by immunofluorescence or electron microscopy that are not visible by light microscopy. In the original (1974) WHO classification, class II had been subdivided into class IIa and IIb according to the absence or presence of mesangial hypercellularity, respectively. In the ISN/RPS classification, the original WHO class IIa has become class I and the original WHO class IIb has become class II.

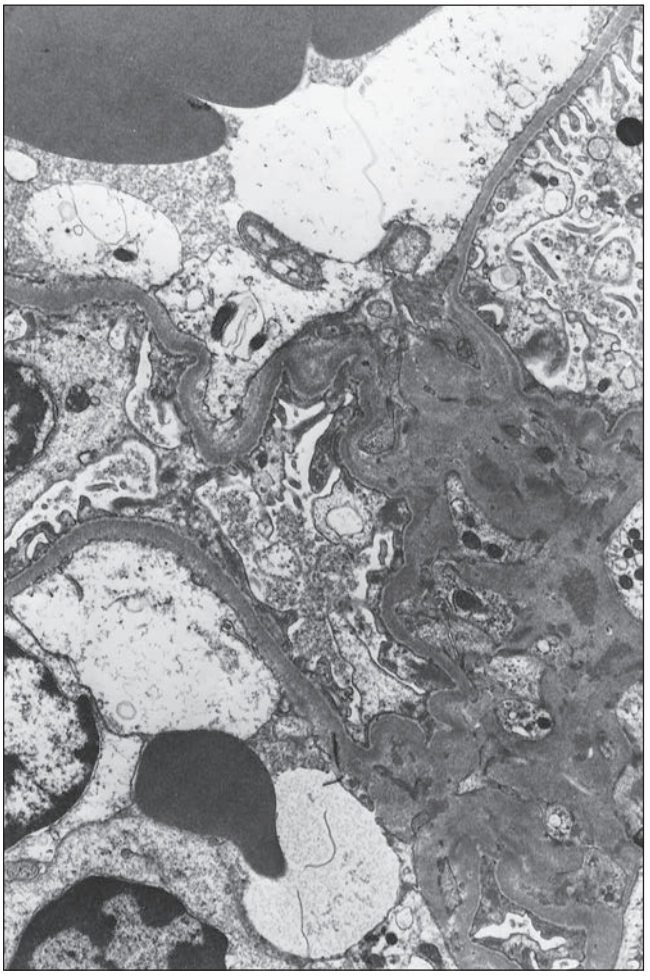


**FIGURE 14.43 Lupus nephritis class I.** The electron micrograph shows small, mesangial, electron-dense deposits. No deposits involve the peripheral GBMs, and foot processes are intact. (×2500.)

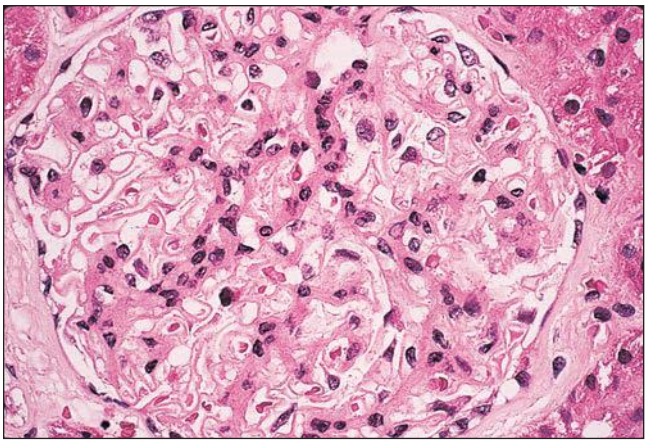


**FIGURE 14.44 Lupus nephritis class II.** There is mild, global, mesangial hypercellularity with thin capillary loops. (H&E, ×500.)

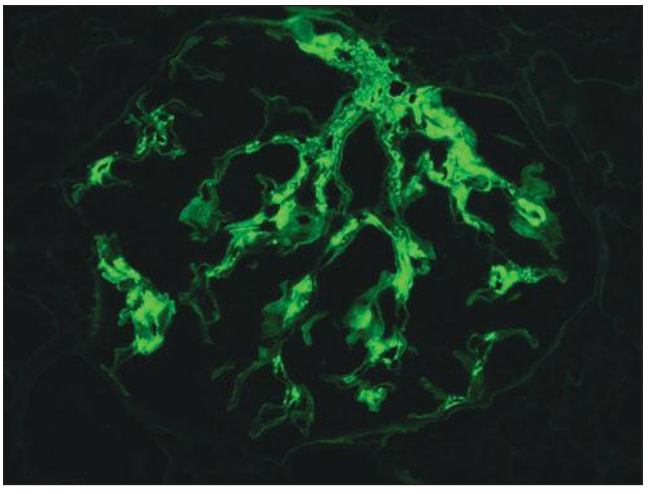


**FIGURE 14.45 Lupus nephritis class II.** Immunofluorescence microscopy shows deposits of IgG confined to the mesangium. (×500.)

By light microscopy, there is mesangial proliferation of any severity (Fig. 14.44). Mesangial hypercellularity is defined as ≥3 mesangial cells in mesangial areas away from the vascular pole, assessed in 3-μm-thick histologic sections. The mesangial proliferation is usually mild to moderate and does not significantly compromise the glomerular capillary lumen. It may vary in distribution from focal to diffuse and may involve the glomerular tuft segmentally or globally. Variable increase in mesangial matrix may accompany the mesangial hypercellularity. Usually, the mesangial deposits are not large enough to be identified by light microscopy. In some cases, however, large mesangial deposits expand the mesangium and impart a glassy, hypereosinophilic appearance to the mesangial matrix. Masson trichrome stain may reveal large mesangial immune deposits as fuchsinophilic (red) zones in the blue/green mesangial matrix.

Cases of lupus nephritis with severe, but purely mesangial, hypercellularity, without obliteration of the capillary lumina, may pose difficulties in classification. Proper classification requires careful assessment of the immunofluorescence and electron microscopic findings. If the immune deposits are limited to the mesangium, even cases of severe diffuse mesangial proliferation should be classified as class II. If significant subendothelial deposits are present by immunofluorescence and/or electron microscopy, or if subendothelial deposits are visible by light microscopy, the case should be classified as focal proliferative if the subendothelial deposits are focally distributed and diffuse proliferative if diffusely distributed. Unfortunately, some early series included examples of segmental endocapillary extension of the mesangial proliferation (148,149) or subendothelial extension of paramesangial deposits visible by light microscopy (188) under the rubric of class II nephritis. In modern series, such cases would more accurately be considered examples of mild or early class III.

Immunofluorescence microscopy typically reveals immune deposits of IgG (18,148,150) and more variably IgM and IgA (93,149), as well as C3 and C1q outlining the mesangial axis, with sparing of the peripheral glomerular capillary wall (Fig. 14.45). These mesangial deposits may be sparse and finely granular or large and globular. Usually, the pattern of deposition by immunofluorescence is more diffuse and regular than the distribution of mesangial hypercellularity seen by light microscopy. Glomerular staining for fibrinogen is usually negative.

As determined by electron microscopy, the mesangial deposits range from small to large and may be segmental or global in distribution (Fig. 14.46). In mild cases, they are often confined to the paramesangial region, subjacent to the GBM reflection. When the mesangial deposits are more abundant, they are widely distributed throughout the full thickness of the mesangial matrix.

Most examples of class II lupus nephritis have exclusively mesangial deposits. However, in practice, some cases of purely mesangial proliferative lupus nephritis will manifest rare small subendothelial electron-dense deposits, especially as extensions from the paramesangial region. These cases raise the question of unsampled focal proliferative (class III) lupus nephritis or at least the possibility of imminent transformation to class III or class IV. Such nonconforming cases are

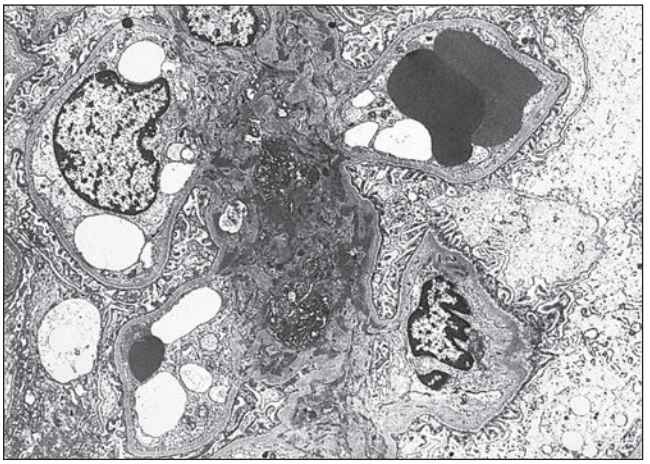


**FIGURE 14.46 Lupus nephritis class II.** The electron micrograph shows mesangial electron-dense deposits accompanied by mild mesangial hypercellularity. There are no deposits involving the peripheral glomerular capillary walls. (×2000.)

problematic and were not adequately addressed by the original WHO classification system. According to the ISN/RPS classification, cases of mesangial proliferative lupus nephritis with rare minute subendothelial or subepithelial deposits (visible only by IF and/or EM) should be classified as class II (21,22). Most practicing renal pathologists deal with these atypical features by indicating in a note that the rare small subendothelial or subepithelial deposits observed in a minority of capillaries suggest the possibility that the glomerulonephritis may evolve into focal proliferative or membranous glomerulonephritis in the near future and that the patient should be carefully monitored. However, the presence of any sizeable (visible by LM) or numerous subendothelial or subepithelial deposits in an otherwise mesangial proliferative glomerulonephritis exceeds what is acceptable in pure class II disease and warrants a designation of class III, IV, or V depending on their distribution and quantity.

Tubular, interstitial, and vascular lesions are typically minimal in class II nephritis. The presence of significant tubulointerstitial or vascular disease should raise the question of a superimposed process, such as interstitial nephritis, hypertensive arterionephrosclerosis, or thrombotic microangiopathy secondary to lupus anticoagulant syndrome or associated TTP-like syndrome.

The clinical renal manifestations of class II lupus nephritis are mild. Fewer than 50% of patients have mild hematuria or proteinuria, which generally does not exceed 1 g in 24 hours (18,149,150,189). The nephrotic syndrome is virtually never observed (unless there is superimposed podocytopathy resembling minimal change disease). Renal insufficiency is uncommon, and mildly reduced creatinine clearance can be demonstrated in less than 15%. Despite the relatively mild glomerulonephritis, serologic tests for SLE may be strongly positive. For example, in two combined series, anti-DNA antibody was detectable in 22 of 28 patients (148,149), with reduced C3 in 6 of the same 28 patients (148,149). Cameron reported C4 to be more regularly reduced than C3 in patients with class II nephritis. Some refinement of these data is available in series in which the mesangial lesions are subclassed as those with and without mesangial proliferation (original WHO class IIb vs. IIa) (18,149,189). Patients with original WHO class IIa nephritis are more likely to have totally normal renal function without proteinuria or abnormal urinary sediment than those with class IIb.

Mesangial lupus nephritis usually has an excellent prognosis, with a 5-year renal survival rate of greater than 90%. Although in many patients it is a stable lesion that may persist for years without change in clinical renal findings, in other patients it may undergo transformation to a more ominous class, which is often heralded by a change in the level of proteinuria, development of a more active urinary sediment, or reduction in renal function. Transformations to diffuse proliferative glomerulonephritis (93,149,162,190–193) are the most common, followed by transformations to focal proliferative or membranous glomerulonephritis (18,149,193). In some patients, a mesangial proliferative glomerulonephritis may represent a phase in the regression of focal or diffuse proliferative lesions, especially after treatment (193).

### Class III: Focal Lupus Nephritis

According to the ISN/RPS and WHO classifications, class III lupus nephritis consists of a focal segmental or global endocapillary or extracapillary glomerulonephritis that affects less than 50% of glomeruli (Fig. 14.47). The endocapillary proliferation is usually focal and segmental. There are typically focal subendothelial immune deposits, with or without mesangial alterations. Lesions may be active or chronic. In determining the percentage of glomeruli affected, both active and chronic lesions must be taken into account. Although most active glomerular lesions are endocapillary proliferative in nature, class III includes glomerular lesions that are membranoproliferative pattern, extracapillary proliferative, or consist of wire-loop deposits without associated proliferation. For this reason, the ISN/RPS classification prefers the broader term "focal lupus nephritis" to the more restrictive term "focal proliferative lupus nephritis" used in the original WHO classification.

The endocapillary proliferative lesions may be an isolated finding. More typically, they occur on a background of mesangial hypercellularity that may be focal or diffuse (Fig. 14.48). Correspondingly, all glomeruli are involved, as detected by immunofluorescence, with generalized mesangial immune deposits, regardless of the focal endocapillary proliferative

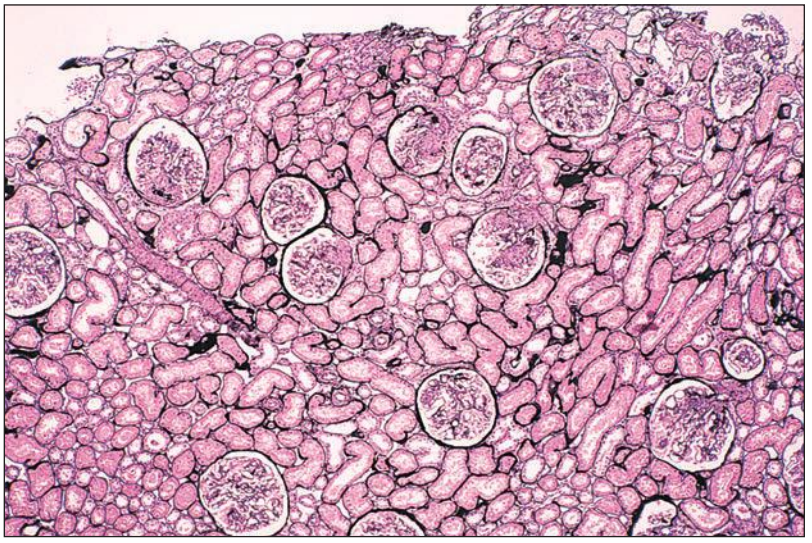


**FIGURE 14.47 Lupus nephritis class III.** A low-power view shows the focal and segmental distribution of the endocapillary proliferation, with some overlying crescents. Endocapillary proliferation affected less than 50% of the total glomeruli in this biopsy. (Jones methenamine silver stain; ×40.)

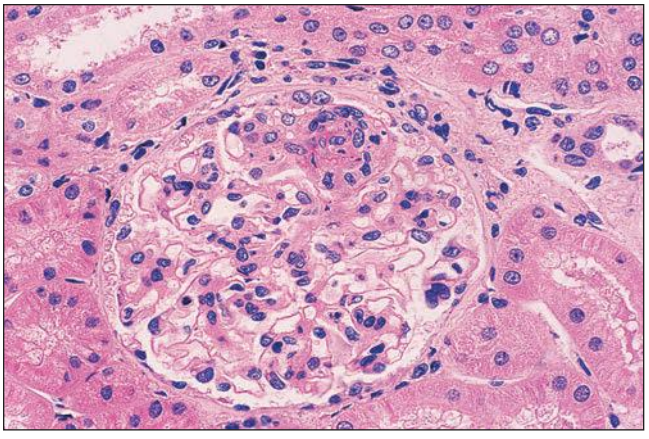


**FIGURE 14.48 Lupus nephritis class III.** There is segmental obliteration of glomerular capillary lumina by endocapillary proliferation, including infiltrating leukocytes, with associated fibrinoid necrosis. The adjacent lobules display mild mesangial hypercellularity. (H&E; ×400.)

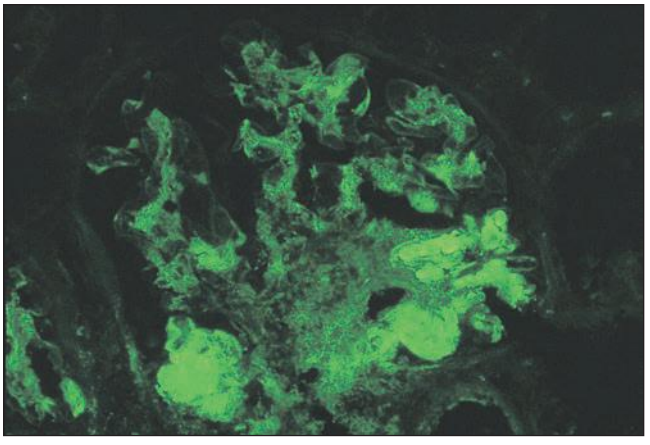


**FIGURE 14.50 Lupus nephritis class III.** The immunofluorescence micrograph shows segmental heavy IgG deposits in several glomerular capillary walls and lumina. The adjacent lobules have mesangial deposits. (×500.)

pattern by light microscopy. The segments with endocapillary hypercellularity exhibit severe narrowing or occlusion of the glomerular capillaries by proliferation of endothelial and mesangial cells with a variable infiltration of mononuclear and polymorphonuclear leukocytes. This may be the only histologic finding in mild cases. More active examples may demonstrate any or all of the histologic features of activity previously discussed in a focal and segmental distribution. These include fibrinoid necrosis, karyorrhexis or pyknosis, rupture of the GBM, wire-loop deposits, intraluminal hyaline thrombi, and overlying cellular crescents (Fig. 14.49). Hematoxylin bodies are sometimes identified in the necrotizing lesions. Typically, foci of focal necrosis heal by progression to segmental or global scars with associated fibrous crescents or small synechiae to the Bowman capsule. Glomerular capillary walls in the "uninvolved" segments are usually thin and delicate.

Class III biopsy specimens often display focal tubulointerstitial disease, including patchy interstitial edema, inflammation, and tubular atrophy. In the chronic phase, tubular atrophy and interstitial fibrosis are more pronounced and usually parallel the distribution of the glomerulosclerotic lesions. Such evolution may be seen on repeat biopsy, during the natural course of disease, or after treatment. In many cases, active and chronic lesions may coexist, especially in chronic cases with recent clinical reactivation.

Immunofluorescence microscopy reveals diffuse and global mesangial deposition in all glomeruli with more focal and segmental subendothelial capillary wall deposits, a pattern that mirrors the distribution of glomerular proliferative lesions by light microscopy (Fig. 14.50). The subendothelial deposits often exhibit a semilinear, comma-shaped appearance with smooth outer contour owing to conformity to the delimiting GBM (Fig. 14.51). Small numbers of subepithelial deposits may appear as more granular, rounded deposits. IgG, IgM, and IgA may be present (18,93,194), but IgG is the most constant and usually the most intense. C3 and C1q are also commonly seen. Strong staining for fibrin is identifiable in the necrotizing lesions and crescents. Immune deposits are frequently identifiable in the tubulointerstitial compartment and arterial walls.

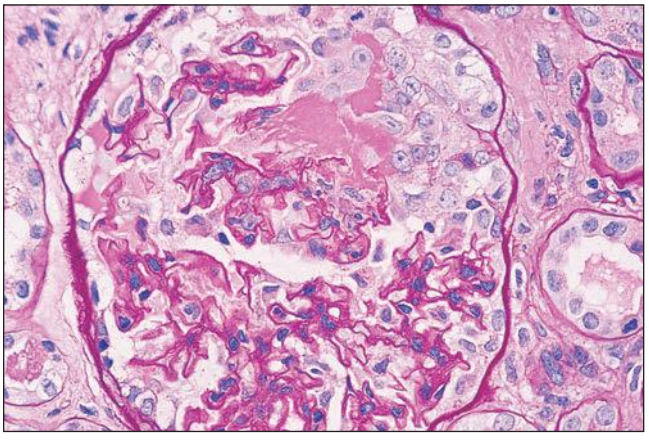


**FIGURE 14.49 Lupus nephritis class III.** There is segmental endocapillary proliferation and necrosis, with focal rupture of GBMs and an adjacent cellular crescent. (PAS, ×500.)

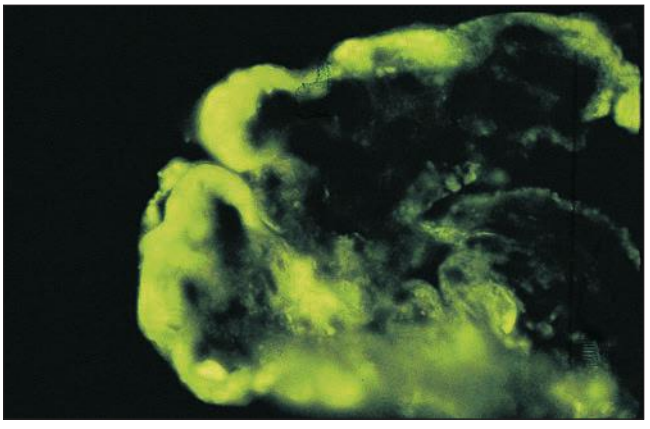


**FIGURE 14.51 Wire-loop deposit.** By immunofluorescence, there is a large subendothelial deposit that conforms to the contour of the GBM, producing a smooth comma-shaped outer contour. (×1000.)

By electron microscopy, mesangial deposits are readily identifiable. Segmental subendothelial deposits are usually demonstrable in the glomeruli with segmental endocapillary proliferative lesions, but they may be absent if the more severely affected glomeruli are not sampled in the tissue processed for electron microscopy. Scattered subepithelial deposits are also frequently seen, often in an irregular distribution. Although there is some disagreement on this point (191), some researchers have suggested that class III biopsies with substantial capillary wall subendothelial or subepithelial deposits are often predictive of future transformation to diffuse proliferative or membranous glomerulonephritis, respectively (192,195).

Some investigators have called attention to a subgroup of class III lupus nephritis with extensive segmental necrosis and crescents but with little or no identifiable subendothelial deposits (196–199). It has been suggested that the glomerular immune complex load is insufficient to account for the severe active lesions, raising the possibility that they may have a natural history and pathogenesis akin to vasculitis and corresponding pauci-immune focal necrotizing and crescentic glomerulonephritis. Circulating ANCA have been identified in some of these patients (198), although ANCA serologies have not been investigated in any systematic way.

Class III lupus nephritis has a heterogeneous clinical picture (200). About 50% of patients have an active urinary sediment (e.g., hematuria, leukocyturia, cellular casts), and 25% to 50% have proteinuria, which may be accompanied by the nephrotic syndrome in up to one third of patients. Renal insufficiency, however, is uncommon, affecting only 10% to 25% of patients. Hypertension occurs in up to one third of patients, initially or over the course of follow-up (18,93,148). Serologic abnormalities are common in this class, with anti-DNA antibody and reduced complements detected in more than one half of patients (18,148,149).

The course and prognosis of class III lupus nephritis are variable. Initial experience suggested a favorable picture with no histologic progression and little renal functional deterioration (185,186,201–203). However, many subsequent studies have reported a 5-year renal survival rate of 85% to 90%, indicating progression to severe, irreversible renal damage in a small percentage of patients (204,205). Repeat renal biopsies have indicated that this poor outcome usually results from progression from class III to class IV, which many consider movement along a disease continuum rather than a real change in the quality of the glomerular lesions. Mahajan et al. (195) and Zimmerman et al. (192) have emphasized that patients with disease that progresses to diffuse proliferative glomerulonephritis often have a higher initial level of proteinuria than those whose disease remains stable. Transformations from class III to class V (membranous) have also been described. These transformations often are heralded by an abrupt increase in proteinuria, sometimes with the development of the nephrotic syndrome.

### Class IV: Diffuse Lupus Nephritis

Class IV denotes diffuse segmental or global endocapillary or extracapillary glomerulonephritis involving ≥50% of glomeruli. Typically, there are diffuse subendothelial immune deposits, with or without mesangial alterations. Lesions may be active or inactive (sclerosing), and both types of lesions should be accounted for when determining the percentage of total

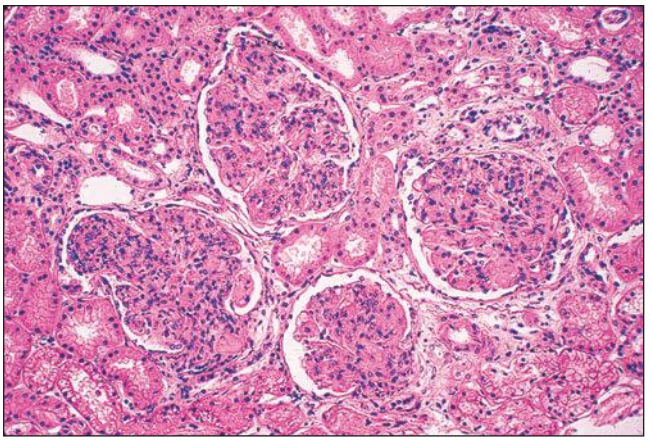


**FIGURE 14.52 Lupus nephritis class IV-G.** There is diffuse and global endocapillary proliferation involving all the glomeruli in this biopsy. (H&E, ×180).

glomeruli affected by glomerulonephritis. As for class III, class IV includes glomerular lesions that are membranoproliferative pattern, extracapillary proliferative, or consist of wire-loop deposits without proliferation, thereby justifying "diffuse lupus nephritis" as the preferred designation over the older term "diffuse proliferative lupus nephritis" used in the original WHO classification. The ISN/RPS classification subdivides class IV into those cases with diffuse segmental (IV-S) versus diffuse global (IV-G) distribution (Figs. 14.52 and 14.53). The designation diffuse segmental (IV-S) is used if greater than 50% of the involved glomeruli have segmental lesions; similarly, diffuse global (IV-G) applies if greater than 50% of the involved glomeruli have global lesions.

Many investigators consider class III and IV lupus nephritis as ends of a pathologic continuum, such that the two classes differ from each other quantitatively but not qualitatively. However, as discussed in detail later in this chapter, some investigators propose that class IV-S is pathogenetically distinct from lupus nephritis that has predominantly endocapillary

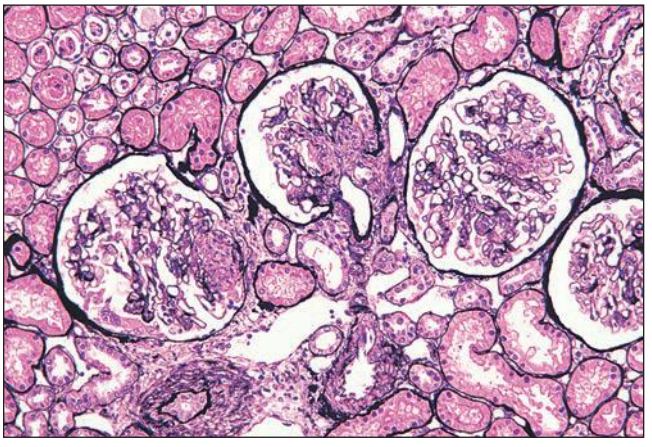


**FIGURE 14.53 Lupus nephritis class IV-S.** There is diffuse segmental glomerular proliferation involving more than 50% of the total glomeruli in this biopsy. (Jones methenamine silver, ×180.)

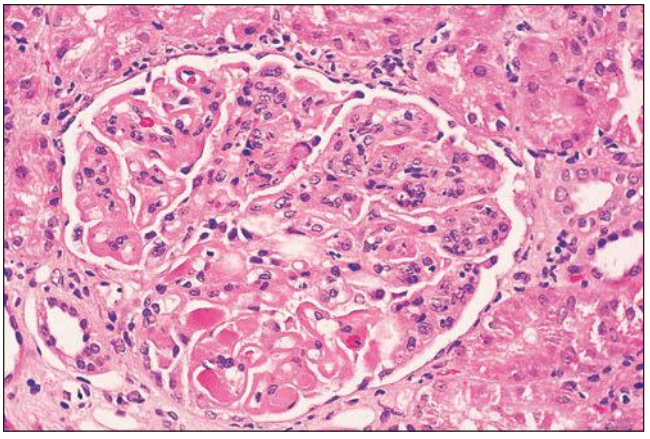


**FIGURE 14.54 Lupus nephritis class IV.** There is global narrowing of glomerular capillaries by mesangial and endocapillary proliferation. Wire-loop deposits and hyaline thrombi are segmentally distributed. (H&E; ×500.)

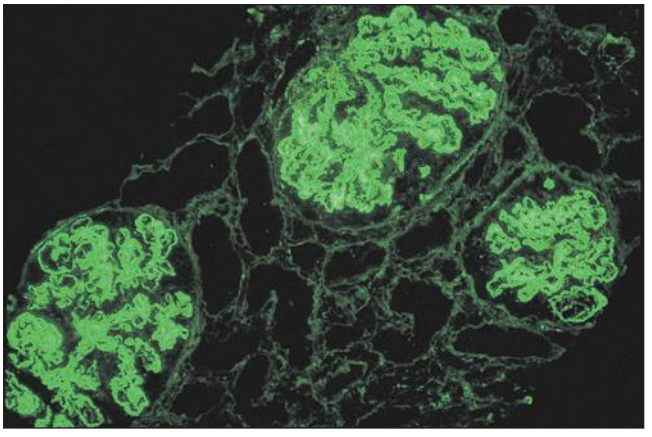


**FIGURE 14.56 Lupus nephritis class IV.** The low-power immunofluorescence micrograph shows intense, diffuse staining for IgG in the glomerular mesangium and peripheral capillary loops, consistent with a subendothelial distribution. (×120.)

hypercellularity rather than predominantly segmental necrosis. All the lesions of active glomerular disease described for class III (e.g., nature of the endocapillary cells, fibrinoid necrosis, karyorrhexis and pyknosis, neutrophil infiltration, wire-loop deposits, hyaline thrombi, hematoxylin bodies, crescents) also apply to class IV. The two classes are distinguished by definition based on the percentage of glomeruli affected.

In most examples of class IV lupus nephritis, especially class IV-G, the lesions tend to be diffuse and global, although segmental lesions may affect some glomeruli (Figs. 14.54 and 14.55). Occasionally, several glomeruli with only mesangial proliferative features among many other severely affected glomeruli are found. Typically in class IV-G, the subendothelial and mesangial deposits are larger and more abundant than in class III and class IV-S and usually stain more intensely by immunofluorescence (Figs. 14.56 and 14.57). As in class III, lobules lacking endocapillary proliferation and subendothelial deposits usually display mesangial deposits and some degree of mesangial hypercellularity. In some patients, proliferation is distributed uniformly throughout most of the glomeruli, but in others, there may be considerable variation in the severity of proliferation from one glomerulus to the next or even between adjacent lobules of an individual glomerulus.

Schwartz et al. (187) described a category of "severe segmental glomerulonephritis" in which the glomerular inflammation was predominantly diffuse but segmental. This category would now be designated as IV-S in the ISN/RPS classification. This category had an outcome measured in short-term renal survival that was intermediate between the classic focal and diffuse proliferative groups (187).

In class IV, all the histologic features of active lupus nephritis reach their most florid expression. In severe examples, there may be abundant wire-loop deposits and necroses, and it is in class IV that hematoxylin bodies are most likely to be encountered. In addition to classic diffuse endocapillary proliferative glomerulonephritis, the modified WHO classification recognized several morphologic variants of class IV that may

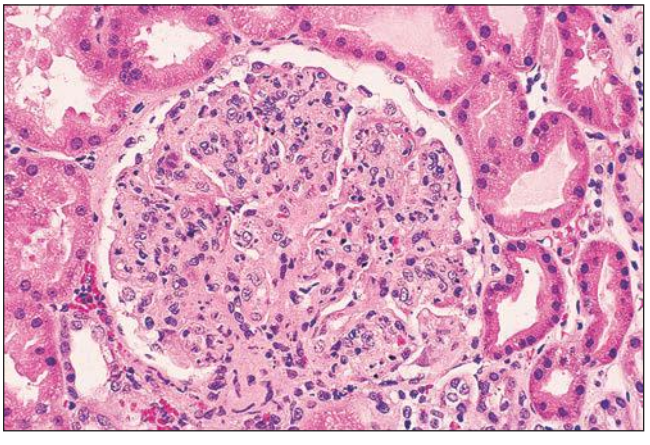


**FIGURE 14.55 Lupus nephritis class IV.** The endocapillary proliferation is global and includes many infiltrating neutrophils. (H&E, ×500.)

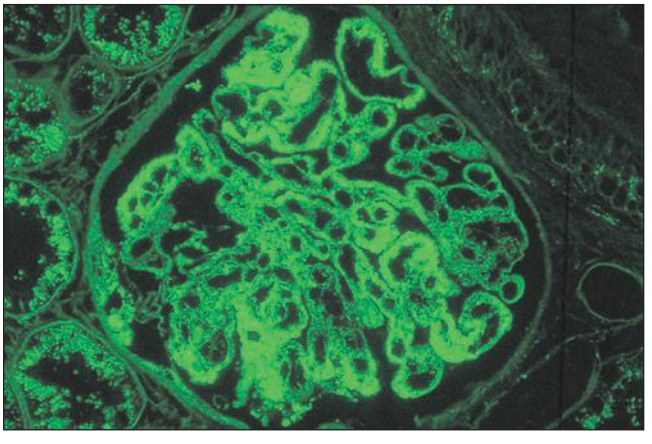


**FIGURE 14.57 Lupus nephritis class IV.** There are abundant deposits of IgG in the mesangium and the peripheral capillary walls. Most of the glomerular capillary wall deposits appear subendothelial, with more segmental subepithelial deposits. (Immunofluorescence micrograph, ×600.)

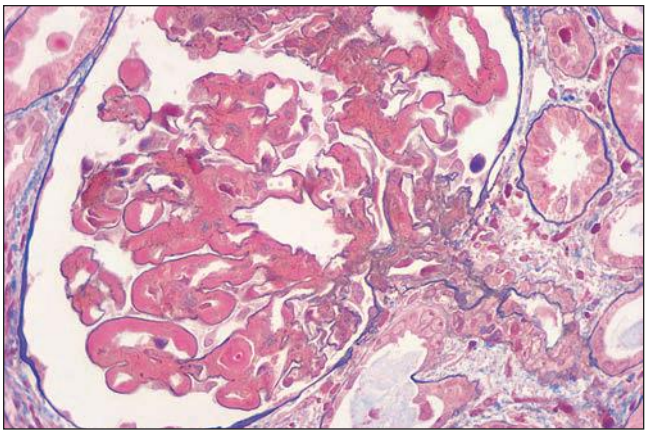


**FIGURE 14.58 Lupus nephritis class IV.** This example has diffuse wire-loop deposits without appreciable endocapillary proliferation. (Masson trichrome, ×600.)

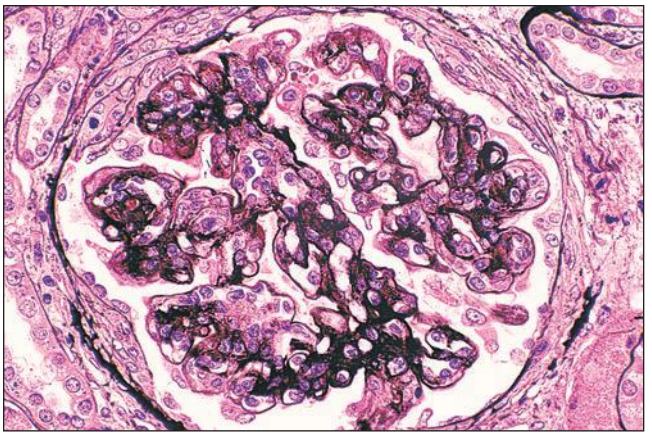


**FIGURE 14.59 Lupus nephritis class IV.** Membranoproliferative variant with lobular accentuation, nodular mesangial expansion, and numerous double contours of the GBM. (Jones methenamine silver; ×500.)

pose difficulty in diagnosis (19,20). These include severe mesangial proliferative glomerulonephritis with diffuse subendothelial deposits and mesangiocapillary (or membranoproliferative) pattern glomerulonephritis and extensive subendothelial deposits with minimal glomerular hypercellularity (Fig. 14.58). Class IV-G disease has diffuse distribution of subendothelial deposits by immunofluorescence and electron microscopy, although the pattern of proliferation that accompanies these deposits varies considerably.

In the severe mesangial proliferative subgroup, which is uncommon, the light microscopic findings frequently suggest class II, and it is not until immunofluorescence and electron microscopy are performed that the true diagnosis of class IV nephritis is apparent. In the variant with diffuse subendothelial deposits but minimal mesangial or endocapillary hypercellularity, the correct designation as class IV is usually apparent even by light microscopy, because the subendothelial deposits are large enough to be visible as extensive wire loops. Diffuse distribution of subendothelial deposits that can be detected by light microscopy, regardless of the pattern of proliferation, is indicative of class IV lupus nephritis.

The membranoproliferative pattern subgroup of class IV is characterized by widespread circumferential or partial mesangial interposition with double-contoured capillary loops owing to subendothelial neomembrane formation (Fig. 14.59). This pattern typically causes accentuation of the glomerular lobularity that is indistinguishable histologically from primary membranoproliferative glomerulonephritis type 1. If numerous subepithelial deposits are also present, the findings closely resemble membranoproliferative glomerulonephritis type 3 of Burkholder (see Chapter 8). It has been suggested that these membranoproliferative variants of class IV lupus nephritis usually lack necrotizing features (18), but others (149) have not found any difference in the frequency of necroses or crescents in this group compared with other examples of diffuse proliferative lupus nephritis.

Immunofluorescence studies reveal diffuse mesangial and widespread (but more irregular) capillary wall staining of immune reactants in class IV, especially class IV-G (86,149,150,162,194,206,207), although there are less immune reactants in class IV-S (214). The capillary wall staining is predominantly subendothelial, but irregularities in the outer contour of the deposits often attest to the presence of scattered subepithelial deposits as well (Fig. 14.60). If the subepithelial deposits are regular and diffuse in distribution (involving at least 50% of the glomerular capillary surface area of at least 50% of glomeruli), a designation of mixed class IV and V (i.e., diffuse proliferative and membranous) lupus nephritis is warranted (21,22). IgG is universally present, although codeposits of IgM or IgA are commonly but less consistently found. In contrast to IgA nephropathy, IgA may be equal to IgG in intensity but is rarely dominant over IgG in intensity (152). Most commonly, the intensity of IgA is less than that of IgG (149,207). In most cases, both C1q and C3 are codeposited, although the intensity of C1q staining is frequently stronger than C3. Properdin is usually present (154) in the same distribution as the other complement components. Staining for fibrinogen is strong in the distribution of crescents and necrotizing lesions, often oblit-

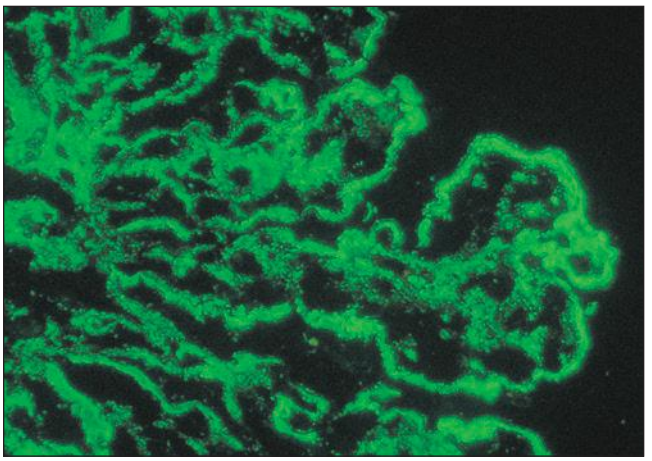


**FIGURE 14.60 Lupus nephritis class IV.** By immunofluorescence, granular deposits of C1q outline the mesangial areas and capillary loops. The peripheral capillary wall deposits are predominantly subendothelial, with minute, more irregular, subepithelial deposits. (×700.)

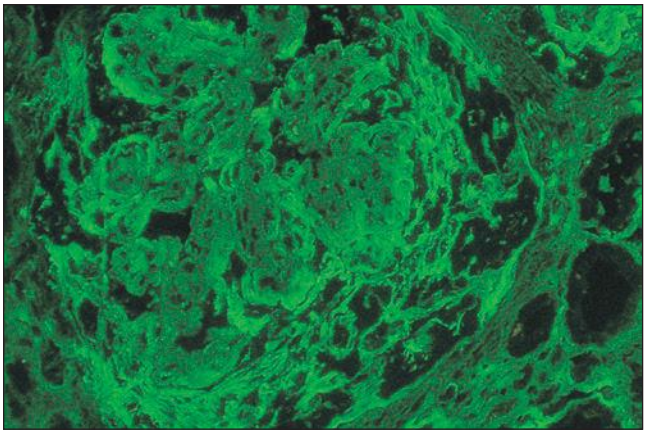


**FIGURE 14.61 Lupus nephritis class IV.** The immunofluorescence micrograph shows staining for fibrinogen in the periphery of the glomerular capillaries and the overlying crescent. (×600.)

erating the tuft architecture (Fig. 14.61). However, more delicate semilinear staining for fibrinogen of about 1+ intensity may be seen more diffusely in areas of nonnecrotizing endocapillary proliferation in active glomerulonephritis.

Electron microscopy confirms and sometimes amplifies the light microscopic and fluorescence microscopic assessment of extensive mesangial and subendothelial deposits, with lesser subepithelial deposits (Fig. 14.62). It is in this form of lupus nephritis that the fingerprint substructure is most commonly identified (208). Fibrin tactoids may be identified in necrotizing lesions (Fig. 14.63).

Vascular lesions occur most frequently in the diffuse proliferative group, although they affect less than 50% of cases. Uncomplicated vascular immune deposits detected by immunofluorescence or electron microscopy are the most common lesion and are usually unaccompanied by detectable vascular changes by light microscopy. These usually consist of granular vascular deposits of IgG, IgM, IgA, C3, and C1q in the subendothelial basement membrane and perimyocyte membranes. Only a subset of patients with severe, active class IV disease has histologically identifiable lupus vasculopathy with obliterative noninflammatory arteriolar lesions containing abundant intimal and intraluminal immune deposits. True arteritis resembling polyarteritis nodosa or microscopic polyangiitis is rare. Thrombotic microangiopathy affecting small arteries, arterioles, and glomerular capillaries may occur in association with a circulating lupus anticoagulant or APL antibody or as a manifestation of TTP-like syndrome.

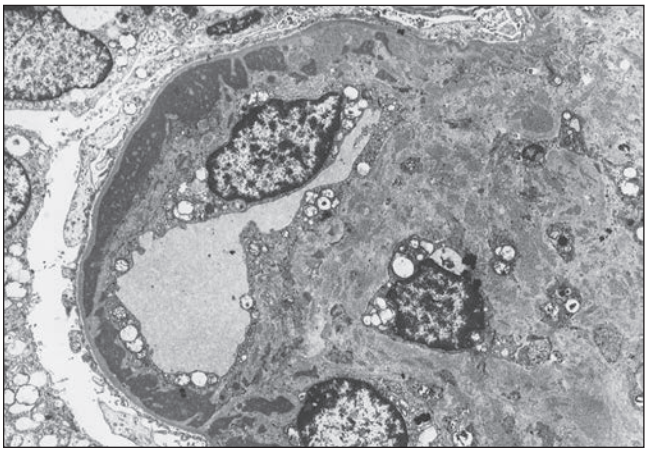


**FIGURE 14.62 Lupus nephritis class IV.** The electron micrograph shows a circumferential, subendothelial, electron-dense deposit that has been incorporated into the glomerular capillary wall by subendothelial neomembrane formation. There is marked mesangial expansion by mesangial proliferation and increased matrix containing many granular, electron-dense deposits. (×2875.)

Tubular and interstitial lesions are nearly universal in diffuse proliferative lupus nephritis. These range from immune deposits detectable only by immunofluorescence and electron microscopy in the interstitium and tubular basement membranes to histologically identifiable interstitial inflammation, edema, fibrosis, and tubular atrophy (Fig. 14.64). The activity and chronicity of these lesions usually correlates with the activity and chronicity of the glomerular lesions. These changes reflect secondary atrophy of the dependent nephron following glomerulosclerosis (i.e., process of nephron dropout) as well as active immunologically mediated tubular damage.

Segmental and global glomerulosclerosis are the inevitable consequence of active necrotizing lesions with crescent formation. Glomerulosclerosis occurs at a slower tempo, even in cases without overt necrotizing or crescentic lesions, as evidenced by repeat biopsies performed to monitor treatment response and prognosis. In such cases, repeat biopsy may show regression to a less active mesangial proliferative form with focal segmental and global sclerosis. At this stage, as detected by immunofluorescence and electron microscopy, small residual capillary wall deposits and ultrastructural basement membrane irregularities such as thickening, lamellation, mesangial interposition, and organized, partially resorbed intramembranous deposits usually attest to the previous presence of more active class IV disease.

Patients with class IV lupus nephritis have the most severe and active clinical renal presentation. Not surprisingly, they constitute the largest percentage of patients in most clinical series of severe lupus nephritis based on renal biopsy. Proteinuria is universal, and up to 50% of patients may have the nephrotic syndrome, initially or manifesting later in the course (18,93). Baldwin's experience (93) is typical in that 41 of 44 patients with diffuse proliferative lupus nephritis had nephrotic proteinuria, in 26 as an initial finding, and in 15 during the course of the renal disease. Hematuria occurs to variable degrees in 80% to 90% of patients (18,93,148), with frequent detection of red blood cell casts and associated leukocyturia in very active cases. Between 30% and 40% of patients has hypertension at disease outset (93,148). Renal insufficiency of various degrees of severity is detected in greater than 50% of patients by determinations of the GFR, although serum creatinine levels may be in the normal range. The serum creatinine levels are typically low in young women with little muscle mass and are a less sensitive marker of renal function than the GFR. Serologic test results for lupus indicate active disease in 50% to 90% of patients (148,149). ANA is detectable in greater than 98%, but anti-dsDNA antibody is less often detectable. Serum levels of complements C3 and C4 are reduced in approximately two thirds of cases (148). Complement levels may normalize with

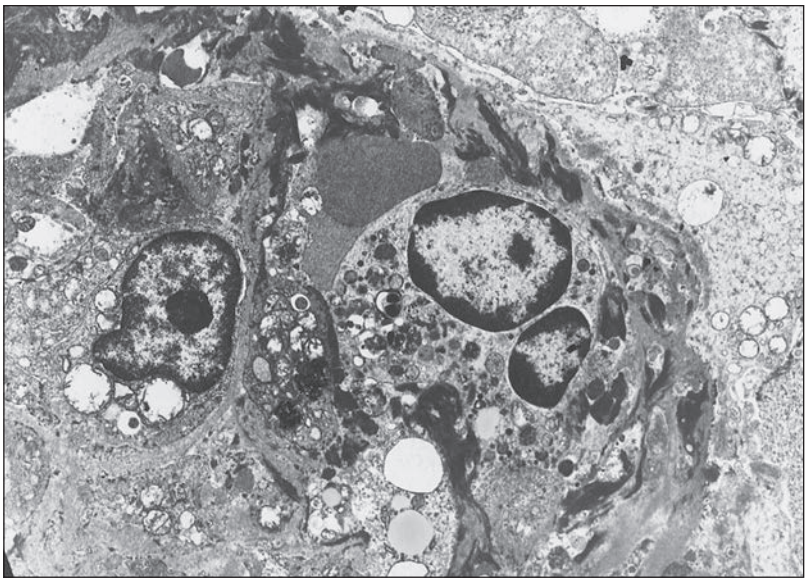


**FIGURE 14.63 Lupus nephritis class IV.** The electron micrograph from a fibrinoid necrotizing lesion shows endothelial necrosis with subendothelial deposition of abundant fibrillar fibrin and scant granular, electron-dense immune deposits. Neutrophils and monocytes infiltrate the glomerular capillary lumen. (×2200.)

steroid therapy, even when brief and inadequate to control the disease. Thus, the percentage of untreated patients with hypocomplementemia is considerably higher, 87% in one series (149). In untreated patients, the quantity of subendothelial deposits was found to correlate roughly with the reduction in serum C3 levels (209).

The series (18,149,150) that separate the membranoproliferative pattern from other forms of diffuse proliferative lupus nephritis are in agreement that the level of proteinuria is greater in the membranoproliferative group. In the series of Sinniah and Feng (150), patients with a membranoproliferative pattern had a higher incidence of the nephrotic syndrome (11 of 19 patients) than those with a usual diffuse proliferative pattern (2 of 15 patients). Appel et al. (18) found that patients with the membranoproliferative variant tend to be persistently hypocomplementemic throughout their course, whereas those with diffuse proliferative lesions of the usual type frequently experience a normalization of the serum complement during therapy. Hill et al. (34) found that patients with a membranoproliferative pattern on first biopsy tended to respond to therapy; however, the persistence of this pattern or its new appearance on repeat biopsy 6 months following therapy was associated with a poor outcome.

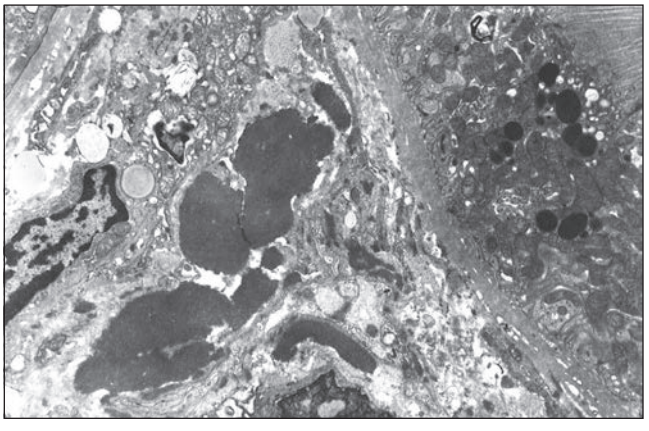


**FIGURE 14.64 Lupus nephritis class IV.** Massive electron-dense deposits are present in the interstitial collagen adjacent to a tubule. (×2000.)

The category IV-S was introduced because of evidence from the Chicago group that this subgroup has worse long-term outcome than IV-G, suggesting important prognostic differences (210,211). Renal survival at 10 years was 75% for IV-G ($n$ = 35) compared to 52% for IV-S ($n$ = 24), which the authors refer to as class III ≥50% (211). During the course of follow-up, 60% of patients with IV-G entered remission compared to only 38% of patients with IV-S (211). The authors proposed that the diffuse segmental lesion, particularly one with necrotizing features unaccompanied by endocapillary proliferation, is a particularly ominous histologic form and may involve pathogenetic mechanisms similar to those in pauci-immune necrotizing glomerulonephritis and vasculitis. Roles for ANCA, antiendothelial antibody, and anticardiolipin antibody have been proposed, but none of these has been studied systematically. The ISN/RPS classification has been used by a number of groups to test the significance of class IV-S versus IV-G (Table 14.8 and summarized in Markowitz and D'Agati (212)). By contrast, no significant differences in outcome were demonstrated by the Boston group after an average follow-up period of 38 months for the IV-S group and 55 months for the IV-G group (213). However, there were some interesting differences in presenting clinical and pathologic features. Greater serologic activity (lower C4 level) was observed in the IV-S group, which also had more frequent segmental fibrinoid necroses and crescents, although the latter did not reach statistical significance. In contrast to the observations by the Chicago group (211), the necrosis in IV-S was accompanied by endocapillary proliferation in most glomeruli, arguing against a distinct pathogenetic mechanism of injury (213). The IV-G group had higher presenting serum creatinine levels, diastolic blood pressures, and more frequent wire-loop deposits (213). Transformation from IV-S to IV-G was observed in two of three repeat biopsies from the IV-S group, suggesting that the segmental phenotype is not immutable, but may evolve into

a more global pattern of involvement over time (213). Hill et al. (214) studied 15 French patients with IV-S and 31 with IV-G and found that at baseline, patients with IV-G have more proteinuria, renal insufficiency, anemia, and hypocomplementemia. Morphologic differences include more membranoproliferative features, wire-loop deposits and hyaline thrombi, greater IF positivity involving the peripheral capillary walls, and less fibrinoid necrosis in IV-G than IV-S. Interestingly, repeat biopsies showed both types of interconversion (IV-S to IV-G in three patients and IV-G to IV-S in seven patients). The French group also observed no significant difference in 10-year survival between class IV-S and IV-G lesions at first biopsy (survival rates 65% vs. 60%, respectively). Interestingly, when repeat biopsies were performed 6 months following therapy, second biopsies with IV-G had a worse outcome than those with IV-S (214). A Japanese study also failed to find significant outcome differences in class IV-S versus IV-G (215). A recent meta-analysis (216) has analyzed the results of eight published studies addressing differences in IV-S versus IV-G (213–215,217–221) and concluded that there was no significant difference in renal outcome (doubling of serum creatinine or ESRD) between these histologic forms. These data call into question the validity of a classification that subcategorizes class IV based on the proportion of segmental and global lesions. Moreover, the IV-S category is relatively infrequent, comprising a minority of class IV biopsies (13% to 34%; see Table 14.8). Based on these data, future iterations of the ISN/RPS classification may opt to eliminate this distinction.

Some patients with class IV lupus nephritis have normal renal function and inactive urinary sediment in the face of biopsy findings of active glomerular lesions, as part of the spectrum of "silent lupus nephritis" (discussed in Other Renal Manifestations of SLE below) (222–226).

### Class V: Membranous Lupus Nephritis

Class V designates membranous lupus nephritis, which is defined by subepithelial immune deposits or their morphologic sequelae as seen in the various stages of primary membranous glomerulonephritis. According to the INS/RPS classification, a diagnosis of class V is based on the presence of global or segmental continuous granular subepithelial immune deposits (21,22). The membranous alterations may be present alone or on a background of mesangial hypercellularity and mesangial immune deposits. Any degree of mesangial hypercellularity may occur in class V. There may be few small subendothelial immune deposits identified by immunofluorescence and/or electron microscopy, but not by light microscopy. Because scattered subepithelial deposits may also be encountered in class III and class IV lupus nephritis, the threshold for an additional diagnosis of membranous lupus nephritis in a proliferative class is membranous involvement of greater than 50% of the tuft of greater than 50% of the glomeruli by light microscopy or immunofluorescence (21,22).

**TABLE 14.8 Comparison of ISN/RPS class IV subgroups**

| Reference | N | Frequency | | Clinical presenting features and pathology | Response to treatment or outcomes |
|---|---|---|---|---|---|
| | | IV-G | IV-S | | |
| Mittal (213) | 33 | 66% | 33% | IV-G had higher sCR and HTN<br>IV-S had more fibrinoid necrosis and lower C4 | No difference |
| Yokoyama (215) | 23 | 74% | 26% | IV-G had lower CH50 levels | Trend to more ESRD in IV-S ($P = 0.1495$) |
| Hill (214) | 46 | 67% | 33% | IV-G had more renal insufficiency, lower C3 and CH50, more proteinuria, more immune deposits, less fibrinoid necrosis | No significant difference in 10-year renal survival |
| Kim (1073) | 42 | 71% | 29% | IV-G had higher proteinuria and lower anti-dsDNA antibody titers | IV-S had higher complete response rates to cyclophosphamide (67% vs. 33%) |
| Hiramitsu (219) | 55 | 75% | 25% | IV-G had more nephrotic syndrome (81% vs. 43%) | Trend to worse outcomes in IV-G, $P = 0.68$ (related to chronicity) |
| Kojo (221) | 65 | 69% | 31% | IV-G had more endocapillary proliferation and wire-loop deposits | Trend to worse outcomes in IV-G, $P = 0.433$ |
| Grootscholten (218) | 72 | 79% | 21% | IV-G had more HTN, lower C3 and C4, higher sCR | No difference |
| Yu (220) | 172 | 87% | 13% | IV-S had less proteinuria, lower sCR, higher C3 more ACL, more ANCA, less anti-C1q, more fibrinoid necrosis | No difference |
| Schwartz (217) | 93 | 66% | 34% | Not examined | No difference |

N, number of patients; sCR, serum creatinine; HTN, hypertension; ESRD, end-stage renal disease.

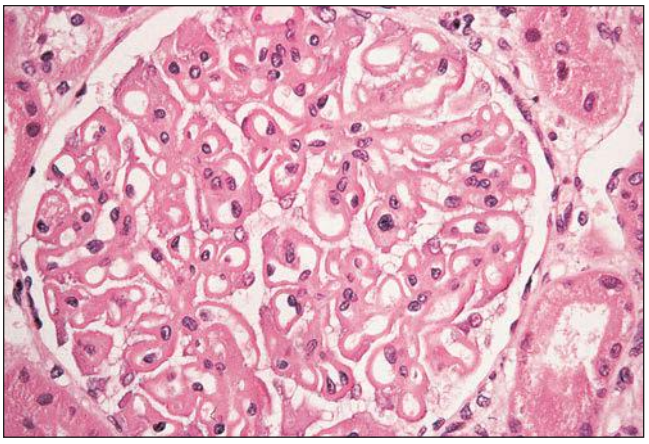


**FIGURE 14.65 Lupus nephritis class V (modified Va).** There is global thickening of glomerular capillary walls without mesangial proliferation. (H&E, ×500.)

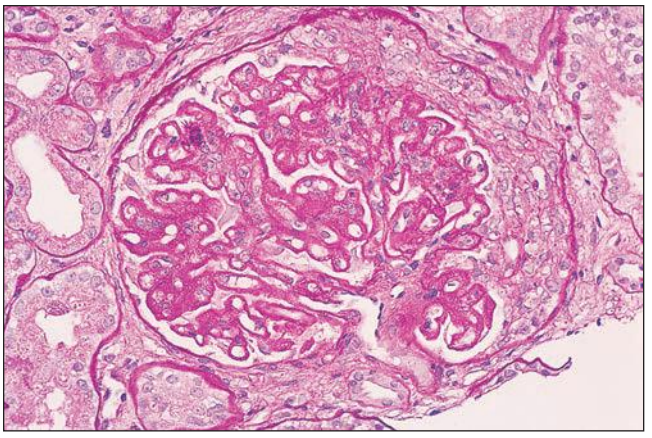


**FIGURE 14.67 Lupus nephritis classes III and V (modified Vc).** There is segmental endocapillary proliferation with an overlying crescent. The patent glomerular capillaries display chain-like thickenings of the glomerular capillary walls typical of membranous glomerulopathy. (PAS, ×400.)

In the modified (1982) WHO classification, membranous lupus nephritis was subdivided into four subclasses, designated Va through Vd (19). It is important to be familiar with these categories because older outcome studies frequently employ these designations. Class Va denotes pure membranous lupus nephritis without associated mesangial proliferation, a glomerular lesion indistinguishable morphologically from primary membranous glomerulonephritis (Fig. 14.65). Class Vb can be considered a form of class Va plus class II in that the lesion manifests the typical peripheral capillary wall features of membranous glomerulonephritis together with mesangial alterations. The latter may consist of mesangial deposits alone without mesangial hypercellularity (superadded IIa) or mesangial deposits accompanied by histologically identifiable mesangial expansion by increased mesangial cell number or matrix (superadded class IIb) (Fig. 14.66). By contrast, the ISN/RPS classification does not subdivide membranous glomerulonephritis into subgroups based on mesangial hypercellularity, and any membranous glomerulonephritis is designated simply as class V irrespective of the severity of mesangial hypercellularity or the presence of mesangial immune deposits (21,22).

The modified (1982) WHO classification also recognized class Vc (combined classes V and III) (Fig. 14.67), in which there are typical features of focal and segmental endocapillary proliferative glomerulonephritis superimposed on the membranous pattern, and class Vd (combined classes V and IV), in which there is superimposed diffuse endocapillary proliferative and membranous lupus nephritis (Fig. 14.68). A major disadvantage of this classification was its placement of classes Vc and Vd lesions under the membranous heading. This placed undue emphasis on the membranous component by implying that it is the dominant and most clinically relevant lesion and detracted from the more serious proliferative component. For this reason, classes Vc and Vd were eliminated from the 1995 Revised WHO classification (20). This approach is amply supported by clinical-pathologic studies

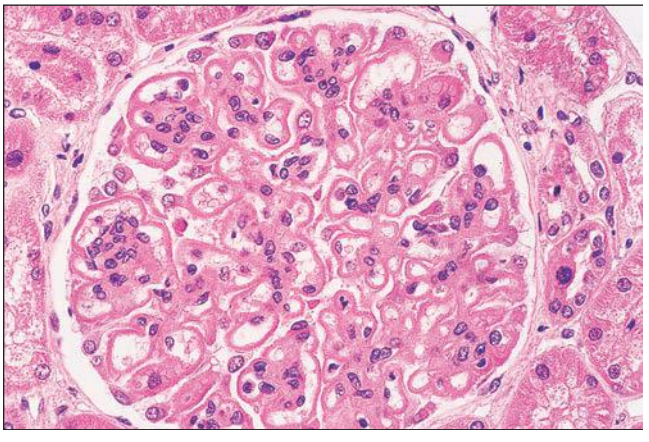


**FIGURE 14.66 Lupus nephritis class V (modified Vb).** There is regular thickening and rigidity of the glomerular capillary walls accompanied by global mesangial hypercellularity. (H&E; ×500.)

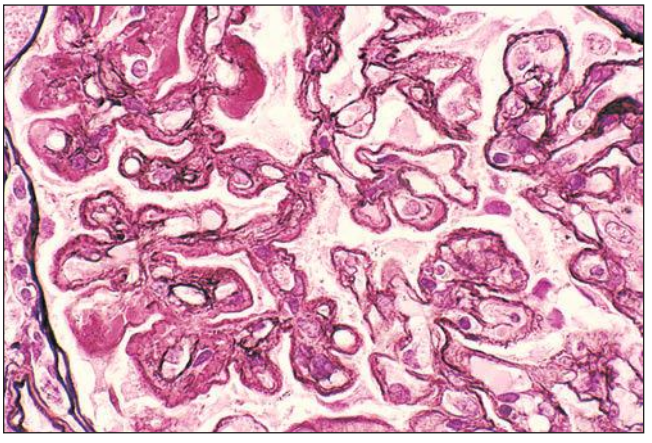


**FIGURE 14.68 Lupus nephritis class IV and V (modified Vd).** There is mixed, diffuse proliferative and membranous glomerulonephritis with complex thickening of the glomerular capillary walls by double contours enclosing subendothelial deposits and well-developed subepithelial spikes. (PAS-methenamine silver-Masson Ponceau stain, ×600.)

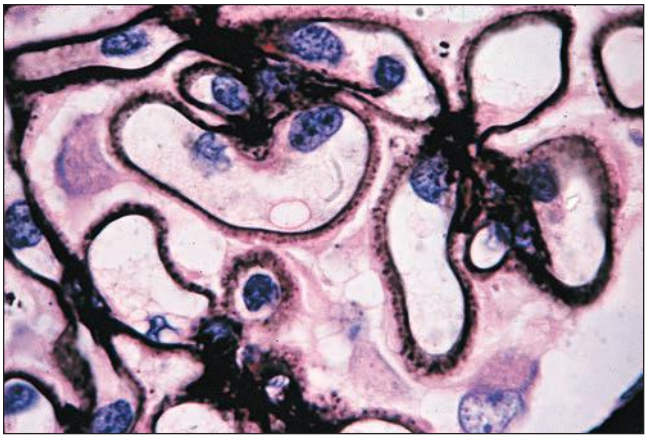


**FIGURE 14.69 Lupus nephritis class V.** Jones methenamine silver stain highlights the spiking of the GBMs. (×1000.)

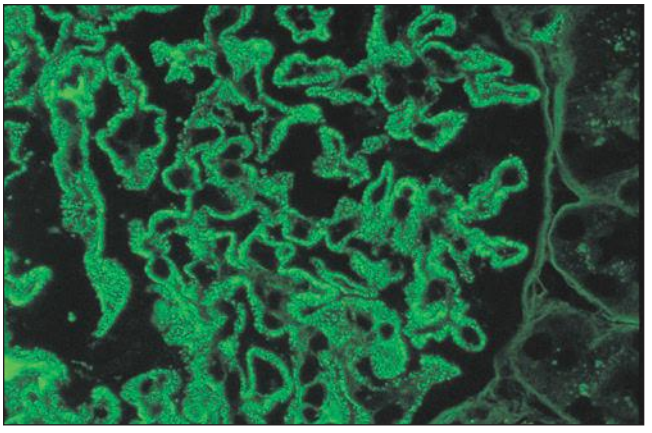


**FIGURE 14.70 Lupus nephritis class V (modified Va).** There are delicate subepithelial immune deposits staining for IgG. No mesangial deposits are observed. (Immunofluorescence micrograph, ×600.)

demonstrating that class Vd has an extremely poor prognosis, even worse than pure diffuse proliferative class IV (187,227,228). According to the ISN/RPS classification, as in the original WHO classification, the designation mixed class III and class V replaces the Vc lesion. Similarly, a designation of mixed class IV and class V replaces the Vd lesion. In the ISN/RPS schema, the additional designation of class V in the setting of class III or IV requires membranous involvement of at least 50% of glomerular capillary surface area of at least 50% of glomeruli by LM and/or IF.

By light microscopy, the peripheral glomerular capillary wall alterations display a spectrum and evolution similar to primary membranous glomerulonephritis. In early stages, the glomerular capillary walls may appear normal in thickness and texture by light microscopy, and subepithelial deposits may only be detected by immunofluorescence and electron microscopy. At this stage, the glomerular capillaries may have a rigid, ectatic appearance with visceral cell swelling. Well-established membranous lesions are typically characterized by uniform and diffuse thickening of the glomerular capillary walls with well-developed spikes of the GBM that are best demonstrated with the silver stain (Fig. 14.69). In older lesions, the deposits may become largely resorbed and overlaid by neomembrane formation producing a vacuolated GBM profile (analogous to stages 3 and 4 of primary membranous glomerulonephritis). This newly laid down basement membrane seems to have a different composition from the original GBM, consisting only of laminin without type IV collagen (229).

By immunofluorescence, IgG is found in virtually all specimens in the distribution of the subepithelial deposits (Fig. 14.70). Subepithelial deposits also frequently stain for IgM, but staining for IgA is somewhat more variable and fainter (18,149,230). C3 is often demonstrable. One study found membranous lupus nephritis to have stronger and more prevalent staining for C1q compared with primary membranous glomerulonephritis (158), but another study of membranous lupus nephritis in children was unable to identify a difference in C1q staining between cases of primary membranous glomerulonephritis and membranous lupus nephritis (184). A background of mesangial immune deposits is commonly observed (Fig. 14.71). Mesangial deposits are more likely to contain IgM than IgG, and they usually have associated C3, although C3 may be absent in some specimens (227).

By electron microscopy, the subepithelial deposits range from small to large but usually involve the majority of capillaries. In some patients, the membranous changes are well developed but involve less than 50% of capillaries, in which case the term *segmental membranous glomerulonephritis* may be used. As the disease progresses, the same ultrastructural stages seen in primary membranous glomerulonephritis may evolve. GBM spikes often separate the subepithelial deposits. In more chronic cases, the deposits become overlaid by neomembrane and later become resorbed and relatively electron lucent. There is extensive foot process effacement in the distribution of the subepithelial deposits. Mesangial deposits are demonstrable in most cases and vary in quantity (Fig. 14.72). Their presence is helpful to differentiate membranous lupus nephritis from primary membranous glomerulonephritis, in which less than 10% of cases have associated mesangial deposits (231). Scattered, small subendothelial deposits are

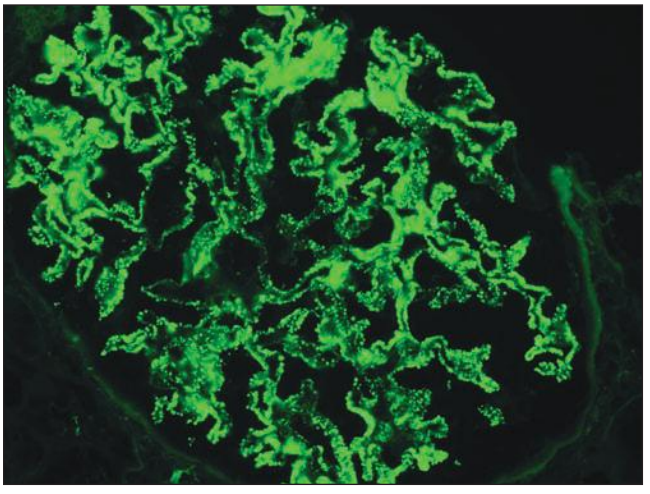


**FIGURE 14.71 Lupus nephritis class V (modified Vb).** There are heavy mesangial immune deposits of IgG with more delicate granular subepithelial deposits. (Immunofluorescence micrograph, ×600.)

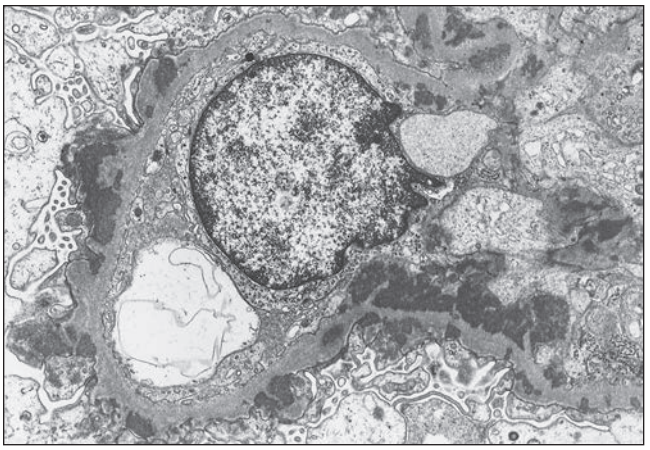


**FIGURE 14.72 Lupus nephritis class Vb (membranous).** The electron micrograph shows numerous subepithelial electron-dense deposits, some of which are surrounded by spiked projections of GBM. There are abundant mesangial electron-dense deposits. The glomerular capillary lumen is patent, and foot processes are extensively effaced. (×3300.)

also common but are not accompanied by endocapillary proliferation. Schwartz et al. (227) found them in eight of nine cases of lupus membranous nephritis (Va and Vb), and the Southwest Pediatric Nephrology Study Group found them in seven of nine cases (184). The presence of subendothelial deposits, which are rare in primary membranous glomerulopathy, is a particularly sensitive ultrastructural feature to distinguish membranous lupus nephritis from the primary form (158). Endothelial TRIs, a common feature of all ISN/RPS classes of lupus nephritis, are readily identified in class V. Tubulointerstitial deposits occur in approximately 25% of cases (64). Tubular atrophy and interstitial fibrosis usually parallel the distribution and severity of the glomerulosclerotic lesions.

Rare cases of lupus nephritis mimicking a membranous pattern by light microscopy demonstrate trapping of podocytic cytoplasmic fragments and cell membrane projections within the GBM. The resulting distinctive ultrastructural appearance has been called "podocytic infolding glomerulopathy" and has been reported primarily from Japan (232,233). Such cases may have weak or no staining for Ig. Although the morphogenesis of this unusual lesion remains unclear, it likely involves remodeling of the GBM following resorption of subepithelial deposits.

Membranous lupus nephritis class V accounts for 8% to 29% of biopsy series using the ISN-RPS classification (193,215,221,234). In older series using the modified WHO classification, virtually all patients with pure membranous lupus nephritis (Va and Vb) have proteinuria at presentation and 59% to 70% have the nephrotic syndrome (18,93,235,236). In some, the nephrotic syndrome is lacking at presentation but supervenes over the course of follow-up (93,230). Hematuria is found in about one half of the patients with red blood cell casts in 10% (230). Hypertension is detectable in about one fourth of the patients. Renal insufficiency is uncommon at the time of presentation with a frequency ranging from 0% in Donadio's experience to 25% in the series of Baldwin (93,230). Renal insufficiency and active urinary sediment are more common in patients with combined endocapillary proliferative and membranous lesions (Vc or Vd) than pure membranous forms (Va or Vb).

It has long been recognized that the membranous form of lupus nephritis differs significantly from the proliferative classes III and IV with regard to presenting serologic findings and multisystem disease manifestations. Patients with class V are more likely to present with renal disease before other systemic features of lupus are apparent. For example, among 60 patients with membranous lupus nephritis biopsied at our center, 32% of patients presented with renal-limited disease manifestations such as proteinuria, whereas 60% presented with predominant extrarenal manifestations. Only 22% of these patients fulfilled four or more ACR criteria for SLE at the time of initial biopsy. Patients with membranous lupus nephritis were more likely to be ANA negative (35%) and normocomplementemic (37%) at presentation than patients with class III or IV lesions. Serum complement levels of $CH_{50}$ or C3 are lowered in 65% to 75% of patients (18,149,230,237). In a significant number of patients with class V lupus nephritis, the renal disease may precede by months or years a clinical diagnosis of SLE (18,149,230,237). Many of these patients are initially diagnosed as having "idiopathic" membranous glomerulonephritis because they lack serologic markers of SLE (238). However, careful review of their renal biopsies often discloses one or more telltale features such as mesangial hypercellularity, mesangial immune deposits, focal small subendothelial deposits, tissue ANA, strong staining for C1q, full house immunofluorescence, TRIs, and, occasionally, tubulointerstitial deposits or vascular immune deposits, betraying a secondary membranous glomerulonephritis related to SLE (158).

Two groups performed a systematic study of the biopsy features that help to differentiate membranous lupus nephritis from primary membranous glomerulopathy (158,184). The presence of subendothelial deposits, tubulointerstitial deposits, and tissue ANA appear to be the most sensitive and specific for SLE, and any combination of features increases the accuracy of a diagnosis of membranous lupus nephritis (158). A positive immunofluorescence stain for anti-PLA2R in the distribution of the subepithelial deposits has not been reported in membranous lupus nephritis (239–241) and strongly favors a diagnosis of nonlupus, primary membranous glomerulonephritis (241).

Patients with membranous lupus nephritis may develop RVT as a complication of their nephrotic syndrome and its associated hypercoagulable state (242,243). It is uncertain whether a circulating lupus anticoagulant may also predispose to this complication (120,123). Some patients have no clinical findings referable to RVT. Others may present with flank pain, gross hematuria, increased proteinuria, reduction in the GFR, oliguria, or signs of pulmonary embolus. The symptoms of pulmonary embolus, including dyspnea, shortness of breath, and tachypnea, may be mistaken for symptoms of lupus pleuritis or infectious pneumonia. The diagnosis of RVT can be confirmed radiographically by the use of renal venography, which demonstrates a filling defect in the renal vein, often extending into the inferior vena cava and with loss of the usual washout (i.e., "streamer effect") of unopacified blood. Doppler ultrasonography and magnetic resonance imaging have greatly facilitated the diagnosis of RVT without

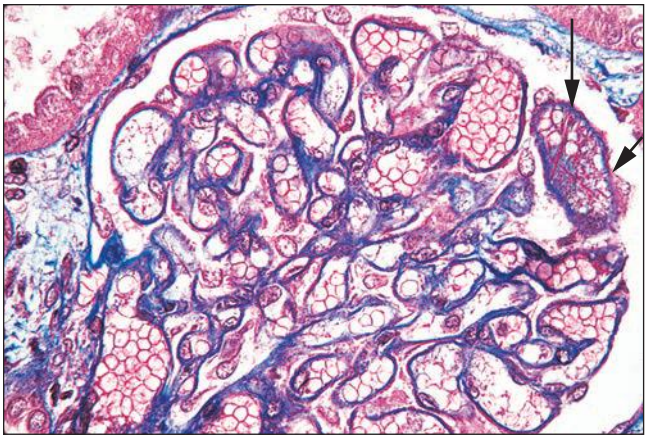


**FIGURE 14.73 Lupus nephritis class V with superimposed RVT.** Histologic features pointing to RVT include the separation of tubules by interstitial edema, marked glomerular capillary congestion, and fibrin strands within a glomerular capillary lumen (*s*). (Masson trichrome stain, ×500.)

subjecting the patient to radiopaque contrast materials, which are potentially nephrotoxic.

Clues to a diagnosis of complicating RVT may be evident in renal biopsies of membranous lupus nephritis. Their astute identification by the pathologist is vital to ensure prompt clinical recognition of RVT and rapid institution of anticoagulation. A diagnosis of possible acute RVT should be suspected in any biopsy specimen with membranous lupus nephritis with diffuse interstitial edema, interstitial microhemorrhage, excessive glomerular capillary congestion, fibrin thrombosis, or neutrophil margination in glomerular capillaries (Fig. 14.73). Chronic RVT may be signaled by the presence of diffuse tubular atrophy and interstitial fibrosis that appears disproportionately severe relative to the degree of glomerular sclerosis.

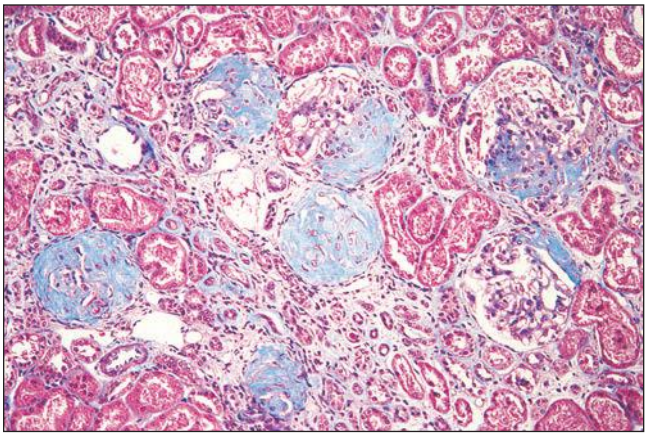


**FIGURE 14.74 Lupus nephritis class VI.** Extensive glomerular sclerosis shows vestiges of fibrous crescents. The global sclerosis affected more than 90% of glomeruli in this biopsy. Several glomeruli pictured here are segmentally sclerotic. Atrophic tubules alternate with groups of compensatorily hypertrophied tubules. (Masson trichrome, ×80.)

### Class VI: Advanced Sclerosing Lupus Nephritis

The modified WHO classification introduced a sixth class in which the findings are those of extremely chronic and advanced glomerulonephritis with widespread glomerular scarring affecting most glomeruli (19,20) (Fig. 14.74). The ISN/RPS schema defines class VI more precisely as advanced sclerosing lupus nephritis with global glomerular sclerosis affecting ≥90% of glomeruli without residual activity (21,22). These examples of class VI lupus nephritis undoubtedly represent the advanced phase of class IV nephritis in most cases. Severe tubular atrophy, interstitial fibrosis, inflammation, and arteriosclerosis usually accompany the glomerular sclerosis. In some of these cases, the changes are so advanced and nonspecific that it is difficult to ascertain (other than by the clinical history of SLE or documented findings in a prior renal biopsy) a diagnosis of chronic lupus nephritis by objective morphologic criteria. In such cases, glomeruli with the less advanced sclerosis often display residual features of mesangial hypercellularity. Discontinuities in the Bowman capsule associated with subcapsular fibrous proliferations are helpful to identify old fibrous crescents.

Small granular immune deposits are usually still detectable by immunofluorescence or electron microscopy in the thickened and sclerotic GBMs, in the fibrotic tubulointerstitial compartment, or in vessel walls.

In advanced lupus nephritis, it is common to observe focal segmental sclerosing features and visceral epithelial cell reactivity (e.g., hypertrophy, hyperplasia, intracytoplasmic protein resorption droplets) that mimic the changes seen in primary FSGS. These changes probably represent the effects of podocyte depletion, glomerulosclerosis, and nonimmunologic progression of renal disease mediated by hyperfiltration in remnant nephrons. This interpretation is supported by the almost invariable presence of glomerular hypertrophy in the remaining glomeruli.

Patients with class VI lupus nephritis have severe renal insufficiency, with variable subnephrotic proteinuria and relatively inactive urinary sediment. Hypertension is common. At this stage, lupus serologies may be inactive (i.e., "burnt-out" lupus). In this phase, it is inappropriate to treat the renal disease with immunosuppressive therapy, and maneuvers designed to reduce intraglomerular pressures, such as by the administration of angiotensin-converting enzyme inhibitors, are usually initiated to allay nonimmunologic progression of renal disease. Preparation for inevitable renal replacement therapy should be initiated.

### Transformations in World Health Organization Class

Lupus nephritis is not static but has the capacity to transform from one class to another, spontaneously or after treatment (41). Because of this potential for transformation, which may occur unpredictably at any time in the course of disease, patients with SLE must be monitored closely. Transformations often are heralded by sudden worsening of proteinuria, development of a nephrotic syndrome, increased activity of the urinary sediment, or a sudden decrease in the GFR (18,191,192,195,244). Some investigators have observed that patients who transform are often younger (18) and often have worse lesions than most other patients in their class of lesions (18,195).

**TABLE 14.9 Class tranformations on repeat biopsies in 427 cases of lupus nephritis**

| | | Repeat biopsy class N (% of reference biopsy class) | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | N | II | III | IV | V | III + V | IV + V | VI |
| Reference biopsy class in first biopsy | | | | | | | | |
| I | 2 | — | — | 1 (50%) | 1 (50%) | — | — | — |
| II | 45 | 3 (7%) | 11 (24%) | 14 (31%) | 6 (13%) | 7 (16%) | 4 (9%) | — |
| III | 59 | 6 (10%) | 9 (15%) | 20 (34%) | 8 (14%) | 7 (12%) | 1 (2%) | 1 (2%) |
| IV | 185 | 24 (13%) | 22 (12%) | 69 (37%) | 22 (12%) | 17 (9%) | 10 (5%) | 19 (10%) |
| V | 53 | 2 (4%) | 2 (4%) | 8 (15%) | 21 (40%) | 13 (25%) | 3 (6%) | 3 (6%) |
| III + V | 37 | — | 1 (3%) | 9 (24%) | 8 (22%) | 14 (38%) | 5 (14%) | — |
| IV + V | 42 | 2 (5%) | 4 (10%) | 7 (17%) | 9 (21%) | 4 (10%) | 11 (26%) | 1 (2%) |

N, number of biopsies.

Refs. (193, 245, 1074, and 1075).

Transformations are relatively common, as outlined in Table 14.9. They occurred in 11 of 56 patients followed by Appel et al. (18), 15 of 88 patients reported by Baldwin et al. (93), and 14 of 90 patients reported by Mahajan et al. (195), for an average transformation rate of 13%. Recent series suggest higher rates of transformation. Seshan et al. (193) found maintenance of the same ISN/RPS class in only 9% of biopsies that were initially diagnosed as class II, 28% of class III, 28% of class IV-G, 33% of class IV-S, and 30% of class V, with the remainder transforming. A recent Chinese series of 156 patients described a change in histologic pattern in 58% of biopsies with pure proliferative pattern (class III or IV), 50% of biopsies with pure membranous pattern (class V), and 60% of biopsies with mixed proliferative and membranous pattern (classes III and V or classes IV and V) (245). Virtually all directions of transformation have been reported, including focal to diffuse (191–193,244), focal to membranous (193,195), diffuse to membranous (162,193,206), diffuse to mesangial (193,246), membranous to diffuse (149,193,227,230), and membranous to membranous with focal proliferative lesions (18). Probably the most commonly reported transformation is class III to IV lupus nephritis, which many prefer to consider movement along a disease continuum rather than a true transformation. Approximately 30% to 40% of class III cases have been reported to transform to class IV (18,191–193,195,244). Patients with extensive subendothelial deposits appear to be particularly at risk. Transformation of class IV to class V after treatment has also been described (204). After treatment, diffuse proliferative lupus nephritis frequently transforms to a mesangial proliferative pattern, although ultrastructural examination usually discloses residual irregularities of the peripheral GBM consistent with resorbed, organized subendothelial deposits (193,246) (Fig. 14.75).

## Activity and Chronicity Index

From the earliest days of steroid therapy, it has been known that immunosuppressive agents are capable of reducing the amount of immune deposition in the kidney and the degree of glomerular necrosis and proliferation (162,185,202,247–249). However, it was equally appreciated that reduction in the histologic activity of the lesion was not always accompanied by

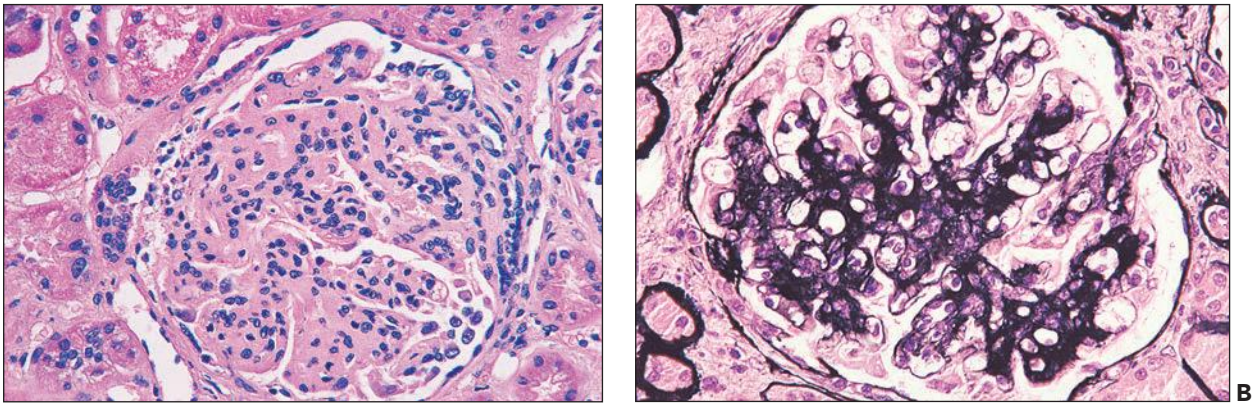



**FIGURE 14.75** The initial renal biopsy of diffuse proliferative glomerulonephritis with marked endocapillary proliferation **(A)** regressed to a mesangial proliferative pattern with focal synechiae **(B)** following aggressive therapy. (**A**, H&E; **B**, Jones methenamine silver, ×500.)

clinical improvement (249,250). It was presumed that there were some lesions, notably glomerular sclerosis, tubular atrophy, and interstitial fibrosis that are irreversible and may progress despite improvement in the proliferative and necrotic lesions. For more than five decades, investigators have attempted to analyze renal biopsy specimens of lupus nephritis with respect to active and chronic features as predictors of outcome and guides to therapy. The rationale is a simple one and based on the premise that active lesions are potentially treatable, whereas chronic lesions represent irreversible damage.

Pirani et al. (185) were the first to attempt to systematically separate "active" lesions from sclerosing lesions. Morel-Maroger et al. (244) carried this concept further to evaluate the efficacy of corticosteroid therapy in a large series of lupus patients with repeat biopsies. They classified the following as active lesions: fibrinoid necrosis, endocapillary proliferation, cellular crescents, nuclear debris, hematoxylin bodies, wire loops, hyalin thrombi, acute tubular lesions, and necrotizing angiitis. Chronic lesions included primarily glomerular sclerosis and interstitial fibrosis. From the number and severity of the different histologic lesions, they were able to develop an activity and chronicity index. Among 20 patients with exclusively active lesions on initial biopsy, 15 showed marked clinical and morphologic improvement after treatment. In contrast, the sclerosis worsened in 14 of 15 patients who had significant chronic features on initial biopsy. High-dose steroids in this latter group were ineffective in slowing the progression of sclerosing lesions in 8 of the 14 patients, although treatment did diminish the active lesions. This tendency for active lesions to be more amenable to therapy than chronic lesions was confirmed in a later study by Striker et al. (203).

The concept of activity and chronicity indices (AI and CI) was adopted in the studies of Austin et al. at the NIH (35,36,251). They modified the features of activity used by Morel-Maroger by adding glomerular leukocyte exudation and interstitial inflammation and deleting renal vasculitis (35). Their CI included fibrous crescents and tubular atrophy in addition to glomerular sclerosis and interstitial fibrosis (35) (Table 14.10). According to their system, the activity index is graded on a scale of 0 to 24 by calculating the sum of individual scores (0 to 3+) for each of six histologic parameters, including glomerular endocapillary proliferation, glomerular neutrophil infiltration, wire-loop deposits and hyaline thrombi, glomerular karyorrhexis and fibrinoid necrosis, cellular crescents, and interstitial inflammation. Glomerular features (including endocapillary proliferation, wire-loop deposits, necrosis, and cellular crescents) are graded as follows: 0, absent; 1+, less than 25% of glomeruli affected; 2+, 25% to 50% of glomeruli affected; 3+, greater than 50% of glomeruli affected (35,36). Neutrophil exudation is defined as more than two neutrophils per glomerulus and scored as mild (1+), moderate (2+), and severe (3+). Fibrinoid necrosis and cellular crescents are weighted double because of their more ominous prognostic importance. For interstitial inflammation, the scoring is as follows: 0, absent; 1, mild; 2, moderate; and 3, severe. A similar score for chronicity is computed by summing the individual scores (0 to 3+) for each of the following: glomerular sclerosis, fibrous crescents, tubular atrophy, and interstitial fibrosis. For glomerular sclerosis, segmental or global glomerulosclerosis in less than 25% of glomeruli is graded as 1+, in 25% to 50% of glomeruli as 2+, and greater than 50% as 3+. Similarly, fibrous crescents involving less than 25% of glomeruli are graded as 1+, 25% to 50% as 2+, and greater than 50% as 3+. Interstitial fibrosis and tubular atrophy are graded as mild (1+), moderate (2+), or severe (3+).

**TABLE 14.10 Activity and chronicity indices**

| | |
|---|---|
| Index of activity (0–24) | |
| Endocapillary hypercellularity | (0–3 +) |
| Leukocyte infiltration | (0–3 +) |
| Subendothelial hyaline deposits | (0–3 +) |
| Fibrinoid necrosis/karyorrhexis | (0–3 +) × 2 |
| Cellular crescents | (0–3 +) × 2 |
| Interstitial inflammation | (0–3 +) |
| Index of chronicity (0–12) | |
| Glomerular sclerosis | (0–3 +) |
| Fibrous crescents | (0–3 +) |
| Tubular atrophy | (0–3 +) |
| Interstitial fibrosis | (0–3 +) |

Austin HA III, Muenz LR, Joyce KM, et al. Prognostic factors in lupus nephritis. Contribution of renal histologic data. *Am J Med* 1983;75(3):382–391; Austin HA III, Muenz LR, Joyce KM, et al. Diffuse proliferative lupus nephritis: identification of specific pathologic features affecting renal outcome. *Kidney Int* 1984;**25**(4):689–695.

Using these indices in a group of patients with diffuse proliferative disease, Austin et al. (36) found the AI to be moderately predictive of outcome, with an AI greater than 12 associated with a 60% 10-year survival. However, none of the elements of AI was individually predictive. In their hands, the CI was more predictive of renal outcome than AI, such that a CI of ≤1 had a 100% 10-year survival, a CI of 2 or 3 had 68% 10-year survival, and a CI of ≥4 indicated a 32% 10-year survival. These findings suggested that higher CI has a graded effect on outcome and that even low levels of chronicity have prognostic significance. In contrast to AI, all the elements of CI were individually predictive of renal failure, particularly tubular atrophy. The NIH group found the combination of cellular crescents and moderate to severe interstitial fibrosis to be a particularly sensitive predictor of the subgroup at risk to double serum creatinine levels (251).

Hill et al. (252) proposed a more comprehensive biopsy index that incorporates histologic features of activity and chronicity involving all renal compartments, as well as immunofluorescence findings. This detailed index consists of four components: glomerular activity index (total possible score 24), tubulointerstitial activity index (total possible score 21), chronic lesions index (total possible score 15), and immunofluorescence index (total possible score 96). This index is unique for the inclusion of many features of tubulointerstitial activity (such as tubular cell flattening, nuclear activation and intraluminal macrophages) and scoring of glomerular monocytes. The comprehensive biopsy index proposed by Hill et al. (252) demonstrated a statistically higher correlation with clinical presenting features and outcome than the NIH activity index or chronicity index. Correlations with outcome were even greater when the index was computed for second biopsies performed 6 months following treatment of diffuse lupus nephritis, indicating its ability to identify recalcitrant disease activity that has not responded to therapy. While the index is useful for

research purposes, many consider it to be too intricate and time consuming to be applied to routine biopsy work-up.

The value and reproducibility of renal AI and CI in lupus nephritis is controversial. Several groups have found good correlations between CI and renal outcome (253–255). Wallace et al. (255) confirmed the earlier observations of Morel-Maroger (244) and found that AI improved in 84% of patients after treatment, whereas the CI worsened in 71%. They also observed that normalization of C3 levels was associated with reduction in AI but that prolonged depression of C3 was associated with worsening of the CI.

Other investigators have failed to corroborate a strong predictive value for the AI and CI (256,257). Appel et al. (204) found no correlation between these indices and outcome in a series containing all classes of renal lesions, although they conceded that these indices might be of greater value if only patients with diffuse proliferative disease were considered. Schwartz et al. (256), in a large study of patients with diffuse proliferative disease, found that the AI did not distinguish between those with eventual renal failure or adverse outcome and those without. They also found that there was no single value of CI that separated those destined to progress to renal failure from those with good outcome. Their data reveal that increasing CI is a measure of increasing probability of poor renal outcome but that minimal levels of chronicity cannot reliably predict adverse outcomes. Schwartz et al. (258) also found that there was poor interobserver and intraobserver reproducibility of the AI and CI, suggesting that it is influenced by subjectivity. When four highly experienced, university-based renal pathologists scored 83 biopsies of lupus nephritis, the mean activity indices ranged from 9.64 to 12.89 and mean chronicity indices from 2.84 to 4.61. The AI, which factors six variables, was consistently less reproducible than the CI (258). Wernick et al. also studied the reproducibility of the AI and CI used by the NIH group among five experienced pathologists, including four community hospital based and one from a university medical center (259). Pairs of pathologists gave scores within 1 point for chronicity and within 2 points for activity index in only 50% of cases. Moreover, repeated readings by the same pathologist at an interval of 8 to 9 months yielded CI scores that were greater than 1 point discordant in 45% of cases and AI scores that were greater than 2 points discordant in 43% of cases.

These studies underscore that there is no cutoff for AI and CI that reliably predicts outcome and that absolute reproducibility of these indices is not possible. Nevertheless, these data should not detract from the general observation that active and chronic renal lesions behave differently, especially in response to immunosuppressive therapy, and should be factored into any formulation of appropriate treatment. Although the value of these indices is controversial, most nephrologists find an overall assessment of activity and chronicity useful, especially when repeat biopsies are performed in individual patients to monitor evolution and response to therapy. In addition to the ISN/RPS classification, our biopsy reports routinely include an AI and a CI and designate in the microscopic description the percentage of glomeruli with active and chronic lesions. More important than the actual numerical value is the description of the type of active lesions and proportion of glomeruli affected. Most pathologists agree that certain lesions, such as necrotizing lesions and cellular crescents, are less reversible than other active lesions, such as subendothelial wire loops or neutrophil infiltration. The ISN/RPS classification specifies that the proportion of glomeruli with active and sclerosing lesions, and specifically those with fibrinoid necrosis or cellular crescents, should be indicated in each biopsy report. It also encourages, but does not mandate, the use of the NIH (or other) index for systematic quantitation of activity and chronicity (21,22). The ISN/RPS classification also incorporates designations of activity and chronicity into the diagnostic line of the renal biopsy report by applying the symbols (A) for active, (A/C) for mixed active and chronic, and (C) for chronic after diagnoses for lupus nephritis class III or IV. In this way, a diagnosis of lupus nephritis class IV (A/C) indicates a diffuse proliferative and sclerosing glomerulonephritis with both active and sclerosing features, whereas a designation of class III (A) indicates focal proliferative lupus nephritis that is purely active and (C) indicates focal sclerosing lupus nephritis that is purely chronic.

## Reproducibility of the ISN/RPS Classification

Several studies have confirmed the improved interobserver reproducibility of the ISN/RPS classification compared to earlier WHO classifications (215,260). A large study involving 20 centers in the UK classified cases of lupus nephritis by the ISN/RPS classification and the modified WHO (1982) classification (260) and concluded that the ISN/RPS classification was more reproducible. They ascribed this to clearer separations between the classes and the elimination of subgroups of class V. Interestingly, the percentage of cases of class IV increased from 23% by the WHO 1982 classification to 46% by the ISN/RPS classification, with fewer diagnoses of class III and class V. This increase could be attributed primarily to the elimination of class Vd as a subgroup of membranous lupus nephritis and the inclusion of sclerotic glomeruli in the assessment of total glomeruli affected. Other studies have shown that the classification is useful to predict outcome based on its incorporation of modifiers reflecting activity (A) and chronicity (C) (219).

## Extrarenal Clinical Manifestations

SLE is extremely diverse in its clinical manifestations, which affect a large number of organ systems with highly variable initial presentations and evolution over time (261). Constitutional symptoms such as fever, malaise, and weight loss are common presenting features. Fever secondary to active disease was documented in over 80% of patients with SLE in the early 1950s, but only 41% of patients in a study conducted from 1980 to 1989 (262), possibly reflecting earlier disease recognition and greater use of antipyretic medications in the modern era.

Involvement of the skin and mucous membranes occurs in 55% to 90% of patients (262,263) and includes butterfly rash over the cheeks and bridge of the nose, oral or nasal ulcers, discoid lupus, and subacute cutaneous lesions. The butterfly rash, detectable in approximately one half of patients, often appears after sun exposure. Presumably, ultraviolet B radiation exposure damages cellular proteins and DNA, releasing subcellular antigenic particles such as nucleosomes (264). Release of cytokines such as interleukin-1 (IL-1) in areas of sun damage may potentiate the immune response. Moreover, ultraviolet light promotes binding of anti-Ro, anti-La, and anti-RNP to keratinocytes (265). Discoid skin lesions occur in approximately 25% of patients and must be differentiated from discoid lupus erythematosus, a purely cutaneous condition. Discoid lesions differ from the diffuse erythematous lesions of classic butterfly rash in having rounded, annular contours, frequent plaque formation, and follicular plugging, leaving depressed scars and

areas of hypopigmentation. In SLE, lesions may occur on the face, scalp, neck and upper chest, or back. Pathologic examination of the skin reveals mononuclear inflammation of the dermis, follicular plugging, edema of the basal epidermis, and hyperkeratosis. Granular deposits of IgG and C3 are often detectable at the dermal-epidermal junction, corresponding to complexes of nucleosomes and antinucleosomal antibodies (266). Alopecia occurs in up to 70% of patients and may affect the eyebrows, eyelashes, beard, and scalp. Nail lesions, ridging and pitting in character, may also affect up to one fourth of SLE patients. Other skin lesions such as periungual erythema, telangiectasia, Raynaud phenomenon, and livedo reticularis are also common. The latter is particularly associated with a circulating lupus anticoagulant and/or APL antibodies. The occurrence of vasculitis may be manifested by splinter hemorrhages of the nailfold capillaries, small microinfarcts of the fingertips, and erythematous indurated lesions on the palmar thenar eminences (i.e., Janeway spots) and fingertips (i.e., Osler nodes).

In a large number of series, arthralgias and arthritis are the most common presenting symptoms, occurring in up to 95% of patients at some time in the course of the disease. Joint complaints may precede by months or years other systemic symptoms. The arthralgias commonly affect the proximal interphalangeal joints, wrist, and knees and may be accompanied by morning stiffness. Rarely is the arthritis deforming, although soft tissue laxity may be seen in the late stages. Muscle complaints such as myalgias, muscle tenderness, and weakness may occur in greater than 50% of patients at some point in the course of disease evolution. Muscle biopsies reveal perifascicular and perivascular mononuclear infiltrates.

Among the pulmonary manifestations of SLE, pleuritis is the most common, affecting 40% to 60% of patients, sometimes with associated pleural effusions. Lupus pneumonitis is far less common, affecting less than 5% of patients (267). Pathologically, it consists of an interstitial pneumonitis with predominantly interstitial mononuclear infiltrates. In severe cases, acute alveolitis, hyaline membranes, and alveolar hemorrhage may occur. Inoue identified deposits of IgG, C3, and DNA antigen in alveolar capillary walls and septa, with corresponding electron-dense deposits seen by electron microscopy (268). Clinically, lupus pneumonitis is often difficult to differentiate from infectious pneumonia. Pulmonary function test abnormalities associated with lupus include mild impairment in diffusing capacity and reduced lung volume. Pulmonary embolus may occur as a complication of RVT associated with the nephrotic syndrome or deep vein thrombosis associated with circulating lupus anticoagulant and/or APL antibodies.

Cardiovascular manifestations are most frequently related to pericarditis, which is detectable in one fourth of patients clinically and in up to two thirds at autopsy (269). In the acute phase, an electrocardiogram reveals tall T waves and elevation of the ST segment. Less common is myocarditis, which was detectable clinically in 8% of patients in two large series (270,271) and up to 40% at autopsy (269). Histologic features include interstitial mononuclear inflammation and fibrosis. Arteritis affecting the major coronary arteries and intramyocardial arterioles is rare. An increasingly recognized cardiac complication of lupus is valvular disease, which correlates highly with the presence of APL antibodies. Valvular thrombotic (sterile) vegetations are most commonly encountered on the mitral valve and rarely the aortic valve. Previously called Libman-Sacks endocarditis, it consists of a fibrinous or fibrosing verrucous lesion that may cause clinically significant valvular stenosis or incompetence.

The neurologic manifestations of lupus are diverse and often pose difficulties in differentiation from steroid-induced psychosis. They include cognitive dysfunction, headache, altered consciousness (ranging from stupor to coma), seizure, stroke, optic neuritis, and peripheral neuropathy. Pathologically, a correspondingly diverse array of morphologic lesions has been described, ranging from cerebral perivascular inflammation to thrombosis, arteritis, cerebritis, hemorrhage, and infarction. A role for antineuronal antibodies (272) and immune complex deposition, identifiable in the choroid plexus, has been proposed. Thrombotic central nervous system disease often is mediated by lupus anticoagulant and/or APL antibodies and may have vasculitic features.

Hematologic abnormalities are common and include lymphadenopathy in 50% of patients. Anemia, affecting about one half of patients, may be hemolytic and produce a positive direct Coombs test result. Lymphopenia and thrombocytopenia are commonly observed. The lymphocytopenia is probably mediated in part by cold-reactive, complement-fixing IgM antilymphocyte antibodies. Severe thrombocytopenia may be a manifestation of immune thrombocytopenic purpura, and platelet counts of less than 20,000 may be associated with clinical evidence of bleeding, including cutaneous petechiae, purpura, or epistaxis. Prolongation of the activated partial thromboplastin time and a false-positive VDRL result are usually manifestations of APL antibodies. Antibody binding to the phospholipid component of the prothrombin activator complex (consisting of factors Xa, V, calcium, and phospholipid) prolongs the in vitro partial thromboplastin time. Antibodies to cardiolipin, the phospholipid component of beef heart, which is admixed with phosphatidylcholine and cholesterol in the VDRL assay, is responsible for the falsely positive biologic test result for syphilis detected in 29% of patients.

A classification of SLE was proposed in 1982 by the ACR (273) and was further revised in 1997 (274). This system attempts to accommodate the diverse clinical manifestations of SLE, while accounting for its highly variable disease evolution affecting different systems over time, with periods of remission and exacerbation. This classification proposes 11 defining clinical and laboratory criteria, including malar rash, discoid rash, photosensitivity, oral ulcers, arthritis, serositis, renal disorder (defined as persistent proteinuria greater than 500 mg/d or 3+ by dipstick testing or cellular casts of any type, including red cell, granular, tubular, or mixed), neurologic disorder, hematologic disorder (including anemia, leukopenia, lymphopenia, or thrombocytopenia), immunologic disorder (including positive test for anti-dsDNA antibody, anti-Smith antibody, APL antibody, or lupus anticoagulant), and positive ANA. The presence of any four of these criteria sequentially or simultaneously has 96% sensitivity and specificity for SLE (273) in the patient database from which it was derived. Subsequent studies have confirmed a more modest 80% to 95% sensitivity and specificity for these criteria in clinical rheumatologic practice (275).

## Management of Lupus Nephritis

### Overview

The management of lupus nephritis has evolved over many decades, with the introduction of new agents and therapeutic strategies following multiple clinical trials (276–279). In brief, corticosteroids have been the mainstay of therapy for lupus nephritis since the 1950s (185) and continue to be used

in most patients with clinically significant kidney disease. In the 1980s, landmark studies from the NIH demonstrated that aggressive immunosuppression with cyclophosphamide in conjunction with corticosteroids was more effective than corticosteroids alone in producing remissions in most cases of lupus nephritis (280–282). However, this gain was accompanied by significant side effects from long-term immunosuppressive therapy, notably infections, gonadal toxicity, and bladder toxicity (283). Subsequent studies demonstrated that lower doses and shorter courses of cyclophosphamide, followed by maintenance therapy with mycophenolate mofetil (MMF) or azathioprine (AZA), were equally effective at preventing relapses and sustaining remissions as the higher doses of cyclophosphamide used in the original NIH protocols (284–288). More recently, MMF has emerged as a suitable alternative to cyclophosphamide for both proliferative and membranous lupus nephritis and may even be the treatment of choice in patients of African descent or Hispanic ethnicity (284,289–293). In addition, the use of calcineurin inhibitors in lupus nephritis is supported by several clinical studies (294–296). In the 21st century, novel biologic agents that target B-cell and T-cell function and cytokines have been employed in patients with SLE. Some of these drugs have entered the therapeutic armamentarium for lupus nephritis, whereas others remain under active study.

The ACR (27) and the European League Against Rheumatism (EULAR) (297,298) have both issued evidence-based guidelines for the management of lupus nephritis, based on renal biopsy findings using the ISN/RPS classification. Therefore, the treatment of lupus nephritis will be reviewed in the context of the ISN/RPS classes.

## Mesangial (Class I and II) Lupus Nephritis

Patients with purely mesangial disease (class I or II) require conservative treatment only. Blockade of the renin-angiotensin-aldosterone system (RAAS) with angiotensin-converting enzyme inhibitors and/or angiotensin receptor blockers is recommended in all patients with hypertension and/or greater than 0.5 g/d proteinuria (27,298), as this may prevent the development of more severe glomerular disease (299). In addition, blood pressure levels should be targeted to ≤130/80 mm Hg, and statins should be employed to maintain low-density lipoprotein (LDL) cholesterol levels less than 100 mg/dL (27). Finally, hydroxychloroquine is recommended for all patients with lupus nephritis, as this reduces renal flares and lowers the risk of subsequent renal damage (27,298).

## Focal and Diffuse (Classes III and IV) Lupus Nephritis

Cases of inactive focal lupus nephritis III (C) require conservative management only. Patients with very mild class III disease lacking substantial subendothelial deposits or necrotizing features may be managed with steroids alone. Class III cases with more severe active features, including extensive focal proliferative lesions, necrotizing lesions, crescents, or abundant subendothelial deposits, are usually treated with vigorous therapy comparable to that recommended for active diffuse proliferative lupus nephritis class IV.

In the current era, most cases of lupus nephritis (class III, class IV, and class V) are treated in two phases: induction and maintenance. The induction phase involves an initial period of intense immunosuppression with the goal of producing a remission, or clinically significant response, as defined by improvement or normalization of GFR, reduction of proteinuria, and elimination of active urinary sediment (277,300,301). This is followed by a maintenance phase with less intense therapy, with the goals of consolidating remission, preventing relapse and nephritis flares, suppressing smoldering disease that could lead to chronic kidney disease, and minimizing the side effects of long-term therapy (27,300). The duration of both induction and maintenance phases must be individualized based on clinical response. In general, the induction phase lasts at least 6 months and the maintenance phase lasts at least 2 years but may need to continue indefinitely.

### Induction therapy for Class III and Class IV Lupus Nephritis

Current expert guidelines recommend that most cases of class III and class IV disease receive cyclophosphamide or MMF in conjunction with intravenous high-dose steroids for 3 days, followed by daily or alternate-day high-dose oral steroids (tapering after 8 weeks) (27,298). Steroids alone are not adequate therapy for proliferative lupus nephritis (281,302). The efficacy of cyclophosphamide for inducing remission of lupus nephritis was established in several NIH-sponsored trials (280–282). Following six to eight monthly boluses of cyclophosphamide plus steroids, most (83%) patients with lupus nephritis will have entered remission (287). Intravenous administration of cyclophosphamide is preferable to oral therapy because of the lower incidence of premature ovarian failure and hemorrhagic cystitis (302). Two induction protocols include the use of cyclophosphamide: the "NIH protocol" (six monthly pulses of intravenous cyclophosphamide 0.5 to 1 g/m$^2$) and the lower dose "EuroLupus protocol" (500 mg intravenously every 2 weeks for 6 doses) (288). The EuroLupus Nephritis Trial in 90 European subjects (who were predominantly white) demonstrated equally good responses in the initial 3-year study period (288) and over 10 years of follow-up in patients who received either lower or higher doses of cyclophosphamide, followed by maintenance therapy with AZA and low-dose corticosteroids (303). However, the suitability of this regimen for populations at risk for more severe renal disease (i.e., those of African or Asian race or Hispanic ethnicity or with severe crescentic glomerulonephritis) remains uncertain.

MMF has emerged as an alternative to cyclophosphamide for induction therapy and may be more advantageous in African Americans and Hispanics (27,289,291). MMF is an inhibitor of inosine monophosphate dehydrogenase, an enzyme involved in de novo purine synthesis, which effectively blocks DNA synthesis in lymphocytes. Whereas cyclophosphamide has significant risk of major infection and gonadal toxicity, the major side effects of MMF are diarrhea, nausea, and minor infections. A groundbreaking trial by Chan et al. (304) in Hong Kong showed comparable remission rates at 1 year in patients with diffuse lupus nephritis treated with MMF for 12 months versus oral cyclophosphamide for 6 months followed by AZA for 6 months. A controlled study in the United States found MMF to be superior to IV cyclophosphamide as induction therapy (291). Among 140 patients receiving treatment for 24 weeks, complete remissions were achieved in 22.5% of patients receiving MMF versus 5.8% of those receiving IV cyclophosphamide, with more favorable safety profile in the MMF group (291). The Aspreva Lupus Management

Study (ALMS) demonstrated similar short-term response rates and safety in patients randomized to receive cyclophosphamide (0.5 to 1.0 g/m$^2$) or MMF plus corticosteroids for 24 weeks induction therapy (289). Importantly, patients of Asian descent may respond to lower doses of MMF (305) and may be more susceptible to side effects (289,306). Thus, the ACR guidelines suggest that Asian patients may be adequately treated with up to 2 g of MMF per day, whereas non-Asian patients and those with more active disease should receive 3 g/d (27). There are few long-term data for MMF or to support its use in severe lupus nephritis. Therefore, cyclophosphamide remains the treatment of choice in most cases with adverse clinical or histologic features, such as rapidly deteriorating kidney function and extensive crescents (298).

Oral AZA plus methylprednisolone was compared to intravenous cyclophosphamide in one Dutch study (307), with similar rates of remission in the first 2 years. However, follow-up studies revealed higher chronicity indexes in the AZA group (308) and higher rates of relapse and doubling of serum creatinine (309).

In addition, there is preliminary evidence, mainly in Asian populations, that either tacrolimus (295,310–313) or cyclosporine A (314) may be as effective as cyclophosphamide or MMF for inducing remission of proliferative lupus nephritis. A study of 31 Czech patients with lupus nephritis showed that cyclosporine A was effective in inducing remission, both as first-line therapy and as salvage therapy in cases that were refractory to cyclophosphamide (315). In a subsequent trial, the rates of remission and renal response were similar in patients who randomly received either cyclosporine A or cyclophosphamide in both the induction and maintenance phases (316). The cyclosporine-treated group demonstrated increase in blood pressure and reversible decline in GFR. A novel induction therapy for combined proliferative and membranous lupus nephritis (class IV + V) involves the use of a calcineurin inhibitor together with MMF or AZA plus corticosteroids (multitarget therapy). In a randomized study of 40 patients with class IV + V disease who received steroids and MMF plus tacrolimus or intravenous cyclophosphamide, intention-to-treat analysis showed a higher rate of complete remission for the multitarget group compared with the cyclophosphamide group, at 6 and 9 months (50% vs. 5% and 65% vs. 15%, respectively) (317). In addition, adverse events were less frequent in the multitarget group (317).

At Columbia University, we have found that repeat renal biopsies in patients completing a 6-month course of monthly intravenous cyclophosphamide typically demonstrate markedly reduced activity, but chronic features are often increased (318). These findings indicate that not all active lesions can be fully reversed to permit restoration of normal glomerular architecture. The more profoundly damaging lesions, such as fibrinoid necrosis and circumferential crescents, lead inevitably to some renal scarring. Subendothelial deposits, glomerular endocapillary proliferation, neutrophil infiltration, and interstitial inflammation appear to be far more reversible lesions.

In summary, cyclophosphamide and MMF (both in conjunction with high-dose steroids) have shown similar efficacy for induction of remission in most cases of severe class III or class IV proliferative lupus nephritis. With cyclophosphamide, the lower-dose EuroLupus protocol may be preferred, as it has fewer side effects than the standard NIH protocol. In clinical practice, MMF has emerged as the treatment of choice in most patients, but cyclophosphamide may be preferred in cases with severe crescentic disease. The choice of therapy must be tailored to the individual patient, in concert with the renal biopsy findings. In the absence of a clinically significant response after 6 months, the induction regimen should be switched from cyclophosphamide to MMF, or vice versa, accompanied by high-dose corticosteroids. Alternative agents such as belimumab, rituximab, or calcineurin inhibitors may be effective in cases resistant to standard induction therapy (see below).

### Maintenance therapy for Class III and Class IV Lupus Nephritis

Following the induction of remission, patients will require maintenance therapy for an indefinite period, but typically lasting at least 2 years. MMF, AZA, or a calcineurin inhibitor may be used, in conjunction with steroids (tapered to as low a dose as possible). In a study from Miami, Contreras et al. (287) compared MMF to cyclophosphamide as maintenance therapy over approximately 2 years, following an initial 4 to 7 months of monthly IV cyclophosphamide as induction therapy. Patients assigned to MMF maintenance therapy had fewer relapses, less mortality, and less drug-related toxicity compared to those randomized to quarterly IV cyclophosphamide pulses as maintenance therapy. In a randomized controlled study of 64 Chinese patients with class IV disease and a median follow-up of 63 months, MMF plus prednisolone was as effective as cyclophosphamide plus prednisolone as combined induction-maintenance therapy (290). Notably, the MMF group had fewer severe infections.

The use of MMF versus AZA for maintenance therapy was compared in two large, well-designed randomized multicenter trials of patients who had undergone successful induction therapy, with different conclusions (284,285). The MAINTAIN trial (A Randomized Multicenter Trial Comparing MMF and AZA as Remission-Maintaining Treatment for Proliferative Lupus Glomerulonephritis) included 105 predominantly white (79%) subjects from 27 European centers, who had class III (31), class IV (58%), and class V (10%) lupus nephritis (285). All subjects underwent induction therapy with cyclophosphamide using the lower-dose "EuroLupus" protocol before being randomly assigned to MMF or AZA maintenance therapy. Importantly, successful induction was not a requirement for entry to the maintenance study phase. Renal flares were observed in 13 (25%) AZA-treated and 10 (19%) MMF-treated patients. The two groups showed equal rates of remission, steroid withdrawal, and disease flares after at least 3 years of follow-up. In addition, follow-up protocol biopsies at 2 years showed no significant differences between the two treatment groups (319). On the other hand, the maintenance phase of the ALMS trial consisted of 227 patients in 88 worldwide centers (44% white, 34% Hispanic ethnicity), who had achieved a clinical response with MMF or cyclophosphamide (0.5 to 1.0 g/m$^2$) (284). The primary end point in this study was time to treatment failure, defined as renal flare (proteinuric or nephritic), sustained doubling of serum creatinine, initiation of rescue therapy, ESRD, or death. The ALMS study reported that MMF was superior to AZA in maintaining renal response and preventing relapse, regardless of induction therapy and irrespective of race, gender, or region, and might be better tolerated than AZA in high-risk non-Caucasian patients (284). The different conclusions probably reflect differences in

patient and disease characteristics, as well as the end points examined in these two studies.

Recent data suggest a possible role for calcineurin inhibitor as maintenance therapy. Chen et al. (294) reported that tacrolimus was equally effective as AZA in maintaining remissions in lupus nephritis and had fewer side effects. Moroni et al. (296) reported that cyclosporine A and AZA were equally effective in maintaining remissions in a study of 75 Italian patients with diffuse lupus nephritis who were followed for 4 years after receiving induction therapy with cyclophosphamide plus corticosteroids. Although concern exists about the nephrotoxic potential of cyclosporine A, this should not be significant with the doses used for maintenance therapy (i.e., 2 mg/kg/d).

In summary, both MMF and AZA are well tolerated and highly effective in maintaining a sustained remission response over 3 to 4 years in most patients with moderately severe proliferative lupus nephritis. MMF may be preferable as first-line therapy in non-Caucasian patients, whereas AZA may be appropriate in patients who wish to become pregnant. Calcineurin inhibitors may be a suitable alternative in Asian and Caucasian patients, but these agents carry a potential risk of nephrotoxicity and hypertension.

## Membranous (Class V) Lupus Nephritis

Conservative antiproteinuric therapy (e.g., with RAAS blockade and statin) may be sufficient in patients with class V lupus nephritis who have minimal proteinuria (less than 2 g/d), due to the low risk of progression to ESRD (278). Steroids continue to be the mainstay of treatment for membranous lupus nephritis and steroid monotherapy, or a calcineurin inhibitor may be appropriate in cases with significant (greater than 2 g/d but still mild proteinuria.

Patients with class V disease who are at high risk for progression (i.e., those manifesting more severe proteinuria, hypoalbuminemia, and reduced GFR) should receive aggressive immunosuppression for at least 6 months in order to induce remission of the nephrotic syndrome (27,298,320). For induction and maintenance therapy, MMF is as effective as cyclophosphamide, irrespective of the initial level of proteinuria (289,291,321). In a small, uncontrolled study, continuous MMF combined with RAAS inhibition and a statin produced sustained remission of proteinuria in 9 of 13 patients with pure membranous lupus nephritis at a mean follow-up of 16 months (322). In addition, cyclosporine A and AZA (in conjunction with steroids) have both been used successfully to induce partial or complete remissions of proteinuria in patients with class V lupus nephritis (323–325). Calcineurin inhibitors (tacrolimus (326) or cyclosporine A (327)) are both superior to steroids alone, and cyclosporine may induce faster remissions than cyclophosphamide, but with a higher frequency of relapses (327). The ACR and EULAR guidelines both recommend the use of corticosteroids and MMF as first-line therapy for class V disease with nephrotic proteinuria (27,298).

Rituximab has been effective salvage therapy in cases of membranous lupus nephritis that were refractory to other therapies, with markedly improved proteinuria and resolution of electron-dense deposits demonstrated by electron microscopy (328). As noted previously, multitargeted therapy (calcineurin inhibitor plus MMF plus steroid) may be beneficial in cases with combined proliferative and membranous disease (class IV plus class V) (317).

## Advanced Sclerosing (Class VI) Lupus Nephritis

Patients with class VI disease should be prepared for renal replacement therapy.

## Other Therapies for Lupus Nephritis

A variety of novel biologic agents that target B cells, T cells and cytokines have been studied in SLE, with varying results in patients with lupus nephritis. These include agents that target B cells (rituximab, belimumab, ocrelizumab), T-cell costimulation (abatacept), cytokines (infliximab, atacicept), and anti-dsDNA antibodies (abetimus sodium).

### Rituximab

Rituximab, an anti-CD20 monoclonal antibody that depletes B cells, has been used successfully to treat lupus nephritis that did not respond to other therapies (328–332). Two meta-analyses showed complete or partial responses of 67% to 69% following use of rituximab in cases that were refractory to other therapies (331,332). However, two randomized controlled trials in which rituximab or placebo were added to standard immuosuppressive therapy failed to demonstrate superiority over placebo (333,334). The Exploratory Phase II/III SLE Evaluation of Rituximab trial tested the efficacy and safety of rituximab versus placebo in 257 patients with moderately-to-severely active extrarenal SLE, but without lupus nephritis (333). The Lupus Nephritis Assessment with Rituximab (LUNAR) trial consisted of 140 patients with severe lupus nephritis who were randomized to rituximab or placebo, together with MMF (up to 3 g/d) and tapering doses of corticosteroids. Although the rituximab group achieved more remissions (complete or partial) and greater reductions in anti-dsDNA and serum complement levels, there was no statistically significant difference in clinical outcomes after 1 year (334). Importantly, these studies may have been underpowered to demonstrate a benefit from rituximab (276). Although these findings do not support the routine use of rituximab in lupus nephritis, this agent may still have a role in cases that are refractory to other therapies (27).

### Belimumab

Belimumab, a humanized monoclonal antibody that targets the B-cell growth factor B lymphocyte stimulator (BLyS) protein, is the only new agent in the past 50 years to have been approved by the United States Food and Drugs Administration (FDA) for treatment of SLE (335). Although its role in lupus nephritis has not yet been formally investigated, a pooled post hoc analysis of two phase III studies (comprising 1684 patients) identified 267 subjects who had baseline renal involvement. A subset of belimumab-treated patients who also received MMF or had serologic activity at baseline showed a reduced number of renal flares and proteinuria compared to placebo (336). These finding suggest that belimumab may have a role as adjunctive therapy in lupus nephritis.

### Abatacept

Abatacept, a CTLA4Ig fusion protein, competes with endogenous CTLA4 expressed on activated T cells for binding to CD80 (B7.1) and CD86 (B7.2) expressed on antigen-presenting cells and B cells, thereby inhibiting the CTLA4 costimulatory pathway. Preliminary studies in murine lupus showed promise (337). In one randomized placebo control trial, abatacept failed to achieve the primary outcome

(defined as urine protein:creatinine ratio less than 0.26 and estimated GFR within 10% of the screening/preflare value, on two consecutive visits). However, if less stringent definitions of response were employed (e.g., those used the LUNAR and ALMS trials), the abatacept-treated group would have shown significant better renal responses than the placebo group (338). A second clinical trial is ongoing.

#### Leflunomide

Leflunomide, an inhibitor of the mitochondrial enzyme dihydroorotate dehydrogenase that is required for pyrimidine synthesis, is well tolerated and efficacious in rheumatoid arthritis (RA). In one observational study of 110 Chinese patients with ISN-RPS class III or IV lupus nephritis (22 of whom had concomitant class V disease) who received either leflunomide ($n$ = 70) or cyclophosphamide ($n$ = 4) (both with corticosteroids) for induction therapy, similar rates of complete or partial remissions were observed at 6 months (21% and 52% vs. 18% and 55%, respectively), with similar rates of side effects (339).

#### Plasmapheresis

Several carefully controlled multicenter trials have failed to demonstrate any additional benefit of plasmapheresis over steroid and immunosuppressive regimens (340). In addition, this modality has been associated with life-threatening viral and bacterial infections (341). However, plasmapheresis may be appropriate in the subset of lupus nephritis cases with thrombotic microangiopathy (e.g., due to antiphospholipid antibodies [APLAs]) or coexistent ANCA vasculitis (342).

#### Intravenous Immunoglobulin

The benefit of intravenous immunoglobulin (IVIG) as a potential steroid-sparing therapy for class IV lupus nephritis is not yet proven in controlled trials (343). One small, uncontrolled study of nine patients with resistant class IV disease reported improvement in clinical and histologic disease activity after receiving IVIG (344). In a small study of seven patients with treatment-resistant membranous or diffuse proliferative lupus nephritis, IVIG was effective in inducing remissions of proteinuria (345). This effect is mediated by immunomodulating interactions with Fcγ receptors on immune effector cells, causing down-regulation of activating FcRIIA and FcRIIC while promoting up-regulation of inhibitory FcRIIB (343). The beneficial effects are usually prompt but short-lived, requiring repeated monthly infusions. However, other studies suggest that in some patients, IVIG may have the paradoxical effect of heightening disease activity (346). Proof of efficacy will require larger controlled trials.

#### Other Drugs

Immunoablation followed by autologous stem cell rescue (using the patient's own CD34-positive hematopoietic stem cells) has shown efficacy in inducing long-term clinical and serologic remissions in patients with SLE refractory to conventional immunosuppressive therapy (347).

Infliximab, a chimeric anti-TNF-α antibody, administered to six lupus patients (of whom four had lupus nephritis) was found to reduce proteinuria and inflammatory manifestations of systemic lupus but resulted in elevated levels of autoantibodies to dsDNA and cardiolipin (348). Short-term (but not long-term) use of anti-TNF agents may be appropriate in patients who have lupus nephritis that is refractory to standard therapies (349). Oligonucleotide-based inhibitors of toll-like receptor (TLR) signaling hold promise for inhibition of interferon-α production (350).

Randomized control trials of several other agents were discontinued due to lack of efficacy or an increased number of adverse events. Abetimus sodium (previously called LJP 394), a B-cell toleragen composed of four dsDNA helices attached to an inert scaffold, causes sustained reduction in the level of circulating anti-dsDNA. Early trials of abetimus in lupus nephritis showed promising results (351,352). However, there was no significant prolongation in time to renal flares in a randomized controlled phase III trial, which was terminated after interim efficacy analysis indicated that continuation would be futile (353). Ocrelizumab, a fully humanized anti-CD20 monoclonal antibody, was investigated in a phase III randomized, placebo-controlled safety and efficacy study that included 381 patients with ISN/RPS class III (20%) or class IV (80%) lupus nephritis. Significantly higher renal response rates were seen in patients who received ocrelizumab in addition to standard therapy compared to placebo (354). However, all clinical trials with ocrelizumab were discontinued in 2010 due to an unexpectedly high rate of serious opportunistic infections. Finally, blockade of costimulatory pathways with monoclonal antibody directed to CD40 ligand (CD154) showed improvement in serologic activity and hematuria in patients with diffuse proliferative disease (355), but another study with this agent was halted due to fatal thrombotic events (356).

In coming years, it is likely that novel biologic agents will be developed to inhibit specific pathogenic cytokines and interleukins.

## Clinical Course and Prognosis

The overall survival of patients with SLE has improved over the past several decades, reflecting better control of the underlying disease and improved management of the side effects of drug therapy (26,204,357,358). In industrialized countries, 5-year patient survival has improved from less than 10% in patients with diffuse lupus nephritis (treated with low-dose steroids) in the 1950s (185) to greater than 98% in the current era (359). The natural history of untreated lupus nephritis is unknown, but the closest approximation comes from the study by Muehrcke et al. (360) in 1957, which found a survival rate of less than 10% at 2 years for patients with diffuse proliferative lupus nephritis who received only low-dose corticosteroids. Following the broad use of high-dose corticosteroids, 5-year survival rates in the 1970s averaged 73% to 78% (148,203,244). In the early 1980s, the introduction of cyclophosphamide for diffuse proliferative lupus nephritis led to patient survivals in the range of 85% at 5 years (33,36), with further improvement to 87% at 10 years by 1987 (361). In the decade of 1990–2000, 5- and 10-year patient survivals in the range of 90% to 95% were achieved (283,287,288,362–365). In a German study, Fiehn et al. (364) found that the outcome of patients with newly diagnosed lupus nephritis was significantly better in the decade of 1990–2000 than the decade 1980–1989, which they attributed to earlier diagnosis and treatment in the later decade. More recently, 5-year survival rates of greater than 98% have been reported in adults (359) and children (366) with lupus nephritis.

In contrast, the incidence of ESRD from lupus nephritis has remained relatively constant over the past four decades (367). Renal survival rates at 15 years average 70% to 75% (368), reflecting the fact that as many as 40% of patients fail to respond to induction therapy. In addition, relapses (also referred to as "nephritic flares") occur in up to 45% of patients following remission and are associated with significantly worse long-term prognosis (210,277,369,370). In an Italian study, there was a much higher risk (relative risk 6.8) of doubling serum creatinine in patients who experienced a nephritic flare (370). The risk of a flare is greater in those who only achieve partial remission (369). The induction of re-remission after a renal flare is successful in only two thirds of patients and those who do not remit have a high rate of progression to ESRD (277).

Not surprisingly, treatment response is an important prognostic indicator (211,287,368,371). Houssiau et al. (372) showed that remission response within 6 months of induction therapy was the best predictor of good renal outcomes at 73 months and 10 years follow-up (303). In the Collaborative Study Group, 86 patients with diffuse proliferative lupus nephritis who all were treated initially with standard (high dose) cyclophosphamide were followed for more than 10 years (371). Patient and renal survivals at 10 years were 95% and 94%, respectively, for those with complete remission, 76% and 45% with partial remission, and 46% and 19% with no remission. In addition, the patient survival without ESRD at 10 years was 92% for complete remission, 43% for partial remission, and 13% for no remission (371).

The long-term benefit of newer strategies for proliferative lupus nephritis is largely undetermined, but treatment failure, flares, and progression to chronic kidney disease continue to occur. In the Euro-Lupus Nephritis trial comparing low-dose and high-dose cyclophosphamide for induction therapy followed by AZA for maintenance therapy, the rates of remission, treatment failure, and renal flare in the low-dose and high-dose groups were 71% and 54%, 16% and 20%, 27% versus 29%, respectively (288). After 10 years of follow-up, the rates of death (11% vs. 4%), sustained doubling of serum creatinine (14% vs. 11%) and ESRD (5% vs. 9%) were substantial, albeit not significantly different among the two treatment groups (303). The ALMS maintenance trial reported a 3-year composite end point (renal relapse, doubling of serum creatinine, death, or transplantation) in 16% of patients receiving MMF and 32% in those receiving AZA (284). Treatment failure occurred in 16.4% and 32.4% of MMF and AZA groups, respectively. The MAINTAIN trial reported a similar 3-year prevalence of worse renal outcomes (nephrotic proteinuria, 33% increase in serum creatinine, or threefold increase in proteinuria or hematuria) in patients treated with MMF (19%) or AZA (25%) (285). Importantly, these clinical trials underestimate the actual prevalence of poor outcomes as they excluded patients who had previously failed therapy and/or had renal failure at presentation.

As a group, patients with class V lupus nephritis tend to have a favorable short-term course, comparable to that of patients with class II disease, but the subgroups of patients with persistent nephrotic syndrome or mixed proliferative and membranous disease have a worse long-term prognosis (204,236). A study from Chicago that included 36 patients with WHO class Va and class Vb lupus nephritis reported 5- and 10-year-survival rates of 86% and 72%, respectively (236). Our experience at Columbia University with a group of 60 patients with pure class V lupus nephritis diagnosed over a 30-year period has yielded similar renal survival rates of 90% and 73% at 5 and 10 years, respectively (Vivette D'Agati, 2014, unpublished data). The Chicago and the New York groups both found significantly worse outcomes for patients who had mixed membranous and proliferative disease at the time of initial biopsy or that developed during following up (187). At 3.5 years of follow-up, 60% of patients with mixed membranous and proliferative disease had an adverse renal outcome, compared with only 36% of the patients with pure class IV disease (187). Adler et al. (373) found that renal failure occurred in only 1 of 7 patients with class V, compared with 7 of 11 patients with class V + III and V + IV lesions. In the Columbia experience, individual predictors of poor renal outcome in class V lupus nephritis include higher serum creatinine levels, higher urinary protein excretion, lower serum albumin levels, and hypertension at the time of renal biopsy. By multivariate analysis, the most significant predictor of adverse renal outcome was black race, similar to the negative prognostic impact of black race in other classes of lupus nephritis (374–376). Some investigators have identified nephrotic-range proteinuria as a risk factor for ESRD in membranous lupus nephritis (377), whereas others have not (236,378).

It is likely that more aggressive immunosuppressive therapy for patients with membranous lupus nephritis who are at high risk for progression has led to improved outcomes in the modern era. Pasquali et al. (235) reported a 100% 10-year survival rate for 26 patients with pure membranous lupus nephritis. The researchers attribute this excellent renal survival to the use of more aggressive therapy, because all patients were treated with steroids and more than one half received a second immunosuppressive agent (i.e., AZA, cyclophosphamide, or chlorambucil). Similarly, in a follow-up study of 38 Asian patients with pure class V disease treated with glucocorticoid and AZA and followed for 12 ± 5.8 years, 24% had doubling of serum creatinine or decline of creatinine clearance by ≥30% but none reached ESRD (378).

According to a large study of 166 patients by the NIH group, clinical predictors of poor renal outcome in class IV lupus nephritis included black race, higher serum creatinine levels, and lower hematocrit values (251,379). Black patients were more likely than whites to have high-risk histologic features, including extensive (greater than 50%) cellular crescents and moderate to severe interstitial fibrosis. In a study of 89 patients with diffuse proliferative lupus nephritis by the North Carolina Glomerular Disease Collaborative Network, 5-year renal survival was 58% among blacks, compared with 95% among whites (376). This poor outcome among blacks appeared to be independent of age, duration of SLE, history of hypertension, adequacy of control of hypertension, or the AI or CI at renal biopsy (376). Similarly, in a retrospective study of 213 patients with lupus nephritis, the frequencies of ESRD or doubling of serum creatinine were significantly higher compared in black patients and Hispanic subjects compared to white patients (31% vs. 18% vs. 10%, respectively) (380). It is unclear to what extent genetic or sociocultural factors (e.g., follow-up or compliance) account for these differences, but an outcome study from New York suggested a major role for socioeconomic factors (375). Poverty and lack of medical insurance conferred increased risk of disease progression

among African American and Hispanic patients (375). A large Japanese study of 515 females and 51 males with SLE treated from 1972 through 1991 found males to have a significantly worse renal survival (381).

In a study of greater than 400 patients with lupus nephritis seen at Mayo Clinic from 1964 to 1986, predictors of progressive renal failure by multivariate analysis included impaired renal function, nephrotic-range proteinuria, anemia, and younger age (382). Interestingly, WHO class was not a significant predictor of outcome when adjusted for these four clinical variables (382). In a study of 85 patients with lupus nephritis diagnosed between 1967 and 1983, the most powerful predictor of renal failure and death due to lupus nephritis was change in proteinuria within 1 year of treatment (383). In the same cohort of patients (384,385), delay between the clinical detection of onset of renal disease and renal biopsy had an extremely negative impact on renal survival. There was significant deterioration in renal function and urinary protein excretion in the interval from renal disease onset to biopsy, and renal biopsy specimens often had higher AI and CI values. These important data underscore the need for prompt renal biopsy and initiation of therapy in SLE patients with any clinical evidence of renal involvement.

The prognostic significance of renal vascular disease in lupus nephritis was demonstrated in a retrospective study of 341 Chinese patients with lupus nephritis (classes II to VI), 279 of whom had one or more vascular lesions, including isolated immune complex deposits (253 cases), atherosclerosis (82 cases), thrombotic microangiopathy (60 cases), noninflammatory vasculopathy (13 cases), and vasculitis (2 cases). Patients with thrombotic microangiopathy had the poorest outcomes, but including vascular lesions in a modified chronicity index was a significant predictor of renal outcome in all cases (386).

## Dialysis and Transplantation

Overall, approximately 5% of all SLE patients and 20% of those with lupus nephritis develop ESRD (387). The incidence of ESRD from lupus nephritis remained unchanged between 1975 and 2005 in a United Kingdom cohort (387). In the United States, the incidence of ESRD increased from 1.16 cases per million person-years in 1982 to 3.08 cases per million person-years in 1995 and has subsequently stabilized but not declined (367,388,389). African American and Afro-Caribbean patients have higher rates of ESRD from lupus nephritis than Caucasians and Asians (380,387). Of note, patients with ESRD due to SLE frequently experience a remission of their extrarenal manifestations and improvement in lupus serologic results such that all immunosuppression can be withdrawn (390). The cause for this improvement is uncertain but may be related to the immunosuppressive effects of uremia itself. Increased suppressor cell activity and reduced mitogenic response of peripheral blood leukocytes to uremic serum are thought to mediate this effect (391).

The survival of SLE patients on dialysis (both hemo- and peritoneal) appears to be comparable to that of non-SLE patients (392). Peritoneal dialysis is associated with better graft survival in adult SLE patients (393) but not in pediatric patients (394). The optimal length of dialysis in lupus patients with ESRD prior to transplantation is controversial. Roth et al. (395) suggested that patients with longer dialysis periods (greater than 2 years) before transplantation achieved better graft function than patients on dialysis for ≤1 year. However, Bumgardner et al. (396) were unable to confirm these findings. Moreover, successful preemptive live donor transplantation has been described in patients with ESRD due to SLE who never underwent dialysis (138). Nonetheless, a waiting period of 3 to 6 months prior to transplantation appears prudent, as 10% to 28% of patients with SLE and chronic kidney failure undergoing dialysis may show spontaneous recovery of kidney function (390).

Transplantation is well tolerated in patients with SLE, with best results seen with preemptive transplantation and living donor kidneys (392,397). Although several studies have shown worse allograft outcomes in lupus patients (398,399), the overall trend suggests graft survival rates comparable to other causes of ESRD (400,401). One retrospective single-center study found worse 1-year graft survival in patients with SLE (402). Graft failures in SLE allograft recipients have been attributed to thrombosis associated with APL antibody, recurrent disease (392), and higher rates of rejection (399). By contrast, a study conducted within the United States Renal Data system of all patients transplanted between 1996 and 2000 found no differences in patient and graft survivals between those with and without lupus after controlling for confounding variables (401). Five-year graft and patient survivals in lupus patients were 68% and 85% for deceased donor recipients and 78% and 92% for living donor recipients. However, among pediatric patients, those transplanted for end-stage lupus nephritis exhibit lower patient survival than their nonlupus counterparts, despite comparable graft survival (403). Several risk factors for poor graft survival have been identified, including number of HLA mismatches. The risk of graft failure and patient death is higher in African American recipients, possibly reflecting socioeconomic factors and higher rates of deceased donor transplantation, delayed graft function, HLA mismatches, early rejection episodes, and recurrent lupus nephritis in this population (393,404–406).

The reported incidence of recurrent lupus nephritis ranges from 1% to 54% (402,405,407–412), with higher rates (30% (410) and 54% (412)) in studies that employed immunofluorescence and electron microscopy. By contrast, a review of the United States United Network for Organ Sharing files found a prevalence rate of 2.44% for recurrent lupus nephritis defined clinically (not by renal biopsy) among 6,850 patients who received a transplant between 1987 and 2006 (413). In this study, recipients with recurrent lupus nephritis had a fourfold greater risk for graft failure compared to control subjects without rejection; however, the major cause of graft failure was rejection (43%), and recurrent lupus caused only 7% of graft losses. Risk factors for recurrence included black race, female gender, and younger age (413). The higher risk of recurrence in African Americans was also demonstrated in another study (405). Recurrent lupus nephritis may be diagnosed at anytime from the first week to 16-year posttransplantation (392,413). All classes of lupus nephritis have been reported in the allograft, with a predominance of milder forms (classes I and II) (405,410–412). One series of 20 patients with recurrent lupus nephritis noted a predominance of mesangial disease in allograft biopsies performed for cause (60%), whereas the native kidneys showed predominantly proliferative (50%) or membranous (30%) lesions (405). We have observed class II lupus nephritis diagnosed as an incidental finding in biopsies performed for graft dysfunction caused by acute rejection. In a study from Norway

of 41 SLE patients who underwent allograft biopsy, including 38 per protocol and 3 for cause, recurrent lupus nephritis was identified in 22 subjects (54%) at a mean time of 8-year post-transplantation (412). Recurrent lupus nephritis was associated with more proteinuria (albeit mild) and lupus anticoagulant. The 22 biopsies showed predominantly class I or class II disease (10 and 7 cases, respectively), but two had class III (including one with class V), one had class IV, and the remaining two had class V lupus nephritis. Thus, it is likely that subclinical recurrence is more common than previously thought and would be detected more frequently if immunofluorescence and electron microscopy were applied systematically to all renal transplant biopsies. Rarely, lupus nephritis has been reported to recur in two successive renal allografts (414). There are also rare reports of donor kidneys with unsuspected crescentic lupus nephritis being transplanted into nonlupus recipients, with variable outcomes (415). Recurrent lupus nephritis is a risk factor for graft loss but appears to have limited impact on patient survival (405) (see also Chapter 29).

A serious complication of ESRD in lupus patients is persistently positive APL antibodies with the attendant increased risk of thromboembolism and thrombocytopenia. Some of these patients develop thrombosis in the renal allograft that may be difficult to differentiate from the vascular manifestations of calcineurin inhibitor toxicity or antibody-mediated rejection (416). Patients with moderate to high titer APL should probably receive prophylactic anticoagulation in the perioperative period (398). Nonlupus populations with ESRD on hemodialysis have also manifested a high (10% to 30%) prevalence of APL antibodies, which does not appear to be related to age, gender, or duration of dialysis (417–419). The prevalence of APL antibodies appears to be greater in patients on hemodialysis than on peritoneal dialysis and in those with arteriovenous grafts compared with those with arteriovenous fistulas. The reasons for these associations remain unexplained.

## Other Renal Manifestations of SLE

### Silent Lupus Nephritis

Renal pathologic abnormalities without clinical evidence of renal disease (silent lupus nephritis) has been identified in up to one third of patients with SLE (225,420). In a review of 204 cases reported in the literature up to 2006, and excluding 38 cases that were originally classified as WHO class I (i.e., with no immune complex glomerular disease), the patterns reported include WHO class II (51%), III (19.3%), IV (24.1%), V (5.4%), and VI (less than 1%) (421). Most patients with silent lupus nephritis appear to have a benign clinical course (422). However, in one study of 31 Japanese subjects with silent lupus nephritis (WHO classes: I, 4 cases; II, 23 cases; IV, 3 cases; and V, 1 case), 8 patients (28%) subsequently developed overt renal disease, at a mean follow-up time of 58 months (226). This event was associated with persistently elevated anti-dsDNA antibodies and hypocomplementemia (226). These findings suggest that lupus nephritis may have a preclinical phase in the early stage of disease. Because renal biopsies are rarely performed in patients without clinical disease, the optimal management of this condition is uncertain.

### ANA-Negative Lupus Nephritis

Renal disease may rarely occur in patients lacking the usual serologic evidence of SLE (ANA-negative systemic lupus) (423,424). The incidence of ANA-negative lupus has been reduced since the replacement of rodent tissues with human carcinoma cell lines as substrate for the ANA immunofluorescence test. Nonetheless, even in the modern era, there are rare reports of renal biopsy findings highly suggestive of lupus nephritis in subjects who do not fulfill ACR diagnostic criteria for SLE at the time of biopsy or after years of follow-up (425).

### Lupus Nephritis With ANCA Positivity

Several cases of lupus nephritis with coexistent ANCA vasculitis have been described. Nasr et al. (342) reported 10 patients with necrotizing and crescentic lupus nephritis, all of whom had positive P-ANCA titers and/or antimyeloperoxidase antibodies (MPO-ANCA). Biopsies showed fibrinoid necrosis (mean 29% of glomeruli) and crescents (mean 31% of glomeruli) and lupus nephritis ISN-RPS class II (one case), III (four cases), IV-G (1 cases), IV-S (one case), or V (three cases). However, as distinct from typical proliferative lupus nephritis, only sparse peripheral capillary wall deposits were seen. One biopsy also had necrotizing arteritis. All patients received cyclophosphamide and prednisone: three died of infectious complications, six had complete or near-complete remission (including one with a subsequent relapse), and one had no response to therapy. Three of the ten patients may have had drug-induced ANCA (hydralazine in 2, thioridazine in 1), as ANCA titers declined after drug discontinuation. More systematic testing for ANCA in lupus nephritis is needed to define potential associations with the necrotizing and crescentic phenotype.

### Lupus Podocytopathy

Some patients with SLE present with abrupt onset of nephrotic syndrome accompanied by pathologic findings of minimal change disease or FSGS, with or without coexistent mild lupus nephritis (426–430). At least some of these cases, and possibly a majority, may be a form of "podocytopathy" induced by the dysregulated cytokine milieu of SLE, rather than coincidental occurrence of idiopathic minimal change disease or idiopathic FSGS (426–428). The diagnostic feature is diffuse foot process effacement in the absence of significant peripheral capillary wall immune deposits. A majority of these cases had coexistent class I or II lupus nephritis (426,427,429,430). Five of seven cases of minimal change disease reported from our group displayed mesangial electron-dense deposits, with or without associated mesangial proliferation, consistent with underlying lupus nephritis class I or II (429). The rapid remission of nephrotic syndrome with steroid therapy supported a clinical diagnosis of minimal change disease (429). In a retrospective study of 11 patients with SLE and abrupt onset of nephrotic syndrome with profound hypoalbuminemia, Hertig et al. (426) described 4 cases of minimal change disease and 7 with FSGS. Immune deposits were confined to the mesangium and could not account for the severe foot process effacement and full nephrotic syndrome (426).

Collapsing variant of FSGS (collapsing glomerulopathy) has been reported in more than 50 SLE patients (430–437). In the largest series of 19 cases with detailed clinical-pathologic information, 11 had no evidence of lupus nephritis (430). The other eight cases had mesangial (four), focal proliferative (one), or membranous disease (three) (430). SLE was present before the diagnosis of collapsing glomerulopathy in 11 patients (duration 1 to 20 years) and was diagnosed simultaneously with collapsing glomerulopathy in eight subjects. Most

patients (16/19) had active extrarenal lupus symptoms at the time of biopsy. Similar to idiopathic collapsing glomerulopathy, most patients were of African descent, all but one subject had nephrotic syndrome and 16 of 17 patients with available data had renal insufficiency at presentation. Among the 13 patients with follow-up data, 2 received no treatment and the other 11 were treated initially with pulse corticosteroids, together with MMF (7 cases) or AZA (1 case). At 21-month follow-up, progression to ESRD occurred in seven patients, one patient had complete remission, and persistent proteinuria and renal insufficiency was seen in the remaining five subjects.

Whether these cases represent a particularly aggressive variant of lupus podocytopathy or coincidental primary collapsing glomerulopathy is unclear. In support of the former possibility are the presence of active lupus symptoms and the simultaneous diagnosis of SLE and collapsing glomerulopathy in many cases. Of note, Larsen et al. (431) reported a strong association with apolipoprotein L1 (APOL1) risk alleles in 26 African American patients who had SLE and collapsing glomerulopathy, supporting a genetic predisposition in patients of African descent.

### Other Renal Diseases in SLE Patients

A variety of renal diseases may occur in patients with SLE, as either an isolated renal biopsy finding or superimposed on lupus nephritis. Amyloidosis has been described in approximately 20 patients with SLE (438–440). In most cases, this is secondary amyloidosis (AA amyloidosis) related to chronic inflammation, as confirmed by immunostains for serum amyloid A (SAA) protein. Most of the reported cases had longstanding SLE (range 1 to 35 years, median 5 years). In the modern era, the overall incidence of secondary amyloidosis has decreased, most likely due to more effective immunosuppressive therapy. There are several reports of fibrillary glomerulonephritis in patients with SLE (441–443) and a single report of concurrent renal sarcoidosis and lupus nephritis (444). We have also observed several cases of lupus nephritis superimposed on diabetic nephropathy, reflecting the increasing incidence of diabetes in the general population.

Patients with SLE have been reported to develop a variety of other renal diseases not directly related to their autoimmune condition (445). Among 252 biopsies performed on 224 patients with SLE at Baylor College of Medicine over a 25-year period, 13 had nonlupus nephritides (445). In addition to cases of focal segmental sclerosis, IgM nephropathy, and amyloidosis, there were also examples of thin basement membrane disease, hypertensive nephrosclerosis, and allergic tubulointerstitial nephritis. Such cases underscore the importance of renal biopsy in this population to identify nonlupus conditions that carry different prognostic and therapeutic implications.

## Drug-Induced Lupus

Although most cases of SLE are idiopathic, up to 10% of patients have drug-induced lupus (DIL) (446,447). A diagnosis of DIL requires three conditions: absence of a history of SLE before drug therapy, positive ANA status and at least one other clinical feature of SLE after starting the drug, and evidence of improvement in clinical symptoms and serologic tests for lupus after drug withdrawal. A fourth condition, recurrence of DIL symptoms if the patient is rechallenged with either the same drug or a similar one in the same class, provides additional evidence but is rarely formally tested in cases of suspected drug toxicity.

Since the first report in 1945 by Hoffman (448) of probable DIL in a 19-year-old man treated with the antibiotic sulfadiazine, up to 80 drugs have been reported to cause a lupus-like syndrome or exacerbate preexisting lupus (446,447). Most of these cases manifest positive ANA and other lupus serologies, a much smaller percentage develop symptoms of lupus and less than 5% exhibit renal involvement. Although much lower than the estimated 50% incidence of renal involvement in idiopathic SLE, the large number of patients exposed to these drugs makes DIL and complicating nephritis an important consideration for any patient who has a biopsy for apparent lupus nephritis, especially older men under treatment for other underlying medical conditions. DIL should be distinguished from precipitation or exacerbation of underlying idiopathic SLE, which may occur following exposure to sulfonamides (449), carbamazapine (450), nonsteroidal anti-inflammatory drug (NSAID) (451), and exogenous estrogens in postmenopausal women (452). Contrary to DIL, idiopathic SLE does not reverse following drug discontinuation.

A host of drugs have been reported to cause DIL (446,447). Although their chemical structures are quite diverse, many share hydrazino, amino, or sulfhydryl groups. The two most common offenders are procainamide and hydralazine, but DIL has also been linked to isoniazid, methyldopa, quinidine, propylthiouracil, chlorpromazine, carbamazapine, minocycline, estrogen, progesterone, α-interferon, and TNF-α blockers (453–456). Approximately 20% of patients treated with procainamide for at least 1 year develop DIL (457). The incidence of DIL in hydralazine-treated patients ranges from 2% to 21%, depending on the dose and duration of therapy (454,458–461). Particularly at risk are patients who are "slow acetylators" or have other genetic predispositions, such as C4 null allele and HLA DR4 (462–464).

### Serologies

More than 99% of DIL patients are ANA positive, but only a minority has anti-DNA antibody or hypocomplementemia. A helpful serologic finding is a high incidence (greater than 95%) of antihistone antibodies, compared with approximately 70% in idiopathic SLE. Conversely, anti-dsDNA antibodies are found in less than 5% of patients with DIL versus 50% of those with idiopathic SLE (465). Higher rates of anti-dsDNA positivity are seen in anti-TNF-α-induced DIL (greater than 50% (466)) and interferon-related DIL (8% (467)). Low C3 and C4 are typically rare, except in cases related to D-penicillamine (23%) and quinidine (33%). APL antibodies are detected in 5% to 20% of patients with DIL related to procainamide and hydralazine and up to 33% of those with DIL related to minocycline (447). However, these APL antibodies are mostly IgM and are not associated with thrombotic complications. ANCA, most commonly perinuclear (P) ANCA with myeloperoxidase specificity (MPO-ANCA), are seen in up to 50% of patients with propylthiouracil-induced DIL and 67% to 100% of cases of minocycline-related DIL (447).

### Clinical Features

DIL is equally common in males and females and is more common in older subjects and Caucasians. Whether these demographic characteristics reflect true differences in underlying

predisposition or simply greater exposure (e.g., older males are more likely to be treated with procainamide and hydralazine) is uncertain. The onset of DIL is usually gradual with symptoms typically developing after several months of drug exposure. The spectrum of DIL ranges from asymptomatic-positive lupus serologies (the most common manifestation) to severe organ involvement in rare cases. Common clinical features include fever, myalgias, arthralgias, and serositis, but malar rash, central nervous system, and renal involvement are unusual. A subset of patients presents with predominantly cutaneous manifestations (subacute cutaneous SLE), characterized by erythema and scaling on sun-exposed areas. Resolution of clinical symptoms after drug cessation usually takes days to weeks, but serologic resolution may take several months (277).

Glomerular disease been described with D-penicillamine (468,469), hydralazine (470), TNF blockers (471), and propylthiouracil (472). Renal manifestations are diverse, ranging from asymptomatic hematuria and proteinuria to overt nephrotic syndrome and acute renal failure. The most commonly reported patterns of glomerular involvement are focal proliferative and diffuse proliferative glomerulonephritis, sometimes associated with crescents. Mesangial proliferative and membranous patterns have also been described. Some patients with DIL caused by hydralazine or propylthiouracil develop positive ANCA status and may manifest systemic vasculitis and pauci-immune forms of segmental necrotizing and crescentic glomerulonephritis, with or without coexistent lupus nephritis (342,473,474). Diagnosis of DIL at the time of renal biopsy requires a high index of suspicion and careful drug history.

### Pathogenesis

The pathogenesis of DIL is largely unknown but likely involves genetic, epigenetic, and environmental factors (e.g., older age, female gender, drug exposure, and cumulative dose). Genetic factors include slow acetylator status for procainamide and hydralazine, HLA-DR4 for hydralazine, and possibly complement C4 null allele (277). Procainamide and hydralazine both cause defective DNA methylation in vitro, which may promote T-cell autoimmunity via dysregulated gene expression (475). A role for neutrophil activation and transformation of these drugs to cytotoxic intermediates has also been proposed (476).

Treatment of biopsy-proven lupus nephritis depends on the pattern of lupus nephritis. Discontinuation of the offending agent is usually coupled with immunosuppressive therapy administered in a regimen similar to that for idiopathic lupus nephritis of the same class.

## Lupus Nephritis Associated With HIV Infection

Although the most characteristic glomerular lesion associated with HIV infection in the United States is the collapsing variant of FSGS, also known as HIV-associated nephropathy (HIVAN), this entity is rare in patients who lack African ancestry. HIV-infected patients may also develop immune complex glomerulonephritis, particularly in the setting of HCV coinfection (see also Chapter 11). In addition, cases of lupus and lupus-like nephritis in HIV-infected adults with diverse risk factors and children with perinatal HIV infection have been described (181,477–479).

Systemic lupus and HIV infection share many clinical features, including anemia, leukopenia, multiorgan involvement, serositis, and renal disease. Although many patients with HIV infection have high levels of circulating immune complexes, polyclonal gammopathy, and autoimmune phenomena (including low-titer ANAs), a serologic diagnosis of systemic lupus in HIV patients is unusual (480). Screening of greater than 150 patients with acquired immunodeficiency syndrome (AIDS) or AIDS-related complex found 19 to be ANA positive, but only two in high titer (480). No patient was found to have double-stranded anti-DNA antibodies and very few had low serum complement levels. Interestingly, remission of SLE symptoms may follow HIV infection, and antiretroviral therapy may lead to lupus flares (481–483).

Strauss et al. reported two male HIV-infected infants with lupus nephritis who were ANA and anti-dsDNA positive (484). D'Agati and Seigle described a 2.5-year-old boy positive for ANA (1:320 titer) and anti-dsDNA antibodies and who had a low serum C3 level; the child had membranous glomerulonephritis with electron-dense deposits in the mesangium, subepithelially, and along the tubular basement membranes, as well as numerous TRIs (485). Two patients from Miami with HIV infection and clear documentation of positive lupus serologic status were reported (486). One had biopsy features suggesting lupus nephritis, and the second, despite serologic status suggesting lupus nephritis (e.g., positive ANA, low serum complement level, high anti-dsDNA antibody titer) had HIVAN.

We reported the clinical manifestations and renal biopsy findings in four subjects with concurrent HIV infection and serologic evidence of SLE and compared them with seven previously reported cases in the literature (477). In this cohort of 11 patients, most were black (91%) and male (73%), and approximately half (55%) were children with perinatal HIV infection, demographics that differ sharply from those of idiopathic SLE. Renal presentations included proteinuria, hematuria, and renal insufficiency. Renal biopsy findings in 10 cases included all classes of lupus nephritis (class II in two cases, class III in one case, class IV in three cases, class V in three cases, class III and V in one case), two of which also displayed overlapping features of HIVAN. One case had isolated findings of HIVAN (477). In a subsequent review of the literature, Palacios et al. identified 30 reported cases of coexistent SLE and HIV infection, with renal biopsy findings of lupus nephritis alone ($n = 13$), HIVAN ($n = 1$), or combined lupus nephritis and HIVAN ($n = 2$) (479). In 15 patients (50%), SLE preceded the diagnosis of HIV infection; in 12 cases (40%), HIV was diagnosed prior to SLE, and in 3 subjects (10%), SLE and HIV infection were diagnosed simultaneously (479).

In summary, patients with coexistent SLE and HIV infection manifest a variety of renal lesions, including lupus nephritis in most cases and HIVAN in a few cases. It appears that the majority of these cases represent chance occurrence of HIV infection and idiopathic SLE. These cases of concurrent HIV infection and SLE must be differentiated from the numerous reports of "lupus-like" immune complex glomerulonephritis occurring in HIV-infected patients who lack lupus serologies or other systemic features of SLE (487–490).

## Pathogenesis of Systemic Lupus Erythematosus and Lupus Nephritis

The pathogenesis of SLE is still not fully understood and is the subject of ongoing intensive study in multiple fields, including genetics, immunology, and cell biology. Details of this complex

topic are beyond the scope of this chapter and are summarized in several recent reviews (277,491,492). The prevailing paradigm of lupus pathogenesis invokes the interaction of multiple genetic predisposing factors with environmental triggers, which result in loss of tolerance to autoantigens and formation of autoantibodies to double-stranded (ds) DNA and other self-antigens. The pathogenesis of lupus nephritis involves deposition of these autoantibodies in the kidney, which triggers an inflammatory response that can lead to chronic fibrosing injury and irreversible organ damage. This section focuses on the key genetic, molecular, and cellular events involved in the pathogenesis of lupus nephritis.

## Murine Models of SLE

Our understanding of the pathogenesis of lupus nephritis has been informed considerably by the study of spontaneous and induced murine models of lupus (493). The classic models of spontaneous SLE are NZB, New Zealand White (NZW), (NZB × NZW) $F_1$ (NZB/W), New Zealand Mixed (NZM)2410 and NZM2328, (Swiss Webster [SWR] × NZB) $F_1$ ($SNF_1$), BXSB/MpJ, and MRL/lpr. Induced models of SLE include chronic graft-versus-host disease (cGVHD) and injection of hydrocarbon oils, such as pristane. Numerous transgenic and knockout models have also been developed to investigate the role of specific genes. The inbred stains all produce high levels of ANA and develop variable features of lupus nephritis and other features of SLE, including joint manifestations, lymphadenopathy, autoimmune cytopenias, and vasculitis. Although none of these strains model human SLE completely, there is considerable genetic and functional overlap in the murine and human disease (494).

### NZB/W and NZM Models

Both NZB and NZW mice develop only mild autoimmunity, whereas NZB/W hybrids and NZM develop a disease that more closely mimics lupus nephritis (494). The disease in NZB/W mice is of greater severity and earlier onset in females and is characterized by high titers of ANA, anti-dsDNA IgG (mostly IgG2a and IgG3), and antibodies to ssDNA, dsRNA, tRNA, histones, and polynucleotides; elevated levels of circulating immune complexes; and hypocomplementemia. Immunologic abnormalities include B-cell hyperactivity which is T-cell dependent, elevated type 1 interferon-induced gene expression, elevation of B-cell–activating factor (BAFF), and defective Fc-mediated clearance of immune complexes. The most severe clinical manifestation is a diffuse proliferative glomerulonephritis associated with deposits of IgG (predominantly complement-fixing IgG2a) and C3 in GBMs, mesangium, tubular basement membranes, and interstitium. The development of clinical nephritis is preceded by mesangial deposition of antichromatin (i.e., antinucleosomal) antibodies, and transition to more severe nephritis is associated with selective down-regulation of renal DNAse-1 (495). Nephritis is heralded by the development of proteinuria at 5 to 7 months of age, followed by uremia leading to death by 8 to 12 months of age (496,497). Other disease manifestations such as lymphadenopathy, splenomegaly, and autoimmune hemolytic anemia are less prominent than the nephritis. The disease in NZM2410 strain displays equal severity of nephritis in males and females, whereas the NZM2328 strain has a female predominance and develops renal disease in two stages, acute and chronic, which are regulated by different genetic factors (498).

Antibodies eluted from the kidneys have variable characteristics, with a predominance of IgG anti-DNA (499–501), anti-RNA polymerase (502), and anti-gp70 of the murine leukemia virus (503,504). Antibodies to dsDNA appear to be the most nephritogenic, because passive transfer of monoclonal IgG2a anti-dsDNA antibodies from NZB/W into normal BALB/c mice can induce nephritis (505). Polyclonal B-cell activation depends on T cell help from $CD4^+CD8^-$ and $CD4^-CD8^-$ cells. The renal phenotype in both sexes can be modulated by hormonal manipulation (506–508). For example, orchiectomy or administration of estrogens to male NZB/W mice causes an earlier switch from IgM to IgG anti-DNA antibodies with the development of more rapid and severe nephritis. Conversely, females administered androgens or submitted to oophorectomy have dampened nephritis and prolonged survival (506).

Multiple genes involving both MHC and non-MHC loci control the development of autoantibodies, loss of B-cell tolerance, and nephritis. Several susceptibility (*Sle1-3*) and suppressive loci (*Sles1*) for glomerulonephritis have been identified by linkage analysis in NZM mice, some of which are linked to the MHC (493). Importantly, congenic strains carrying the *Sle* loci demonstrate that coexpression of all three major loci is necessary and sufficient for the development of fully penetrant disease, whereas none of these lupus susceptibility loci alone leads to full disease expression (509). Human orthologs of these susceptibility genes include *FCGR2B* and *FCGR3B* (510), complement receptor 2 (*CR2*) (511), signaling lymphocyte activation molecule (*SLAM*) (512), and kallikrein gene variants (513). Deletion of Stat6, a transcription factor that mediates responses to type 2 cytokines such as IL-4, reduces the development of glomerulosclerosis in NZM2328 mice but has no effect on anti-dsDNA levels or renal IgG deposition, whereas germ-line deletion of Stat4 (a transcription factor for type 1 cytokines) suppresses anti-dsDNA IgG production but does not reduce the incidence of nephritis (514).

### $SNF_1$ Model

The (NZB × SWR) $F_1$ hybrid (also known as $SNF_1$) mouse differs from the NZB × NZW hybrid in that one parent, the SWR, is entirely healthy and without evidence of autoimmune serologic abnormalities. This hybrid is an excellent model of lupus nephritis, with a 50% female mortality from uremia by 6 months of age (515). The glomerulonephritis resembles that of the NZB/W morphologically but is distinguished immunologically by the oligoclonality of the IgG anti-dsDNA antibodies deposited in the kidney, which consist predominantly of IgG2b cationic subclasses with restricted idiotypes (516,517). It has been postulated that the cationic charge of these antibodies facilitates binding to the polyanionic glomerular capillary wall (518,519) to initiate nephritis. As in NZB/W mice, B-cell hyperactivity and anti-dsDNA antibody synthesis require T-cell participation from classic helper subsets ($CD4^+CD8^-$) bearing the ($\alpha\beta$) TCR or $CD4^-CD8^-$ ($\gamma\delta$)–positive cells.

### BXSB/MpJ Model

The BXSB/MpJ mouse is an inbred strain that is distinguished by more severe expression in males (520). Fifty percent of affected males die of renal failure by 5 months with severe proliferative glomerulonephritis; females develop a milder form of nephritis with a later onset and 50% mortality rate at 15 months. Castration of males or administration of androgen to females

had little effect on the renal phenotype, indicating that the gender difference was not caused by sex hormones. Instead, this factor (originally termed *Yaa*, for Y-linked autoimmune acceleration) is largely related to duplication of the *Tlr7* gene due to translocation of the telomeric end of the X chromosome to the Y chromosome (521). Following exposure to single-stranded (ss) RNA, the additional TLR7 copy causes release of proinflammatory IFN-α from plasmacytoid dendritic cells and enhanced antibody production by autoreactive B cells. Crossbreeding of BXSB with other lupus-prone mice demonstrates that the accelerated development of SLE in male mice is related to the *Yaa* factor. A recent candidate gene study in Japanese and Chinese subjects with SLE found an association with a polymorphism of the human ortholog, *TLR7*, located on the X chromosome, with a stronger effect in males than females (522).

### MRL/lpr Model

The MRL/lpr strain is autosomal recessive for a spontaneous mutation in the *fas* gene (523), which causes a distinctive phenotype of massive lymphadenopathy, necrotizing arteritis, proliferative glomerulonephritis, destructive inflammatory polyarthritis, and an autoantibody repertoire that includes anti-Sm, rheumatoid factors (RFs), anti-dsDNA, anti-ssDNA, and anti-gp70. The glomerulonephritis is characterized by subacute endothelial and mesangial proliferation with focal crescents and IgG and C3 deposits in capillary walls. The immunoglobulins deposited in kidney are largely IgG2a anti-DNA, with less consistent deposits of anti-RNA polymerase 1 and anti-gp70. Deposits of immunoglobulin and C3 are less consistently detected in the intima and media of arteries, arterioles, and venules. The necrotizing arteritis has a particular predilection for coronary and renal beds (496) with the development of myocardial infarction.

The Fas molecule, also designated CD95, is a member of the tumor necrosis factor (TNF) receptor family that binds Fas ligand (FasL) to mediate apoptosis. In the lpr mouse, the defective Fas receptor product is caused by insertion of a retroviral sequence into an intron of the *fas* gene, leading to reduced expression of Fas on the cell surface. The loss of Fas-FasL interactions prevents normal deletion of autoreactive T-cell clones by apoptosis. Mutations in the human ortholog (*FAS*) cause autoimmune lymphoproliferative syndrome (Evans syndrome), an autosomal dominant disease characterized by failure to eliminate activated lymphocytes following exposure to self-antigens or foreign antigens and characterized by lymphadenopathy, hepatosplenomegaly, and autoimmune cytopenias, but not SLE.

### Chronic Graft Versus Host Disease Model

cGVHD disease is produced by injecting parental lymphocytes into an $F_1$ hybrid that is discordant for MHC at one locus. Injections shortly after birth result in a fatal runting disorder. If injections are delayed until 6 weeks of age, and the animal does not succumb to acute GVHD, a chronic condition resembling SLE results, with greater severity in females. Autoantibodies include anti-dsDNA, anti-ssDNA, and anti-histone, with variable occurrence of glomerulonephritis in mice producing IgG anti-DNA antibody. Essential effector cells in this model are donor $CD4^+$ T cells that recognize a foreign MHC antigen on recipient cells. Some parental offspring hybrids produce anti-DNA antibodies in high titer but will not develop nephritis in the absence of a susceptible haplotype in the MHC class II genes 1-A and 1-E (corresponding to DQ and DR in humans). Animals without nephritis have mesangial IgG deposits only, whereas those with clinical nephritis also have capillary wall deposits.

### Susceptibility Genes Identified in Mouse Models

The genetic basis of murine models of spontaneous lupus has been studied intensively via the generation of congenic mice by backcrosses and intercrosses. Over 100 susceptibility loci have been identified in New Zealand mice on chromosomes 1, 3, 4, 7, 12, 13, and 17, and the corresponding susceptibility genes in human disease have been identified for some of these loci (494). *Fcgr2b* is the ortholog of FCGR2B in human SLE and is involved in immune complex clearance. *Slamf6* (*Ly108*), located in the *sle1b* susceptibility focus on chromosome 1, has been directly implicated in B-cell tolerance (524) and corresponds to the signaling lymphocyte activation molecule family (*SLAMF*) gene in humans. Variants of *SLAMF3* and *SLAMF4* have been identified in families with SLE and are associated with an increased number of $CD8^+$ memory T cells and decreased proportion of $CD4^+$ naive T cells and activated T cells (512). Kallikrein genes are underexpressed in NZW compared to B6 mice and have been associated with spontaneous lupus nephritis in a mouse model (513). An association between polymorphisms in *KLK1* and the *KLK3* promoter in humans with SLE or lupus nephritis has been reported (513). Mutations in *Coro1A*, the gene for a filamentous actin regulator protein Coronin-1A, leads to altered T-cell activation and protects MRL-*lpr* mice from disease (525). Its role in human disease remains to be determined.

Other experimental approaches involve single-gene manipulations that lead to lupus-like disease through gene knockouts or transgenic models. Such gene targeted approaches have been directed to products that cause defective apoptosis (such as Fas, FasL, and Bcl-2), defective clearance of apoptotic cells or immune complexes (such as serum amyloid P component, DNAse, secreted IgM, and C1q, and C4), abnormal maturation and persistence of autoreactive B cells (such as BAFF (526)), dysregulated lymphocyte activation caused by receptors and their ligands (such as FcγRIIB, CD22, and TGF-βRII) or intracellular signaling molecules (such as SHP-1 and PKC-d), cytokine production abnormalities (such as TGF-β, IL-10, IFN-γ, and IL-4), and defective hormone signaling (such as estrogen receptor [ER]α) (527–530). Evidence suggests that even these single-gene lupus models are genetically complex, requiring the interaction of other strain-specific background loci for the development of the lupus phenotype.

Studies with transgenic or knockout mice have identified genes that were subsequently identified in human SLE (e.g., Fc gamma receptor 2B [*FCGR2B*] (510) and interleukin-10 [*IL-10*] (531)). Conversely, orthologs of many of the genetic loci identified in human SLE have roles in immune function in murine models (494).

## Genetic Factors

The importance of genetic factors in human SLE is supported by several lines of evidence including epidemiologic data, rare mendelian risk factors with high disease penetrance, and findings from linkage and association studies, which together have identified more than 40 distinct genetic associations with SLE (531–543) (Table 14.11). As with other complex polygenic

**TABLE 14.11 Human genes associated with SLE**

| Pathway[a] | Chromosomal location | Gene | Gene product | Genetic variation | Odds ratio for SLE | Function | Reference |
|---|---|---|---|---|---|---|---|
| Innate immunity | 7q32.1 | *IRF5* | Interferon regulatory factor 5 | SNP | 1.88 | Interferon regulation | (532,535,543,1076) |
| | 11p15.5 | *IRF7* | Interferon regulatory factor 7 | SNP | 0.78 | Interferon regulation | (535,543) |
| | 16q24.1 | *IRF8* | Interferon regulatory factor 8 | SNP | 1.17 | B-cell differentiation | (542) |
| | 2q24 | *IFIH1* | Interferon-induced helicase C | SNP | 1.23 | Interferon α production | (542) |
| | 19p13.2 | *TYK2* | Tyrosine kinase 2 | SNP | 1.16 | Interferon response genes | (542) |
| | 6q23.3 | *TNFAIP3* | TNF-α–induced protein 13 | SNP | 1.48 | TNFR and NF-κB signaling; ubiquitination | (543,1076) |
| | 2q32.2 | *STAT4* | Signal transducer and activator of transcription protein 4 | SNP | 1.5 | Transcriptional regulator of IFN-γ signaling; apoptosis | (1076) |
| | 4q13 | *SPP1* | Osteopontin | SNP | 1.3 | IFN-α production | (1077) |
| | Xq28 | *IRAK1* | Interleukin receptor–associated kinase | SNP | 1.29 | TLR, IL-1 signaling | (1076) |
| | 9 | *DNAJA1* | DNA J homolog subfamily-1 | SNP | 0.96 | Interferon signaling | (541) |
| | 3 | *KPNA1* | Karyopherin-α1 | SNP | 1.22 | Interferon regulation | (541) |
| | 7 | *PTN* | Pleiotrophin | SNP | 2.02 | Induces inflammatory cytokines | (541) |
| Adaptive immunity | 6p21.32 | *HLA-DR2* | MHC class II cell surface receptor | Haplotype | 1.98 | Antigen presentation | (1082) |
| | 6p21.32 | *HLA-DR3* | MHC class II cell surface receptor | Haplotype | 1.76 | Antigen presentation | (1082) |
| | 1q13.2 | *PTPN22* | Protein tyrosine phosphatase nonreceptor 22 | R620W | 1.35 | B-cell receptor signaling | (1076) |
| | 2q27.3 | *PDCD1* | Programmed cell death protein 1 | PD1.3A; PD1.5C | 1.2 | T-cell signaling | (1078) |
| | 1q25.1 | *TNFSF4 (OX40L)* | TNF superfamily 4 | SNP | 1.46 | T-cell signaling | (531) |
| | 8p23.1 | *BLK* | B lymphoid tyrosine kinase | SNP | 1.22 | B-cell activation | (543) |
| | 4q24 | *BANK1* | Brassica napus kinase 1 | R61H | 1.4 | B-cell activation and signaling | (543) |
| | 11q23.3 | *ETS1* | Protein C-ets-1 | SNP | 1.37 | Lymphocyte development and activation | (540) |
| | 7p12 | *IKZF1* | Ikaros family zinc finger-1 | SNP | 1.23 | Altered Th1/Th2 equilibrium | (542) |
| | 1 | *IL-10* | Interleukin-10 | SNP | 1.19 | Cytokine | (531) |
| | 1q25 | *NCF2* | Neutrophil cytosolic factor 2 | SNP | 1.18 | Increases B-cell differentiation | (542) |
| | 11 | *CD44* | Cell surface glycoprotein | SNP | 0.72 | Lymphocyte activation and homing | (541) |
| | 5q23 | *PPP2CA* | Protein phosphatase 2 catalytic subunit alpha | SNP | 1.3 | Decreases IL-2 production | (572) |
| | 6q21 | *PRDM1* | Positive regulatory domain-1 element | SNP | 1.19 | Represses IFN-β gene expression | (531) |

(*Continued*)

**TABLE 14.11 Human genes associated with SLE (*Continued*)**

| Pathway[a] | Chromosomal location | Gene | Gene product | Genetic variation | Odds ratio for SLE | Function | Reference |
|---|---|---|---|---|---|---|---|
| Immune complex clearance | 1q23.3 | *FCGR2A* | IgG fixed chain receptor 2A | H131R | 1.35 | Phagocytosis | (560) |
| | 1q23.3 | *FCGR2B* | IgG fixed chain receptor 2B | I232T | 2.21 | Phagocytosis | (1079) |
| | 1q23.3 | *FCGR3A* | IgG fixed chain receptor 3A | F176V | 1.6 | Phagocytosis | (1080) |
| | 1q21-23 | *CRP* | C reactive protein | SNP | 1.3 | Phagocytosis | (566) |
| | 16p11.2 | *ITGAM* | Integrin-α M | R77H | 1.62 | Cell adhesion; iC3b receptor | (535) |
| | 1p36.11 | *C1q* | Complement 1Q | Deletion | 10 | Complement | (587) |
| | 6p21.3 | *C4* | Complement 4 | CNV | 6.5 | Complement | (582) |
| | 6p21.3 | *C2* | Complement 2 | Deletion | 5 | Complement | (584) |
| Others: | 22q11.21 | *UBE2L3* | Ubiquitin-conjugating enzyme 2L3 | SNP | 0.78 | Ubiquitination | (535) |
| | Xq28 | *MECP2* | Methyl CpG–binding protein 2 | SNP | 1.2 | DNA methylation | (539) |
| | 5q32-q33.1 | *TNIP1* | TNFAIP-interacting protein 1 | SNP | 1.27 | Unknown | (531) |
| | 7p15 | *JAZF1* | Juxtaposed with another zinc finger-1 | SNP | 1.2 | Transcriptional regulation | (531) |
| | 6q23 | *UHRF1BP1* | Ubiquitin like with PHD and ring finger domains-1–binding protein | R454Q | 1.17 | DNA methylation | (531) |
| | 3p21.31 | *TREX1* | 3′ repair exonuclease 1 | SNP; haplotype | 1.73 | DNA repair | (578,579) |
| | 16p13.3 | *DNASE1* | Deoxyribonuclease 1 | SNP; deletion; nonsense mutations | 1.66 | DNA clearance | (580,1081) |

[a]Some of these genes are involved in more than one pathway; CNV, copy number variant; CpG, cytosine-phosphate-guanine; MHC, major histocompatibility complex; SNP, single-nucleotide polymorphism; TLR, toll-like receptor.

diseases, genetic predisposition to SLE involves the interaction of multiple susceptibility or protective traits, each of which has a small contributory effect (typical odds ratio 1.5).

### Family Studies

The relative risk of SLE occurring in siblings of an affected individual is approximately 8- to 20-fold that of the general population (544), and the concordance of SLE is 10-fold higher in monozygotic twins (40%) than in dizygotic twins or other siblings (2% to 5%). The incidence of SLE and other autoimmune disease is also higher in first- and second-degree family members of patients compared to controls (10% to 12% vs. less than 1%) (544).

### Gender

The higher prevalence of SLE in women (2- to 12-fold) likely reflects X chromosomal factors and exposure to sex hormones (see Role of Sex Hormones below). At least four SLE-associated genes (*IRAK1* (538), *MECP2* (539), *TLR7*, and *TNFS5*, encoding CD40 ligand (545)) are present on the X chromosome, suggesting a possible gene dosage effect (546). In addition, the prevalence of SLE in men with Klinefelter syndrome who carry an extra X chromosome is 14-fold higher compared to XY men (546). The female predominance of SLE may also reflect epigenetic modification of SLE-related genes on the X chromosome (as has been demonstrated for CD40 ligand (545)) and microchimerism due to exposure to fetal stem cells during pregnancy, all of which may predispose to the development of autoimmunity. Although the incidence and prevalence of SLE and lupus nephritis are higher in females (547), males may develop more severe renal disease (548,549). Given that males and females develop similar disease manifestations (albeit with different frequencies of some characteristics (549)), it appears that sex factors in lupus represent a threshold effect that permits emergence of disease, rather than being directly causative.

### Race

The incidence and prevalence of SLE are higher in nonwhite populations compared to whites living in the same geographic area, supporting a role for genetic factors (547,550). The incidence of SLE ranges from 0.4 to 38.6 cases per 100,000 per year (in white American men (550) and African American women (547), respectively), while the prevalence ranges from 20 to 286.4 cases per 100,000 (547,551–554). In a study from Birmingham, United Kingdom, Johnson et al. (555) reported a sevenfold higher prevalence in Afro-Caribbean women compared to the general population. Similarly, in a study of Medicaid enrollees in the United States, African American race was associated with a twofold increase in prevalence and 1.74-fold increase in the incidence of SLE (547). SLE appears relatively rare among blacks in Western Africa but may be more common in individuals from Central or Southern Africa (556). A high prevalence of SLE has also been reported in Eastern Asians (67.4 cases per 100,000 persons in Taiwan) (557). In Hawaii, the incidence of SLE in whites was 5.8 cases per 100,000 whites compared with 17.0 cases per 100,000 Asians (558). Genes that have been linked to SLE in African American patients include those responsible for metabolism of reactive intermediates (e.g., glutathione reductase, NADH dehydrogenase, and nitric oxide synthase (559)) and for FcγR (560). In addition, genome-wide association studies (GWAS) have identified different risk allele profiles in different populations, supporting a role for genetic heterogeneity in the differences in racial prevalence of SLE.

The incidence and severity of lupus nephritis are also greater in non-Caucasian populations, including subjects of African and Asian descent. Genetic risk factors for lupus nephritis include HLA-DRB1*15 and DQA1*01 (561), *STAT4* polymorphisms (in European (562,563) but not in Japanese (564) or Chinese subjects (565)), the *CRP-A* allele (in African Americans and Caucasians) (566,567), *FCGRIIIA* (in Asians (568) and Europeans (567)), *FCGRIIA* (in African Americans (560), Europeans (569), and Brazilians (570)), *IRF7/KIAA1542* (in Chinese subjects (565)), *ITGAM* (in Europeans) (571), *PPP2CA* (in Asians, European Americans, and Hispanic Americans, but not in African Americans) (572), and nonmuscle myosin heavy chain-9 (MYH9) in European Americans but not in African Americans, Asians, Amerindians, or Hispanics (573). Of note, no association with nephropathic apolipoprotein L1 (apoL1) variants was identified in one study that included 407 African Americans (573).

### Evidence From Linkage and Association Studies

Beginning in the 1970s, serologic testing identified an association between SLE and HLA DR2 in all populations and HLA DR3 in Caucasian subjects. Subsequent DNA sequencing studies confirmed that the risk alleles in these regions were DRB1*1501 and DRB1*0301, respectively. DRB*1501 and DQA1*0101 may predispose to lupus nephritis, whereas DQA1*0102 suppresses the nephritogenic effect of DRB1*1501 (561). HLA alleles have consistently shown the strongest association with SLE in GWAS. There is also evidence that genes in the MHC class III region, such as *MSH5* (mutS homologue 5) and *SKIV2L* (535) may predispose to SLE, suggesting that some of the observed associations with DR and DQ might reflect linkage disequilibrium (535).

Since 2008, several GWAS have been reported in patients with SLE (494,532), with identification of more than 40 different genes and susceptibility loci (see Table 14.11) (533–537). Importantly, the frequency of these risk alleles varies between different ethnic populations, some are protective rather than causative, some genes have more than one risk variant, and gene-gene interactions have been described (574). In addition to risk alleles, genetic risk may reflect copy number variation (e.g., for FcγR IIIB and C4), epigenetic modification of gene expression (e.g., in response to environmental factors and genetic modifiers) and altered microRNA levels (575,576). In addition, several of these loci have also been identified in other autoimmune diseases, implying a common immunogenetic pathway. Importantly, the genes identified thus far represent only a fraction (less than 20%) of the total genetic contribution to SLE, indicating that other genetic factors remain to be discovered.

Most of the risk alleles associated with SLE have roles in IFN-α signaling and innate immunity, B and T lymphocyte function, and clearance of immune complexes, consistent with the central pathogenetic role of immune dysfunction in SLE (532). The most consistent risk loci include HLA-DR, interferon regulatory factor (IRF)-5, signal transducer and activator of transcription 4 (STAT4), B lymphoid tyrosine kinase (BLK), and integrin-α M (ITGAM). STAT4 is necessary for interleukin-12 signal transduction and causes increased IFN-γ secretion in natural killer cells and increased Th1 activation. IRFs regulate IFN expression in response to TLR engagement.

Activated IRF5 promotes transcription of IFN-α and IFN-responsive genes, and IRF5 levels are in turn induced by IFN-α, suggesting a possible feedback loop whereby SLE-associated *IRF5* polymorphisms might contribute to the up-regulation of IFN-related genes ("IFN signature") seen in patients with active SLE disease (577). BLK is a member of the src family of tyrosine kinases that transduce signals downstream of the B-cell receptor. The *BLK* risk allele is associated with reduced expression of *BLK* mRNA in transformed B-cell lines (536), which might promote altered tolerance (536). ITGAM, also known as complement receptor type 3, is involved in immune complex clearance and leukocyte activation, adhesion and migration (535,536). Further characterization of genes whose function is as yet unknown may generate novel insights into the pathogenesis of SLE.

### Deficiencies With Mendelian Inheritance in SLE

Mendelian risk factors are rare in SLE but have a high degree of penetrance. These include homozygous deficiencies of early complement pathway components and genes involved in regulation of genomic DNA catabolism (e.g., 3′ repair exonuclease 1 [*TREX1*] (578,579)) and deoxyribonuclease 1 [*DNASE1*] (580)). Inherited deficiencies of complement components in SLE most commonly involve C2 and C4 and may be homozygous or heterozygous. C4A null allele is the most common inherited complement deficiency identified in SLE patients and is present across broad ethnic and racial lines (581). In SLE, 86% of patients have one C4A or C4B null allele, compared with 42% of normal individuals, while 14% of SLE patients have both C4A and C4B null alleles, compared with only 1% of normal subjects (582). Antibodies to Ro are more common in SLE patients with genetic deficiencies of C4 (583). Inherited C2 deficiency in SLE is typically associated with mild disease, primarily confined to skin and joints and sparing the kidney (584). Deficiencies of C1 and CR1 (i.e., erythrocyte receptor for C3b), C3, C5, C6, C7, C8, and C9 have also been identified in a small number of SLE patients, but some of these associations may be coincidental (585). Because complement is encoded by genes on chromosome 6 between HLA-B and HLA-DR, these inherited complement deficiencies may arise through linkage disequilibrium with HLA genes, such as DR3 and DR2. Complement deficiencies are implicated in the pathogenesis of SLE through their regulatory effects on B-cell function, cytokine function, and on scavenging of necrotic and apoptotic cellular debris (586).

Although homozygous C1q deficiency is very rare, with only 40 cases reported, over 90% of patients develop SLE (587). Parenthetically, C1q-deficient mice with restricted genetic backgrounds have been reported to develop spontaneous glomerulonephritis with glomerular immune deposits, resembling lupus nephritis (588,589). A critical role for C1q in clearance of apoptotic cells has been proposed as a possible predisposing factor (588). In the setting of homozygous deficiency of early classical complement pathway components, an accumulation of autoantigen-containing apoptotic cell debris due to defective complement-mediated clearance might lead to inappropriate presentation of these autoantigens to T cells, leading to the development of autoimmunity. Immature dendritic cells produce abundant C1q and are tolerogenic, whereas mature dendritic cells produce less C1q and are immunogenic. Thus, a genetic deficiency in C1q production might predispose to autoimmunity. In addition, complement has an important role in setting the threshold of B- and T-cell reactivity, and the absence of regulatory signals provided by activated complement fragments to B and T lymphocytes might permit self-reactive B cells to escape from negative selection, resulting in the loss of tolerance to self-antigens.

Mutations in *TREX1* were identified in 9 of 417 individuals with SLE and 0 of 1712 controls in one study (578) and in 0.5% of 8,370 patients with SLE in another study (579). Similarly, mutations in *DNASE1* are uncommon (2 of 20 patients in one study) (580) but are highly associated with SLE.

### Findings From Transcriptomal Studies

The application of laser capture microdissection and microarray analysis to human renal biopsies of lupus nephritis has allowed the study of transcriptional profiles at the level of the glomerulus, independent of confounding factors in other renal compartments. We found considerable heterogeneity of gene expression in glomeruli from cases of class IV lupus nephritis (590). Four main gene clusters were identified, linked to B cells, myelomonocytic cell lineage, fibroblast proliferation and extracellular matrix production, and expression of type I IFN-inducible genes. Not surprisingly, the fibrosis-related genes correlated with pathologic evidence of glomerulosclerosis. Interestingly, the expression of type I IFN-inducible transcripts identified biopsies with milder pathologic features. In a murine model of lupus nephritis (12-week-old MRL/lpr mice) nephritis, 567 genes were up-regulated in MRL/lpr glomeruli compared to control congenic mice (591). These genes included complement components, adhesion molecules, chemokines and their receptors, and molecules related to antigen presentation. Over 130 genes were preferentially or exclusively expressed in hematopoietic cell lineages, possibly reflecting leukocyte accumulation, and these changes were accompanied by increased mRNA expression of IFN-γ–inducible genes (591). More widespread use of this technology may help to identify clinically relevant subsets of nephritis.

## Role of Exogenous Factors

The importance of exogenous factors in SLE is supported by the concordance rate of less than 50% in identical twins. Factors that have been implicated in initiating and exacerbating SLE include sex hormone, viruses, ultraviolet (UV) radiation, cigarette smoking, chemicals, and drugs. Patients with SLE have increased circulating Epstein-Barr virus (EBV) viral loads compared to healthy controls and higher titers of antibodies to EBV (592). Anti-EBV antibodies have been shown to precede the development of anti-Ro antibodies in patients with SLE (593). EBV infection may be a triggering event in pediatric SLE (594). Endogenous retroviruses have also been postulated to trigger lupus via structural and functional molecular mimicry, with formation of antiretroviral antibodies that cross-react with nuclear antigens. UV radiation inhibits DNA methylation in $CD4^+$ T cells, potentially causing autoreactivity via up-regulation of lymphocyte function–associated antigen 1 (LFA-1) (595). UV radiation also induces apoptosis of keratinocytes and release of cytokines that promote formation of lupus autoantibodies. Exposure to silica dust has been linked to development of SLE, especially in African American women (596,597). A possible role for cigarette smoking was suggested by a meta-analysis of nine studies (598), but this association

was not detected in two prospective studies (599,600). There is no apparent association with exposure to hair dyes, occupational solvents, or pesticides.

Importantly, environmental agents may contribute to lupus pathogenesis through epigenetic mechanisms that regulate gene expression, such as histone acetylation and DNA methylation, as evidenced most strongly in cases of drug-induced SLE related to hydralazine and procainamide (discussed in Drug-Induced Lupus above).

## Role of Sex Hormones

SLE is more common in females at all ages (2- to 12-fold), but the peak incidence and greatest discrepancy between the sexes occurs in the reproductive years, strongly suggesting that exposure to female sex hormones is a risk factor. Onset or exacerbations of SLE during pregnancy, a period of peak hormonal activity, is common (601). Males with Klinefelter syndrome have a higher rate of SLE that may partly reflect abnormal metabolism of estrogen and excessive 16α-hydroxyesterone and estrol metabolites, resulting in a hyperestrogenic state. Males and females with SLE have abnormal androgen metabolism (602,603), with lower basal levels of testosterone and dehydroepiandrosterone (604,605), the levels of which correlate negatively with disease activity (606). The use of estrogen-containing contraceptives and administration of estrogen to postmenopausal women are both associated with an increased incidence of SLE. On the other hand, sex hormone levels in women with SLE are not increased compared to controls without autoimmune disease, arguing against a direct pathogenetic role (607). Men with SLE may have significantly higher frequency of autosomal risk alleles in the HLA region and in the interferon regulatory factor (*IRF*)-5 gene compared to women (608), indicating that gender differences in the prevalence of SLE may not be solely due to X chromosomal or hormonal factors.

The importance of hormonal effects is supported by murine models of lupus nephritis, in which castration of female NZB/W mice, administration of the estrogen receptor antagonist tamoxifen, or knockout of estrogen receptor -α result in decreased disease and prolonged survival (609). The mechanisms by which sex hormones may influence the immune system are complex and poorly understood. Effects of sex hormones on thymic development and involution, T-cell subsets, T-cell proliferative capacity, immunoglobulin synthesis, and macrophage activation have been proposed.

## Defective Apoptosis in SLE

The loss of tolerance to self-antigens and development of autoantibodies in SLE may reflect abnormal apoptosis. During apoptosis, the organized cleavage of chromatin leads to clustering of nucleosomes on the surface of apoptotic cells. Normally, these apoptotic bodies are efficiently cleared by scavenger cells without triggering an inflammatory reaction. In SLE, excessive and/or impaired apoptosis may promote the formation of nucleosome-specific T cells and autoantibodies (610). This defect may reflect genetic variants of factors involved in apoptosis or clearance pathways (e.g., Fas, C3, C4, or DNAse-1) (611). Impaired apoptosis in SLE leads to secondary necrosis of nonphagocytosed apoptotic cells, resulting in the release of nucleosomal apoptotic fragments containing mobility group box protein 1 (HMGB1). This nonhistone nuclear protein acts as a stabilizing factor inside the cell and as a proinflammatory cytokine when released from dying cells. Serum levels of HMGB1 and anti-HGMB1 antibodies are increased in patients with active lupus and lupus nephritis (612). HMGB1-bound nucleosomes may promote dendritic cell maturation and release of proinflammatory cytokines via their engagement with TLR-2 and receptors for advanced glycation end products (RAGE). It is possible that tissue injury in lupus nephritis provides a source of apoptotic bodies that promote ongoing autoimmune activation.

## Immunologic Abnormalities in SLE and Lupus Nephritis

SLE and lupus nephritis are characterized by a plethora of immunologic abnormalities that include autoantibody production, B-cell hyperactivity, defective T-cell regulation, abnormal dendritic cell function, aberrant cytokine synthesis, and activation of the complement and coagulation systems. Although the molecular and immunogenetic characteristics of these abnormalities have been progressively elucidated, it remains unclear which of these defects are primary and which are secondary, as these systems are intricately interconnected in health and in disease. Some of these abnormalities are likely genetically determined, as discussed above.

### Role of Autoantibodies in Lupus Nephritis

SLE is characterized by the production of a host of autoantibodies, some of which are involved in the pathogenesis of tissue injury (613–615). Many of these autoantibodies are directed to nuclear antigens (ANA) consisting of complexes of proteins and nucleic acids, suggesting that they are synthesized against large macromolecular particles that are processed and presented in concert by antigen-presenting cells. This would explain the existence of "linked sets" of autoantibodies that recognize structurally and spatially related epitopes of larger protein-nucleic acid complexes (616). Of note, autoantibody formation may precede clinical manifestations of SLE by several years.

Lupus nephritis is defined pathologically by the presence of glomerular immune complex deposits, and an abundance of clinical and experimental evidence suggests a central pathogenetic role for autoantibodies with specificity for dsDNA and nucleosomes. Most patients with lupus nephritis have circulating anti-dsDNA antibodies, and these are frequently enriched in the kidney. In experimental animals, injection of anti-DNA antibodies derived from mice with lupus (160) or patients with lupus nephritis (617) can induce the development of lupus nephritis. Immune complexes containing anti-dsDNA may activate the adaptive and innate immune pathways, via engagement of complement receptors and TLR, leading to inflammation and fibrosing injury. Autoantibodies may also have direct effects on intrinsic renal cells (618), inducing cell proliferation and release of proinflammatory cytokines, which contribute to the pathogenesis of lupus nephritis.

On the other hand, not all patients with high titers of circulating anti-dsDNA antibodies develop renal disease (619) and lupus nephritis occasionally occurs in the absence of circulating anti-dsDNA antibodies (620,621). The specific characteristics that make certain anti-dsDNA antibodies nephritogenic remain poorly understood. IgG autoantibody clusters uncovered by glomerular proteome arrays include DNA/chromatin and laminin/myosin/heparan sulfate/vimentin (622).

In a murine model, injection of antibodies that recognize dsDNA in the context of PolIV, a bacterial DNA polymerase, are more pathogenic, suggesting differences in the fine specificity of nephritogenic autoantibodies (617).

### Antibody Specificities in SLE and Lupus Nephritis

The production of ANA is a defining feature of SLE. Historically, the first clinically recognized manifestation of ANA (and the first diagnostic laboratory test for SLE) was the LE cell (10). This consists of neutrophil or monocyte that has phagocytosed a nucleus, producing a purplish inclusion. The in vivo equivalent of the LE body is a "hematoxylin body," sometimes seen in glomeruli and other tissue sites in patients with SLE (Fig. 14.12). Immunofluorescence assay for ANA, which is positive in approximately 98% of patients with SLE, is the major initial serologic screening test for lupus (623). Originally performed using indirect immunofluorescence on rodent liver or kidney, the preferred tissue substrates in the modern era are human cell lines, such as HEp-2 or KB tumor cells. Human cell lines yield a positive ANA test result for up to 98% of SLE patients, compared with 90% to 95% sensitivity using rodent tissues as substrate. Human cell lines have larger nuclei than rodent tissue, allowing greater resolution of the immunofluorescence pattern produced by binding to subcellular organelles, such as deoxyribonucleoprotein (DNP), histone, chromatin, and rarely to DNA. Positive ANA has been found in approximately 32% of putatively healthy subjects at 1:40 dilution but in only 3% at 1:320 dilution (624). In addition, low-titer positivity is common with a variety of nonlupus conditions, such as aging, autoimmune thyroiditis, chronic liver disease, chronic infections, or malignancy.

The pattern of fluorescence (i.e., homogeneous, rim [peripheral], speckled, or nucleolar) in the interphase nucleus gives some clues about the ANA specificity but needs to be more finely characterized by hemagglutination, ELISA, immunodiffusion, or *Crithidia luciliae* immunofluorescence studies for particular nuclear antigens. The peripheral or rim pattern is most specific for SLE and is most commonly observed with antibody to dsDNA and less commonly seen with antibodies directed to ssDNA, DNP, and histones. A speckled pattern can be observed with antibody to U1-ribonucleoproteins (RNP), Smith (Sm), Ro/SSa, La/SSB, and Ku antigens. This speckled pattern is also common in systemic sclerosis and is produced by anti-scl-70 antibody that has specificity for topoisomerase. Anti-Ro/SSa often gives unreliable ANA staining, thereby requiring more sensitive immunodiffusion studies for its detection in the face of a negative ANA screening test result.

Antibodies to dsDNA are the most specific for SLE and are detectable in 50% to 70% of patients. Anti-dsDNA antibodies correlate highly with disease activity in many patients and may precede the onset of clinical disease by several years (625). Indirect immunofluorescence, using the kinetoplast of the hemoflagellate *C. luciliae*, and the Farr radioimmunoassay are both highly specific for anti-dsDNA but have been largely replaced in the modern era by enzyme-linked immunoassays (ELISA) and immunoblotting. High titers of anti-dsDNA antibody have more than 90% specificity for SLE. Rising titers, often associated with falling serum complements, are helpful clues to the occurrence of disease flares. Anti-ssDNA antibodies are present in approximately 90% of SLE patients and are increased during flares; however, they are also found in a host of other rheumatologic diseases and are thus nonspecific.

Autoantibodies to the two major components of nucleosomes—histones and DNP—are present in approximately 70% of SLE patients and typically give a homogeneous ANA pattern. Antihistone antibodies are even more prevalent (70% to 100%) in DIL. Antibodies are directed to all classes of histones, particularly H1 and H2B in primary SLE and H2A-H2B or H3-H4 in DIL. Specificity for SLE is poor, as antihistone antibodies have been detected in up to 80% of patients with RA or juvenile rheumatoid arthritis. It has been suggested that release of nucleosomes in the course of sun exposure, neutrophil apoptosis, or other forms of cell injury may provide an important source of antigen that stimulates antinucleosomal antibody production.

Antibodies to RNP are detected in up to 30% of SLE patients, and one of these, anti-Sm, named after a prototype serum identified by Tan and Kunkel in 1966 (626), is a particularly useful diagnostic marker (627). These antibodies are predominantly reactive with uridine-rich small nuclear ribonucleoproteins (UsnRNP) (628), comprised of complexes of RNA and protein. The most abundant of these are the U1, U2, U4/U6, and U5 RNP, which all share associations with other polypeptides to form larger RNA-protein complexes known as Sm core proteins (629). These are distinct from the intranucleolar RNP of the U3, U8, and U13 series.

The Sm core proteins bind U RNA at a distinct highly conserved binding site known as the Smith-binding site. The Sm snRNP are assembled in the cytoplasm and then enter the nucleus, where they regulate pre-mRNA splicing. Anti-Sm antibodies are detected in 20% to 76% of SLE sera (630,631). Eighty-three percent of patients with anti-Sm antibody have SLE (631), indicating a high specificity for SLE. The presence of anti-Sm has been included in the revised ACR criteria for the diagnosis of SLE (273). In SLE, antibody to U1-RNP rarely occurs alone but is almost always found in association with anti-Sm antibodies (reactive with U2, U4, U5, and U6). Anti-U1-RNP antibodies without anti-Sm are most commonly found in patients with mixed connective tissue disease (MCTD) (632,633), typically in high titers. Anti-U1-RNP have also been described in some patients with scleroderma, Sjögren disease, polymyositis, and RA. Antinucleosomal antibodies have equal specificity (94.9% vs. 94.2%) and higher sensitivity (59.9% vs. 52.4%) compared to anti-dsDNA antibodies for the diagnosis of SLE, but these do not appear to correlate with lupus nephritis (634).

First described by Anderson et al. (635) in Sjögren syndrome (SS) as antibodies to SS A and B antigens, antibodies to Ro (SSA) and La (SSB) are identified in 60% and 24% of SLE patients, respectively (636). These antibodies are helpful diagnostic markers of SLE in patients who are ANA negative by indirect immunofluorescence (637). The 46k-d Ro/SSA autoantigen has homology with calreticulin, a high-affinity, calcium-binding protein located in the endoplasmic reticulum (638). The 48-kd La/SSB polypeptide has some sequence homology with RNAs from EBV, adenovirus, and vesicular stomatitis virus, suggesting a source for molecular mimicry (639). All patients with anti-La also have anti-Ro positivity, suggesting that these are linked autoantibodies (similar to anti-U1-RNP and anti-Sm).

Clinical associations of anti-Ro (SSA) include photosensitive skin rash, interstitial pneumonitis, and thrombocytopenia. Anti-Ro is common in SLE patients with early complement

deficiencies, particularly C2. Anti-La has been associated with the absence of nephritis, although the biologic basis for this protective role is not understood (640). The presence of both antibodies may precede the diagnosis of SLE and has been associated with the development of congenital heart block and dermatitis in infants with congenital lupus caused by transplacental transfer of maternal antibodies.

Serum anti-C1q autoantibodies are found in up to one third of patients with SLE and in two thirds of those with lupus nephritis (641,642). Increased titers of C1q antibody correlate with periods of clinical renal involvement and may predict clinical renal flares (641–646). These antibodies bind epitopes within the collagen region and the globular domain of C1q, inhibiting its binding to CRP and FcγR and leading to impaired immune complex clearance (647). These autoantibodies have been shown to augment renal immune deposits by binding to C1q in situ (648). A meta-analysis has shown that measurement of anti-C1q antibodies is sensitive and specific for the diagnosis of lupus nephritis (58% and 75%, respectively) and for distinguishing active lupus nephritis from inactive disease (75% vs. 77%, respectively) (644). The observation that both congenital C1q deficiency and acquired anti-C1q antibodies that inhibit C1q function are associated with lupus nephritis is intriguing.

Some of the autoantibodies detected in SLE patients have RF or cryoglobulin activity. IgM RFs are detectable in 20% to 60% of SLE patients, mostly at low to modest titers (271). In a study by Miyazaki, RFs of IgM, IgA, and IgG classes could be detected in the sera of five patients with active diffuse proliferative lupus nephritis but were undetectable in membranous lupus nephritis (649). Moreover, fluoresceinated normal human IgG and Fc fragments but not F(ab′)2 fragments bound to the glomerular deposits of patients with diffuse proliferative but not membranous lupus nephritis, suggesting that RF activity might promote accretion of glomerular immune deposits.

Mixed cryoglobulinemia (polyclonal IgM and IgG [type III] or monoclonal IgM and polyclonal IgG [type II]) occur in patients with SLE. In one study of 122 patients with SLE, cryoglobulins were detected in 25% of patients but less frequently (9%) in titers greater than 5%; the latter cases all had type II cryoglobulins. Cryoglobulinemia was significantly associated with RF, hypocomplementemia, hepatitis C virus infection (for subjects with cryocrit greater than 1%), and cutaneous vasculitis (650). Antibodies with cryoglobulin or RF activity may influence the pattern of murine or human lupus nephritis (651,652), due to trapping in the subendothelial region because of their size or physical characteristics or binding to previously deposited immunoglobulin.

Antiendothelial antibodies are detected in up to 80% of patients with SLE (653–656). Administration of antiendothelial antibodies to normal mice induces a glomerulonephritis that resembles lupus nephritis (657). Perry reported a correlation between antiendothelial antibody levels and severity of lupus nephritis (658). One potential endothelial cell autoantigen identified by molecular cloning strategy is ribosomal P protein P0 (659). In vitro studies suggest that some antiendothelial antibodies may be internalized into glomerular endothelial cells by interaction with surface integrins via fibronectin (660). Injection of these antibodies in vivo generates electron-dense deposits in the glomerular endothelial cytoplasm and subendothelial regions of SCID mice and produces large wire-loop deposits in MRL/gld lupus-prone mice (660).

ANCA have been associated with spontaneous and drug-induced SLE (661–665). The incidence of ANCA in SLE ranges from 21% to 39% (661,663,665). ANCA specificities by ELISA include myeloperoxidase, lactoferrin, and elastase. ANCA may be pathogenetically related to a subgroup of lupus nephritis with necrotizing and crescentic features and sparse immune deposits (342).

A host of other autoantibodies occur in SLE patients, some of which are associated with extrarenal disease manifestations and some of which may indirectly promote lupus nephritis. Up to 65% of SLE patients have antierythrocyte antibodies, as indicated by a positive direct Coombs test result (666), although most of these have no clinical evidence of hemolysis. The subset of patients with immune hemolytic anemia primarily manifests warm-reactive IgG antibodies to erythrocytes. Antierythrocyte antibodies of the IgG1 and IgG3 subclass are most deleterious, due to their avidity for macrophage Fc receptors. Antiplatelet antibodies may give rise to a clinical syndrome of immune thrombocytopenic purpura. At least some of these pathogenic antibodies appear to have specificity for the GP IIb-IIIa glycoprotein receptor on the surface of platelets (667). Platelet-associated immunoglobulin is probably heterogeneous in nature, representing a combination of specific antiplatelet antibody, APL antibody, and bound immune complexes. Antineutrophil antibodies do not correlate well with the development of neutropenia. In contrast, antilymphocyte antibody titers correlate with lymphopenia (668). Antilymphocyte antibodies could alter T-cell function through receptor blockade or receptor modulation that stimulates or suppresses cell function (669).

### Immunoglobulin Characteristics in Lupus Nephritis

The specific characteristics that render some anti-dsDNA antibodies nephritogenic are not well understood, but there is evidence from experimental and human studies that anti-dsDNA antibodies in the kidney are qualitatively different from circulating anti-dsDNA antibodies, in terms of heavy chain restriction, oligoclonality, and avidity, supporting a direct pathogenetic role.

Antibodies eluted from the kidney in cases of lupus nephritis demonstrate isotype and subclass restriction and increased avidity compared to circulating autoantibodies. In murine and human lupus nephritis, the predominant immunoglobulin isotype in glomerular deposits is IgG, although other immunoglobulin classes (IgM, IgA, and IgE) frequently codeposit. In humans, the IgG subclasses most commonly identified in proliferative lupus nephritis are IgG1, IgG2, and, IgG3, which comprise the most effective complement-fixing subclasses (670–675). In a study of renal biopsy samples of 80 SLE patients, we have found that IgG1 (81%), IgG2 (89%), and IgG3 (96%) were the predominant subclasses in all classes of lupus nephritis (672). By contrast, IgG4 was detected in only 36% of samples. The frequency of genotypes containing the low-binding IgG2 allele, FcγRIIa-R131, was greater in patients with class III or class IV lupus nephritis and in patients with intense IgG2 deposition, suggesting that this genotype may predispose to disease pathogenesis by impaired removal of immune complexes (672). Iskandar et al. (676) also reported a predominance of IgG3 in patients with proliferative lupus nephritis. Imai confirmed that all IgG subclasses are deposited in the glomeruli of patients with lupus

nephritis, but noted stronger staining for IgG1 and IgG2 (673). Haas et al. reported a predominance of IgG4 in cases of primary membranous glomerulonephritis, whereas IgG1 and IgG3 were seen in cases of membranous lupus nephritis (673,677). There is only a poor correlation between IgG subclass profile in serum and the type or severity of nephritis (671,675,678,679). Overall, IgG1 and IgG3 tend to predominate in the serum of patients with severe nephritis. In humans, a rise in IgG2 antinucleohistone antibodies and IgG1 anti-dsDNA antibodies in the serum has been reported to herald the development of renal relapses (680). On the other hand, a significant rise in antinucleosomal antibodies of IgG3 subclass has been reported in lupus patients experiencing renal flares (681). In MRL/lpr mice, IgG3 appears to be the major subclass detected in kidney eluates, although IgG2a predominates in serum (682). This discrepancy may be related to the propensity for IgG3 to form RF or cryoglobulins that, because of their size, are more likely to become deposited in glomerular capillaries (683).

There are few human studies on the avidity of immune complexes eluted from glomeruli in lupus nephritis (684,685). Asano found the avidity to be higher in patients with subendothelial and mesangial deposits than in those with subepithelial deposits (684). Winfield (685) also found that antibody eluted from human lupus kidneys bound dsDNA more avidly than serum antibodies of the same specificity, but these findings were based on studies of only four patients. In contrast, the antibodies eluted from kidneys of NZB/W mice have included high- and low-avidity antibodies (686).

Studies in human disease and murine models (687) have revealed considerable heterogeneity of charge in lupus autoantibodies, without consistent isoelectric patterns. Monoclonal anti-DNA antibodies of various charges are capable of initiating nephritis when passively transferred to normal mice. Analysis of human anti-DNA autoantibodies has revealed highly cationic germ-line $V_\kappa$ genes that confer the cationic charge, whereas somatic mutations induce affinity maturation (688,689). The nephritogenic potential of lupus autoantibodies appears to reside in the variable region of the heavy chain ($V_H$) gene sequence, with silent and replacement substitutions in the $V_H$ germ-line gene complementarity-determining regions (CDR) that were not present in the patient's germ-line DNA (690). These findings support that B-cell clones undergo somatic mutations in response to exogenous stimuli, whose identity remains to be determined. In summary, these findings suggest that charge characteristics may play a role in the localization of immune deposits in lupus nephritis, but they are not the major determinant of susceptibility to nephritis.

## Mechanisms of Immune Complex Deposition in Lupus Nephritis

Three major mechanisms of immune deposition in the kidney have been proposed: (a) binding of autoantibodies to autoantigens that have been planted in the glomerulus; (b) binding of autoantibodies to intrinsic glomerular antigens; (c) deposition of preformed circulating immune complexes. These mechanisms are not mutually exclusive, and there is evidence for all three pathways in murine lupus nephritis. However, there is little if any evidence to date that deposition of preformed immune complexes plays a major pathogenetic role in human lupus nephritis (277).

### In Situ Immune Complex Formation in Lupus Nephritis

Autoantibodies may bind to nonglomerular autoantigens, such as nucleosomes, that become "planted" within the glomerulus through charge or other physical interactions. Necrosis of glomerular cells is a potential source for these antigens. Once planted in the glomerulus, these antigens bind with circulating autoantibodies to form immune complexes in situ. This hypothesis, initially proposed by Izui (691) in 1976, has been supported by data suggesting an affinity of DNA for collagen, fibronectin, and laminin (692–694), all components of the glomerular matrix, and the demonstration that DNA is concentrated in the glomerular electron-dense deposits of human lupus nephritis (695).

Low molecular weight DNA of less than 200 base pairs (bp) appears to display a particularly high affinity for GBM. The most nephritogenic size of DNA, 120 to 200 bp, which was observed by Fournie (696), falls within the predicted range of the DNA that is bound to histones within nucleosomes. Histones have long been considered an excellent candidate protein for planting of glomerular antigen, because they are highly cationic and have high affinity for anionic components of the glomerular capillary wall such as heparan sulfate (697–701). If histones are first injected through the renal artery, followed by DNA alone and subsequently anti-DNA antibody alone, a membranous pattern with subepithelial deposits is produced (694). By contrast, perfusion of DNA alone into the rat renal artery, followed by the administration of anti-DNA antibody, produced mesangial immune deposits in vivo (694). Stockl et al. (702) were able to demonstrate histone antigen in the glomeruli of 65% of the 48 cases of lupus nephritis but in only 2% of nonlupus controls. Histones have also been demonstrated in the glomerular immune deposits of murine lupus (703). Anti-dsDNA antibodies, anti-ssDNA antibodies, and antihistone/DNA (nucleosomal) antibodies have been detected in the glomerular immune deposits from human and murine lupus, often with higher concentrations in kidney eluates than in simultaneous serum samples (496,500,501,687,704–712). Other autoantibodies that may be enriched in the glomeruli in lupus nephritis demonstrate specificity for Sm, RNP, histone, SSA, SSB, and C1q (713,714). One study found that nucleosome-restricted autoantibodies were detectable before anti-dsDNA antibodies or antihistone antibodies in the serum of MRL/lpr mice and were also detectable in the glomerular eluates, supporting an initiating role for antinucleosomal antibodies (704).

Antinucleosomal antibodies may bind to dsDNA-histone complexes that are liberated into the circulation when cells die through apoptosis. Once liberated from apoptotic cells, nucleosomes bind to negatively charged cell surfaces or matrix components, providing a source for planted nucleosome antigen and in situ immune complex formation (715). In two strains of mouse, the MRL/lpr and BXSB mouse, antibodies specific for nucleosomes can be detected in the circulation before the appearance of anti-dsDNA or antihistone antibodies in the course of lupus nephritis, suggesting that anti-dsDNA antibodies arise as a subset of antinucleosomal antibodies (264,716,717). Antinucleosomal antibodies have been eluted from the kidney (264,717), and nucleosomes, antinucleosome autoantibodies, and nucleosome-Ig complexes alike have been identified in the glomerular immune deposits (610,718,719). Using a colocalization TUNEL and immunoelectron microscopy

technique, Kalaaji et al. (720) have demonstrated that the electron-dense deposits in lupus nephritis contain chromatin and anti-DNA antibodies, supporting a primary role for binding of autoantibodies to planted autoantigens, not cross-reactivity to intrinsic glomerular structures.

### Cross-Reactivity to Intrinsic Glomerular Antigens in Lupus Nephritis

Glomerular anti-DNA antibodies exhibit a broader range of cross-reactivities than serum anti-DNA antibodies, including for polynucleotides, phospholipids, and proteins (687,713). These cross-reactivities include normal glomerular constituents, such as heparan sulfate and chondroitin sulfate proteoglycans (721–723), phospholipids (724–726), laminin (711,727,728), type IV collagen (727,729), podocyte and proximal tubular brush border proteins such as dipeptidyl peptidase IV (727,730), cytoskeletal proteins such as vimentin (731) and α-actinin 4 (732,733), and cell surface antigens on mesangial and endothelial cells (618,734–736). Interestingly, most nephrophilic (glomerular-binding) antibodies in the glomerular deposits of $SNF_1$ mice are polyreactive ANAs that have the potential to bind both dsDNA and glomerular antigens like laminin directly, without the need for nuclear antigenic bridges (711). Circulating cationic anti-DNA antibodies from humans with SLE have also been shown to exhibit cross-reactivity with dextran sulfate, chondroitin sulfate, and hyaluronic acid in vitro (737,738). The levels of antibody reactive with heparan sulfate appear to correlate with periods of active renal disease in human lupus (739).

These findings suggest that lupus autoantibodies may bind in situ to normal glomerular cellular or matrix components and that these cross-reactivities may play a role in the development of nephritis. Krishnan et al. demonstrated that only anti-DNA antibodies that bound to basement membrane could activate complement and induce proteinuria when injected into mice, suggesting that direct binding of anti-DNA antibody to antigens in the GBM or mesangial matrix may be necessary to initiate lupus nephritis (723). In these experimental models, the pattern of glomerulonephritis and localization of immune deposits (i.e., whether mesangial, subendothelial, or subepithelial) may depend on the specificity of these cross-reacting antibodies. For example, in the murine GVHD model, Bruijn et al. (727) demonstrated that early in the course of the disease, there is a predominance of antibodies to laminin and type IV collagen, producing a linear pattern of fluorescence. Later in the disease, a granular subepithelial distribution of immune deposits occurs with the appearance of antibodies to brush border enzyme dipeptidyl peptidase type IV that cross-react with an epitope expressed on the surface of visceral epithelial cells. Based on studies in MRL/lpr mice, Van Bruggen et al. have hypothesized that the initial event in lupus nephritis consists of direct binding of antiglomerular autoantibodies, followed by deposition of antinuclear antibodies that recognize planted antigens (e.g., nucleosomes) (710).

## Role of Complement in Lupus Nephritis

The importance of the complement system in the pathogenesis of SLE and lupus nephritis reflects not only its role in mediating inflammation but also its central role in clearing immune complexes and apoptotic fragments and its regulatory effects on immune cell function (59,585). Complement serves as a bridge linking the innate and adaptive immune responses and is an important amplifier of the inflammatory responses that cause tissue injury in SLE. Decreased serum levels of complement correlate strongly with disease activity and with lupus nephritis, reflecting consumption at sites of immune complex deposition. This phenomenon should be distinguished from the rare occurrence of hypocomplementemia due to homozygous deletions of the early components of the classical complement pathway (notably C1q, C4, and C2), which are discussed above (see section Deficiencies With Mendelian Inheritance in SLE).

In patients with SLE, the complement profile typically reveals depressed $CH_{50}$, with more frequent depressions of early complement components C1 and C4 than C3, although many patients have reductions in all of these. In one study (740) of lupus patients with active nephritis, $CH_{50}$ was reduced in 87%, C1q and C4 in 80%, and C3 in 68%. Complement reductions were less marked in patients without nephritis ($CH_{50}$, 50%; C1q, 50%; C4, 62%; and C3, 37%). Others have observed better correlations of periods of disease activity with C3 than C4 levels (741). Low C1q levels have been associated with proliferative lupus nephritis and may predict the occurrence of renal flares (742).

A variety of catabolic complement components may be elevated in lupus sera, including C2a, C3a, C3d, C4d, Bb, and Ba, and these have been shown to correlate with disease activity and predict lupus flares (743,744). Serial monitoring of complement, anti-dsDNA antibody and circulating immune complex levels may help predict periods of disease exacerbation (586). Of note, more intense glomerular C4d deposition has been associated with the presence of microthrombi in lupus nephritis, both with (745) and without (94) evidence of APL syndrome, suggesting that complement activation via the classical pathway may contribute to endothelial injury and thrombotic microangiopathy in SLE.

## Role of Immune Cells and Soluble Factors in SLE and Lupus Nephritis

B-cell hyperactivity is a key feature of SLE. Immunoglobulin production is increased, reflecting increased numbers of Ig-secreting B cells in the peripheral blood (746), even though the number of total B lymphocytes in the peripheral blood is often reduced. During periods of active disease, the number of immunoglobulin-secreting B cell approaches 50-fold greater than that of controls, and serum immunoglobulin levels are correspondingly increased. Evidence from mice and humans with lupus suggests that this B-cell hyperactivity results initially from antigen-independent polyclonal B-cell stimulation, followed by more selective expansion of a subset of B cells producing particular autoantibodies (747–749). For example, in early murine lupus, mice have a generalized increase in the numbers of B cells and hypergammaglobulinemia before the selective emergence of autoantibody-secreting clones that become progressively more selective over time (750–753). In murine lupus, these autoantibody-secreting B cells undergo a process of affinity maturation, isotype switching, and idiotype selection, as expected in antigen-driven antibody responses. Moreover, the ratio of replacement nucleotides to silent nucleotides in the hypervariable region of the autoantibody combining site suggests a progressive selection of high-affinity clones that is antigen driven. B-cell maturation and survival depend on interactions between two cytokines of the TNF family,

BLyS/BAFF and a proliferation inducing ligand (APRIL), and their receptors on B cells. BLyS is produced by neutrophils, dendritic cells, and macrophages in response to TLR signaling and IFN, both of which are up-regulated in SLE. Excessive levels of BLyS may lead to enhanced survival of autoreactive B cells and contribute to perpetuation of SLE by a variety of mechanisms. Belimumab, a fully human recombinant monoclonal antibody to BLyS, has shown efficacy in cases of SLE that are refractory to standard therapy (see section on treatment above).

B-cell hyperactivity may result in part from failure to eliminate autoreactive B cells due to defective apoptosis. The role of fas in the acquisition of self-tolerance has been described in the MRL/lpr mouse, in which there is a mutation in the *fas* gene, which encodes for the fas cell surface molecule involved in the induction of apoptosis (523). Mice transgenic for *bcl-2*, which enhances cell survival through inhibition of apoptosis, overexpress bcl-2 and display polyclonal B-cell expansion, leading over several months to the development of a lupus-like autoimmune state (754).

A variety of B-cell signaling defects have been identified, including higher fluxes of intracellular calcium and cytosolic protein tyrosine phosphorylation. Expression of complement receptor 2 (CR2) and of the inhibitory receptor FcγRIIIB expression are both decreased on SLE B cells, and this defect may be genetically determined, as a polymorphism causing reduced expression of FcγRIIIB has been identified in SLE patients (755). The expression of the protein tyrosine kinase Lyn is reduced in the cytoplasm of B cells in two thirds of patients with SLE, and this may impair signaling from inhibitory cell surface molecules such as C22 and FcγRIIBI, thereby contributing to B-cell overactivity (756).

In addition to their role in antibody production, B cells serve as antigen-presenting cells for activation of autoreactive T cells through cell-cell contacts that involve MHC class II and costimulatory molecules. B cells also secrete a number of cytokines and chemokines that contribute to inflammatory injury including IL-2, IL-6, IL-10, TGF-β, IFN-γ, and TNF-α (757). Studies have shown a high frequency of charged recurrent motifs in the TCR CDR loops of Th clones from murine and human SLE through presumed recombination events (758,759). These findings suggest that there is selective expansion of particular Th clones recognizing charged autoantigens (759). Through antigen processing and presentation to B cells, these restricted Th clones presumably drive autoantibody production.

Abnormalities of T-cell function in SLE result in excessive B cell help and insufficient suppressor function (491). Absolute numbers of T lymphocytes are reduced in the peripheral blood, with a particular reduction in $CD8^+$ suppressor T-cells (760,761), whereas $CD4^+$ helper T cells are increased. Moreover, $CD8^+$ cells from patients with SLE are often unable to down-regulate polyclonal immunoglobulin production and synthesis of autoantibodies (762). The cause of this suppressor defect is uncertain but may be related to a preponderance of naive $CD8^+$ cells with helper function (763). This relative deficiency in suppressor cell function may be mediated in part by defective $CD4^+$ T-cell function, because naive $CD8^+$ T cells mature in response to various cytokines such as IL-2 and IFN-γ produced by $CD4^+$ T cells (764). The number of double-negative ($CD4^-CD8^-$) T cells is significantly increased in SLE; these cells induce anti-DNA antibody formation by autoreactive B cells, produce IL-17, and are present in the kidney in lupus nephritis (53).

Other T-cell–mediated abnormalities in SLE include altered cytokine synthesis. Decreased IL-2 synthesis reflects an imbalance between two molecules that bind to the *IL-2* promoter region—cyclic AMP–responsive element-binding protein (CREB) and cyclic AMP–responsive element modulator (CREM)—due to enhanced transcription of CREM (a negative regulator). Reduced levels of IL-2 may underlie the impaired number and function of $CD4^+FoxP3^+$ T reg cells in SLE, which are needed to prevent autoimmunity. On the other hand, T cells in SLE produce an excess of the inflammatory cytokine IL-17. Serum IL-17 levels correlate with disease activity, and IL-17–producing T cells (either $CD4^+$ Th17 or $CD4^-CD8^-$ cells) are present in the kidney in lupus nephritis (765). IL-17 recruits inflammatory cells and aids in germinal center formation.

A broad range of signaling abnormalities have been identified in T cells in SLE, including defective TCR zeta (ζ) chain synthesis and increased FcRγ synthesis, increased lipid raft clustering, impaired ERK signaling, increased calcium response, decreased protein kinase C (PKC) activity, decreased protein kinase A (PKA) levels and activity, and reduced DNA methyltransferase (DNMT) 1 (766). Replacement of the TCR ζ chain by FcγR leads to signaling via spleen tyrosine kinase (Syk) instead of the canonical ζ–associated protein (ZAP-70), resulting in decreased IL-2 production and an increased IL-17 synthesis (767–769). This defect in TCR ζ chain expression may be genetically determined (768–770). Kammer et al. (771) demonstrated that the defect in PKA-catalyzed protein phosphorylation was caused by deficient PKA type 1 isozyme in lupus T cells. This was the first enzyme deficiency to be demonstrated in SLE and may be a heritable trait. The net result of these signaling abnormalities is altered T-cell function, with impaired production of regulatory T cells and enhanced inflammation.

There is growing evidence that DNA hypomethylation, via either inhibition of DNMT1 function or suppression of its expression, is associated with overexpression of methylation-sensitive genes in T cells from SLE patients (772). Methylation-sensitive gene products that are overexpressed in lupus T cells include CD11a (*ITGAL*), a component of leukocyte function antigen (LFA)-1, which contributes to T-cell autoreactivity; CD70 (*TNFS7*), a B-cell costimulatory molecule; perforin (*PRF1*), a cytotoxic molecule that is expressed on $CD4^+$ T cells in patients with active but not inactive lupus; and CD40 ligand (*TNFS5*), a B-cell costimulatory molecule that has been shown to stimulate overproduction of autoantibodies in vitro. Of note, CD40L is encoded on the X chromosome gene and therefore relatively overexpressed in women, thereby possibly contributing to the female predominance of SLE.

Recent findings from genetic studies and transcriptomal studies (590) strongly support a role for dendritic cells, type 1 interferon signaling, and the innate immune system in the pathogenesis of SLE and lupus nephritis. Dendritic cells in SLE have a mature phenotype, characterized by increased expression of activating Fcγ receptors and costimulatory molecules that make them more immunogenic and proinflammatory than immature dendritic cells. Engagement of pathogen-associated molecular patterns (such as viral and bacterial RNA

and DNA) with pattern recognition receptors on plasmacytoid dendritic cells leads to release of proinflammatory cytokines that promote the activation of leukocytes and intrinsic renal cells, thereby contributing to lupus nephritis.

Both immune and nonimmune cells express inflammatory cytokines and chemokines in response to activation of TLRs by specific ligands. TLR7 and TLR9, expressed on plasmacytoid dendritic cells, bind dsRNA and unmethylated cytosine-guanosine dinucleotide (CpG) DNA, a stimulatory motif found in bacterial and viral DNA which is rare in mammalian DNA. In SLE, TLR9 expression is increased on peripheral B cells, plasma cells, and plasmacytoid dendritic cells, and abnormal TLR7 and TLR9 signaling (in response to RNA and DNA), respectively, may predispose to formation of autoantibodies by B cells and release of IFN-α by plasmacytoid dendritic cells. In MRL lpr/lpr mice, *Escherichia coli* DNA and synthetic hypomethylated CpG-DNA stimulated a rise in serum anti-dsDNA antibody titers (773). Interestingly, patients with DIL have higher serum levels of hypomethylated CpG-DNA, presumably because these drugs are potent inhibitors of DNMT activity (774).

Inflammatory cells and intrinsic renal cells also play important roles in amplifying the initial inflammatory response and promoting tissue injury in lupus nephritis. Deposition of immune complexes in the glomerulus leads to activation of the complement system and of cytokine networks that promote leukocyte recruitment, cellular proliferation, and matrix production. These pathways are activated via engagement of Fcγ receptors on the surface of macrophages, dendritic cells, neutrophils, and intrinsic renal cells. The importance of renal influx of FcR-bearing effector cells in lupus nephritis has been demonstrated by attenuation in a knockout model (775). NZB/W mice that are deficient in FcγR are protected from the development of severe nephritis, although their glomeruli accumulate immune complex deposits and activate complement (775). In addition, DNA and RNA in these immune complexes (or released from apoptotic renal cells in the form of chromatic particles) are potent self-antigens for TLR signaling and downstream activation of IFN-α (350,773).

Neutrophil and monocyte recruitment to glomeruli are particularly marked in diffuse proliferative lupus nephritis, reflecting up-regulation of chemokine expression. In murine lupus nephritis (776), mesangial colony–stimulating factor type 1 (CSF-1) expression increases in glomeruli before the accumulation of macrophages, and exogenously administered CSF-1 has an inductive effect on the nephritis (777). Monocyte chemoattractant protein type 1 (MCP-1) is present in the urine of patients with active lupus nephritis and likely plays an important role in monocyte recruitment (778). Other chemoattractants, such as colony-stimulating factor, vascular cell adhesion molecule 1, ICAM-1, RANTES, CX3CL1 (fractalkine), and osteopontin, have been identified in the kidneys of MRL/lpr mice (777,779–782). Class II (DR) and intercellular adhesion molecule type 1 (ICAM-1) expression on tubular epithelial cells precedes renal injury in the MRL/lpr mouse (56,57,783). VCAM-1 expression in glomeruli, tubules, and vascular endothelium is also up-regulated in active murine nephritis, suggesting a role in the adherence of infiltrating leukocytes (781). Renal tubular expression of ICAM-1, DR, and costimulatory molecule CD40 has also been correlated with active lupus nephritis in humans (59,784). Up-regulation of MHC class II molecules (DR and DQ) has been demonstrated in the glomerular endothelium of patients with active glomerular lesions (785) and correlated with glomerular activity index and serum levels of IFN-γ. These findings suggest that cytokines play a role in up-regulating MHC class II expression in multiple renal compartments, potentially augmenting autoimmune reactivity and the inflammatory response in human and murine lupus nephritis.

Interstitial infiltration by T cells, B cells, dendritic cells, macrophages, and neutrophils likely contribute directly to the pathogenesis of lupus nephritis via autoimmune reactivity, local autoantibody production, direct cytotoxicity (via T cells, macrophages, and neutrophils), and release of proinflammatory and fibrogenic cytokines. Neutrophils release neutrophil extracellular traps (NET) containing DNA and histones, which activate plasmacytoid dendritic cells and produce type 1 IFN (786). Infiltrating plasma cells may be a source of local autoantibody production in lupus nephritis in NZB/W mice and human lupus (52,787). In a study of 68 biopsies of lupus nephritis, Chang et al. identified three patterns of interstitial T- and B-cell infiltration: (a) diffuse infiltrates (48%); (b) well-circumscribed aggregates of T and B cells (46%), which contained CD138(+)CD20(low/–) plasmablasts; and (c) germinal centers (6%), which contained CD21+ follicular dendritic cells and CD138(–)CD20(+) centroblasts (51). The latter two organized forms were associated with tubular basement membrane immune complex deposits, suggesting a pathogenic role in their synthesis. Winchester et al. described several patterns of T-cell infiltration, including $CD4^+$ dominant periglomerular aggregates, mixed $CD4^+$ and $CD8^+$ periglomerular aggregates, and $CD8^+$ dominant interstitial infiltrates. $CD4^+$ T cells were prominent in nearly two thirds of SLE biopsy samples and were distributed as broad periglomerular aggregates or intermixed with $CD8^+$ T cells forming periglomerular caps (50). Infiltrating T cells showed oligoclonal expansion. $CD8^+$ T cells predominated in cases showing tubulitis or adherence of lymphocytes to the basal portion of tubular epithelium and the Bowman capsule. In addition, $CD8^+$ cells showed features consistent with participation in an adaptive immune response, namely CD28 null memory-effector phenotype, trafficking of the expanded clonotype to different regions of the kidney, and clonal persistent for years in repeat biopsy samples. These findings suggest that the presence of T cells in the kidney in lupus nephritis is not simply a generalized response to inflammation but instead reflects an adaptive immune response, either to glomerular immune complexes or possibly to renal antigens induced by intrarenal inflammation. Interestingly, CD28 null memory-effector T cells are present in the urine of patients with active lupus nephritis but are decreased in the circulation, suggesting migration to the kidney (788). There are increased numbers of T cells expressing CD40 ligand (CD40L) in the kidney, and CD40 is up-regulated on intrinsic renal cells in lupus nephritis. CD40L-CD40 binding promote B-cell clonal expansion, immunoglobulin class switching, and plasma cell differentiation (789). In addition, binding of CD40L to CD40 on tubular cells might promote release of proinflammatory cytokines, including MCP-1, RANTES, and IL-8, as has been demonstrated by in vitro coculture experiments (790).

Cytokines play a major role in amplifying the inflammatory response in murine and human lupus (reviewed in (791)). IL-17 levels and IL-17–producing T cells (double-negative $CD4^-CD8^-$ and $CD4^+Th17^+$) are increased in the peripheral

blood and renal biopsies of patients with lupus nephritis (765). IL-17 is a proinflammatory cytokine that recruits effector cells and promotes survival and proliferation of B cells and formation of germinal centers, thereby possibly contributing to lupus nephritis. The differentiation of IL-17–producing T cells requires priming by TGF-β in the presence of inflammatory cytokines such as IL-6 and IL-21, which are both increased in patients with SLE. Production of IL-2 is defective in T cells of patients with SLE, which may lead to defective regulatory T-cell function. Urinary levels of IL-2 receptor and IL-6 are increased in patients with active lupus nephritis, consistent with origin from the inflamed renal parenchyma (792,793). Moreover, urinary IL-6 levels decline after therapy, suggesting that this may be a useful marker to monitor disease activity.

There is also considerable evidence that TNF-like weak inducer of apoptosis (TWEAK) plays a role in lupus nephritis. TWEAK is synthesized by inflammatory cells and renal tubular epithelial cells, and its levels are increased in the kidney and urine in active lupus nephritis (794). Engagement of TWEAK with its receptor, fibroblast growth factor–inducible 14 receptor (Fn14), activates several pathways, notably NF-κB, in mesangial and tubular cells, with diverse functional effects including release of proinflammatory cytokines and chemokines (795). In lupus nephritis, TWEAK is predominantly expressed on infiltrating inflammatory cells, and Fn14 is up-regulated on mesangial cells, podocytes, endothelial cells, and interstitial fibroblasts. TWEAK/Fn14 signaling may also induce cell proliferation, promote cell survival or cell death, and promote fibrosis. Deficiency of Fn14 (due to gene knockout) or use of a neutralizing anti-TWEAK monoclonal antibody results in reduced renal damage in murine models of lupus nephritis (795).

### Coagulation Abnormalities in Lupus Nephritis

Evidence of coagulation in lupus nephritis includes increased serum and urinary levels of fibrin degradation products (796), increased plasma levels of fibrinopeptide A (797), and enhanced fibrinogen catabolism (798). Monocytes probably contribute to these procoagulant effects through the production of monocyte procoagulant capable of direct activation of prothrombin (799). Reduced fibrinolysis also perturbs this equilibrium, as evidenced by reduced levels of plasminogen activator, increased levels of plasminogen activator inhibitor, and increased levels of α2-antiplasmin (800,801). Renal thromboxane production is increased in murine lupus nephritis (802), and thromboxane $A_2$ synthase inhibition (803), and thromboxane receptor blockade (804) have a beneficial effect on murine lupus nephritis, suggesting a role for thromboxane $A_2$–mediated platelet aggregation. Platelet activation through immune and coagulation pathways has the direct effect of promoting thrombosis and release of vasoactive amines and platelet-derived growth factor, which affects the growth and proliferation of glomerular mesangial cells. Platelet cationic proteins have been detected in glomeruli of patients with lupus nephritis, suggesting local activation (805).

As noted previously, classical complement pathway activation, as evidenced by more intense glomerular C4d deposition immunohistochemically, may have a pathogenetic role in the development of endothelial injury and microthrombosis in cases of lupus nephritis (94,745).

## MIXED CONNECTIVE TISSUE DISEASE

### Introduction

MCTD is a syndrome characterized by overlapping clinical features of SLE, systemic sclerosis (scleroderma) and polymyositis, and autoantibodies to the uridine-rich small nuclear ribonucleoprotein (U1-snRNP) spliceosome complex (806). This entity was first described in 1972 by Sharp et al. (806) in 25 patients who had overlapping clinical features of SLE, scleroderma, and polymyositis and high titer autoantibodies to RNAse-sensitive extractable nuclear antigen (ENA). The antigen was subsequently characterized as U1-snRNP, a complex containing U1 RNA linked noncovalently to three polypeptides, 70 kd, 33 kd (A′), and 22 kd (C) (807). Although characteristic of MCTD, anti-U1-snRNP antibodies are not specific as these may also occur in SLE and scleroderma. Moreover, many cases of MCTD will over time fulfill criteria for a defined connective tissue disease (most commonly SLE or scleroderma and rarely RA or SS) (808,809). However, a majority of patients will continue to have features of MCTD, supporting that this is a distinct clinical entity (810).

The etiology and pathogenesis of MCTD are unknown, but these are presumed to involve autoimmunity to the U1-RNP complex. This process likely involves autoantigen modification (e.g., during apoptosis) leading to increased immunogenicity, engagement of the innate immune system via TLRs, generation of autoantibodies, and activation of B and T lymphocytes (807). Genetic studies have demonstrated that patients with antibodies to a 70-KD component of U1-RNP share a common epitope in the antigen-binding groove of their DR molecules, suggesting that the acquisition of anti-U1-RNP antibodies may be antigen driven (807,811,812). Of note, MCTD is associated with HLA-DR4 and HLA-DR2, whereas SLE is associated with HLA-DR3 and systemic sclerosis with HLA-DR5, suggesting that the clinical phenotype is at least in part genetically determined (813).

Whether MCTD is actually a distinct "disease" remains unproven, given the absence of a specific etiology or a demonstrated pathogenetic role for anti-U1-RNP. However, it is generally accepted that MCTD is a useful concept, as it identifies a subset of patients with connective tissue disease who share similar serologic and clinical characteristics (810,812,814,815).

### Clinical Features of MCTD

MCTD may occur at any age but typically begins in the second or third decade of life. The incidence is 16-fold higher in females than males. The overall clinical course is similar in children and adults, but there may be a greater preponderance of SLE features in children (816). Common clinical features include Raynaud phenomenon, arthralgias, and arthritis (sometimes erosive or deforming); swelling or sclerodermatous changes of the hands; restrictive lung disease (which may be asymptomatic); myositis; and esophageal dysmotility. Other common features include cutaneous lesions (i.e., alopecia, digital and oral ulcers, digital gangrene and vasculitis, discoid lupus, malar erythema, and photosensitivity), mitral valve prolapse, hepatosplenomegaly, serositis, and lymphadenopathy (808,810,817,818). Secondary SS may occur in up to 50% of patients. Trigeminal neuralgia occurs in approximately 25% of cases and may be an early manifestation.

Importantly, these clinical features typically occur at different times during the course of the disease. Central nervous system and renal manifestations affect a minority of patients but may have significant morbidity (815). In the initial report of MCTD, renal disease and CNS manifestations were rare, and most patients had a good initial response to corticosteroid therapy, suggesting a benign clinical course (806). However, later studies (812,819,820) demonstrating major organ involvement, including lung, kidney, and CNS, indicate a more guarded prognosis (812).

There are several different classification criteria for MCTD, all of which include positive anti-U1-RNP and presence of at least three clinical criteria (814). The criteria proposed by Alarcon-Segovia and Cardiel (821) are the simplest, consisting of anti-RNP titer greater than 1:1,600 (by hemagglutination assay) and the presence of at least three of the following clinical manifestations: hand edema, synovitis, myositis, Raynaud phenomenon, and acrosclerosis. These criteria have a reported sensitivity of 62.5% to 99.6% and specificity of 86.2% to 100% for the diagnosis of MCTD (821,822).

Antibodies to U1-snRNP and to heterogeneous nuclear ribonucleoprotein (hn-RNP)-A2 are the hallmark of MCTD and produce speckled ANA of high titer (often greater than 1:1000 or even 1:10,000) and hypergammaglobulinemia (815,823). RF may be present in 70% of patients, but complement levels are usually normal (815). Antibodies to Ku and U2-RNP have identified a group with features of polymyositis and scleroderma, and anti-Jo-1 identifies a group with features of polymyositis and pulmonary fibrosis. Anti-Sm antibodies are associated with SLE features and renal involvement. Anti-Ro/SSA positivity is associated with neurologic and pulmonary involvement; anti-scl70 with esophageal involvement; anticentromere positivity with sclerodactyly; and anti-dsDNA and anti-Ro/SSA with serositis (810). APLAs (usually IgM isotype) may be present in 15% of cases and are associated with pulmonary involvement.

## Clinical Renal Presentation

While initially the incidence of renal disease in MCTD was thought to be low (806), over time this has been revised. Two of the original 25 patients in the series of Sharp et al. (819) died of renal disease, and a third patient had low-grade renal disease. Kitridou et al. (818), in reviewing the published series, described renal involvement in 10% to 26% of adults and 33% to 50% of pediatric cases. In their own series, 12 of 30 (40%) MCTD patients developed proteinuria greater than 0.5 g/d, 9 of whom had nephrotic syndrome (818). In a recent large multicenter study of 161 MCTD patients, approximately 10% developed renal disease over a mean follow-up of 8 years (810).

Renal involvement has variable clinical manifestations and may be asymptomatic. The percentage of patients with cryptic renal disease is unknown but may be higher in children. Ito et al. reported no urine abnormalities in four of six pediatric MCTD cases undergoing renal biopsy and 28% of cases in the literature (824). Kobayashi et al. (825) described four patients with subclinical renal disease, with mesangial proliferative glomerulonephritis in three cases and membranous glomerulonephritis in one. One patient reported by Kitridou et al. had normal kidney function and trace proteinuria but was discovered to have membranous glomerulonephritis at autopsy (818). A few patients present with marked hypertension, microangiopathic hemolytic anemia, and acute renal failure, similar to the clinical presentation of "scleroderma kidney" (see Chapter 18) (826,827).

There is no correlation between the titer of anti-RNP and the development of renal disease. The presence of anti-Sm antibody (810,818,828) or anti-dsDNA antibody increases the likelihood of renal disease (818,828), but significant renal disease may occur in the absence of both. Similarly, some reports suggest that hypocomplementemia is more common in those with renal disease (829), whereas others found that hypocomplementemia is equally common in patients without renal disease (818).

## Pathologic Findings

Most renal biopsies display immune complex glomerulonephritis similar to lupus nephritis (818,824,825). In a review by Bennett of 100 patients with MCTD and renal histologic analysis reported up to 1990, 36% had membranous glomerulonephritis, 35% mesangial disease, 22% severe vascular disease, 15% interstitial disease, 10% proliferative glomerulonephritis, and 12% normal renal histology (830). An autopsy study from Japan reported clinical renal disease in 16 (64%) of 25 cases (831). Membranous glomerulonephritis was the most common finding (11 cases), followed by mesangial proliferative (3 cases), focal proliferative (1 case), and membranoproliferative (1 case) glomerulonephritis. Nine of twenty-five cases had no obvious glomerular lesion. Arterial intimal thickening was a common finding, even in patients without glomerular disease (831).

Similar to lupus membranous glomerulonephritis, MCTD-associated membranous glomerulonephritis may be accompanied by mesangial deposits and mesangial hypercellularity. Immunofluorescence studies reveal granular capillary staining for IgG and C3, and occasionally IgA and IgM (818). IgA and IgM more often show predominantly mesangial staining, with complement components in the same distribution. One study reported that cases of membranous glomerulonephritis in patients with anti-RNP show a predominance of IgG-2 heavy chain, whereas lupus membranous nephritis typically stains for all four heavy chains, suggesting that these conditions have a different pathogenesis (832).

The remaining cases display a variety of proliferative lesions, of which diffuse mesangial hypercellularity accompanied by mesangial deposits of IgG and C3 is the most common pattern (818). Focal or diffuse proliferative changes are accompanied by subendothelial deposits of IgG, IgM, C3, and occasionally IgA. Fibrinoid necrosis and crescent formation are rare (818). Tubular basement membrane deposits have been described, albeit rarely. Probably because MCTD patients usually have very high titers of circulating anti-RNP antibodies, immunofluorescence microscopy often reveals conspicuous speckled ANA staining of nuclei in biopsy specimens. Endothelial TRIs, similar to those in SLE, have been described. If a "fingerprint" substructure is identified, the possibility of cryoglobulinemia should be considered.

As with lupus nephritis, progression from one glomerular lesion to another may be seen in serial biopsies, including from mesangial glomerulonephritis to membranous glomerulonephritis and from membranous glomerulonephritis to focal proliferative glomerulonephritis (818). In addition to immune complex glomerulonephritis, several cases of pauci-immune crescentic glomerulonephritis, often associated with positive

anti-MPO ANCA serologies, have been reported in patients with MCTD (833–836). Rare cases of minimal change nephrotic syndrome (837), collapsing glomerulopathy (838), and secondary amyloidosis (839) have also been described.

Arterial disease is common, even in biopsies with immune complex glomerulonephritis. The predominant change is intimal sclerosis, affecting arcuate to arteriolar-size vessels. Medial hyperplasia may be present, but fibrinoid necrosis is rare. Some patients (819,840) have had severe hypertension or renal failure, apparently on the basis of these lesions. There are also reports of concurrent pulmonary hypertension and renal crisis (841,842), much like the presentation of systemic sclerosis kidney (843). Morphologically, the arterial lesions have much in common with those of systemic sclerosis, with predominant findings of intimal mucoid edema and fibrous sclerosis at the interlobular artery level (Fig. 14.76). As in systemic sclerosis, renal infarcts may also occur (844). Burdt et al. described arterial intimal proliferation and medial hypertrophy in multiple organs in 4 patients undergoing autopsy. Of note, two of these patients had anticardiolipin antibodies, suggesting a possible pathogenetic role for APL antibodies in some cases (812).

To summarize, the glomerular lesions in MCTD parallel those in SLE, with a predominance of membranous glomerulonephritis and mesangial proliferative glomerulonephritis, and the vascular lesions are similar to those in systemic sclerosis (scleroderma). Thus, renal disease in MCTD indeed presents a mixed picture.

### Clinical Course, Prognosis, Therapy, and Clinicopathologic Correlations

Most groups have employed corticosteroids in various doses in patients with MCTD who have significant organ involvement. Steroids are effective in suppressing the inflammatory and often the arthritic symptoms, but they do not usually alter the sclerodermatous-type lesions (819). Cutaneous lesions often progress from the initial edematous, puffy appearance to a progressively tighter, hide-bound appearance. Similarly, Raynaud phenomenon and gastrointestinal motility changes are likely to persist (810,819). Treatment directed to the glomerular diseases is similar to that used for the corresponding class of lupus nephritis. In patients with a systemic sclerosis renal crisis presentation, angiotensin-converting enzyme inhibitors and intravenous prostacyclin are indicated (814).

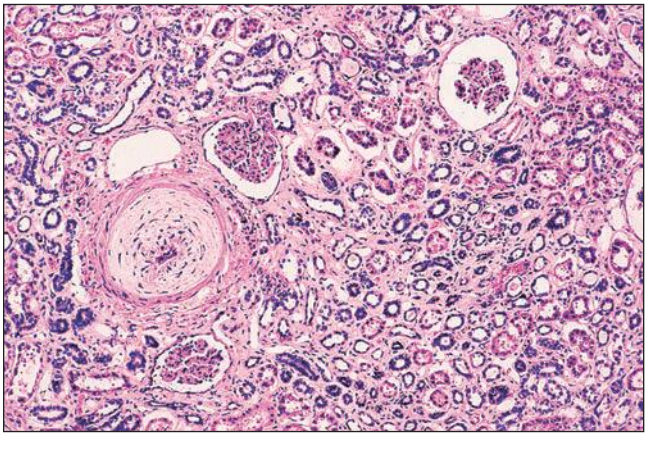


**FIGURE 14.76 Mixed connective tissue disease.** In this patient, the features were primarily those of scleroderma, with onion skin mucointimal thickening of interlobular arteries and ischemic-type glomerular retraction and tubular atrophy.

In some patients, the syndrome of MCTD is a way station in the evolution of connective tissue disease. The longer the disease progresses, the smaller is the proportion of patients whose illness can still be characterized as MCTD alone. Nimelstein et al. (819) found in follow-up of Sharp's original group that only 6 of the 17 patients for whom data were available could still be regarded as MCTD, whereas the others evolved to systemic sclerosis (8 patients), SLE (2 patients), or RA (1 patient). On the other hand, in a study of 161 cases with an initial diagnosis of MCTD, Cappelli et al. (810) reported that over half remained classified as MCTD after a mean follow-up of 7.9 years, supporting that this is a distinct clinical entity. However, using the Alarcon-Segovia classification criteria, only 7.5% of patients had MCTD alone at last follow-up, whereas 42.4% had MCTD plus features of another connective tissue disease (SLE, SS, or RA); the remainder had evolved into SLE (9%), SS (16.9%), or RA (1.6%), had an undifferentiated disease (18.5%), or an overlap syndrome (4.1%) that did not fulfill criteria for MCTD (810).

Although MCTD was initially thought to have a benign course, 8 of the 25 patients in Sharp's original series had died on follow-up assessment, with one death caused by hypertensive crisis related to systemic sclerosis kidney and three others due to cardiovascular disease (819). Other investigators have found mortality rates in the range of 10% to 20% (818,840,845). Pulmonary hypertension is the main cause of mortality (815).

Kitridou et al. (818), summarizing series comprising 76 cases, found an incidence of chronic renal failure of 14%. If the marked intimal vascular lesions in the patients of Singsen et al. (840) are representative, over the years, significant morbidity and mortality likely ensue from these lesions, particularly those of the coronary and intrarenal arteries. Thus, the ultimate prognosis in MCTD must be viewed as guarded.

## SJÖGREN SYNDROME

### Introduction

In 1933, the Swedish ophthalmologist Henrik Sjögren first described the ophthalmologic manifestations of keratoconjunctivitis sicca that characterize the syndrome that bears his name (846). The characteristic features of this condition are a chronic inflammatory cell infiltration of the exocrine salivary and lacrimal glands causing a "sicca complex" of xerostomia and xerophthalmia. SS most commonly occurs as a primary form (70%), while approximately 30% of cases are associated with systemic diseases, such as SLE, RA, or MCTD (847). The reported incidence of primary of SS is approximately four to five cases per 100,000 population (847–849). In most cases of primary SS, inflammation is limited to the salivary and lacrimal glands, but 25% of patients will have other organ involvement, including kidneys, lungs, esophagus, thyroid, stomach, and pancreas (850).

The etiology and pathogenesis of SS are unknown, but these likely involve a genetically determined predisposition and autoimmunity to ribonucleoprotein components Ro/SS-A and La/SS-B. Infection with EBV may also play a role (851,852), as anti-EBV antibody titers are elevated in patients with SS, and

EBV DNA has been isolated from renal proximal tubules in cases with kidney involvement (853).

The reported frequency of SS in other autoimmune diseases ranges from 2% to 30% in SLE, 5% to 31% in RA, 17% to 29% in systemic sclerosis, and 42% to 56% in MCTD (815,854). In patients with SLE, SS is associated with age greater than 25 years and presence of anti-Ro/SSA antibodies (855). Less frequently, this syndrome is associated with other collagen vascular or inflammatory disorders such as polymyositis, primary biliary cirrhosis, Crohn disease, systemic vasculitis, and fibrosing alveolitis (856). Patients with SS are at greatly increased risk for the development of lymphoma (857,858), particularly B-cell lymphomas and Waldenström macroglobulinemia (859,860). Vasculitis of the skin, muscle, and nerve may occur in 10% to 15% of patients with primary or lupus-associated SS, and this may also involve the kidney, albeit rarely (854).

Common serologic abnormalities in patients with SS include hypergammaglobulinemia (present in almost 100% in the older literature and in 22% to 42% in modern series) (854); positive ANA (homogeneous or speckled pattern) in 70% of patients; and anti-Ro/SSA and anti-La/SSB in greater than 60%, especially those with severe extraglandular disease. RF is present in 40% to 60% of primary SS and may have cryoglobulin activity (854). Importantly, the presence of cryoglobulins is associated with extraglandular disease, severe vasculitis, and development of non-Hodgkin lymphoma (854). APL antibodies may be present in up to 20% of primary SS, but fewer than 10% of these patients meet criteria for the diagnosis of APL syndrome (861). In addition, some patients have organ-specific antibodies, including antibodies to thyroid microsome (i.e., thyroid peroxidase), thyroglobulin, salivary duct epithelium, and gastric parietal cells. Antibodies to fodrin, α-amylase, and carbonic anhydrase have also been demonstrated (862). Although there are often high levels of circulating immune complexes, hypocomplementemia occurs in only 10% to 25% of primary SS patients (854) but may be more common in association with SLE and overt lupus nephritis.

Standard criteria for the diagnosis of SS were defined by the American-European Consensus Group in 2002 (863). These include presence of ocular and oral symptoms, objective measures of tear and salivary dysfunction, minor salivary gland histopathology, and positivity for anti-Ro/SSA or anti-La/SSB antibodies (863). A revised definition based entirely on objective tests was recently proposed by the ACR and Sjögren International Collaborative Clinical Alliance (SICCA) in 2012 (864). The ACR/SICCA classification requires the presence of at least two of the following three objective features: (a) positive serum anti-Ro/SSA and/or anti-La/SSB or (positive RF and ANA titer greater than 1:320), (b) keratoconjunctivitis sicca (based on fluorescein and lissamine green stains), or (c) labial salivary gland biopsy showing at least 1 focus with 50 lymphocytes per 4 $mm^2$ of glandular tissue (864). Exclusion criteria include history of head and neck radiation treatment, hepatitis C infection, AIDS, sarcoidosis, amyloidosis, graft versus host disease, and IgG4-related disease (864).

## Clinical Renal Features

The prevalence of overt renal disease in patients with primary SS ranges from less than 1% (865) to approximately 5% (866,867). However, subclinical renal abnormalities may be present in up to 30% of patients (868). In a series of 7276 patients with primary SS followed over a 40-year period at Mayo Clinic, only 24 subjects (0.3%) underwent renal biopsy for evaluation of kidney disease (869). Skopouli et al. described cumulative incidences of 10% for tubulointerstitial nephritis and 2% for glomerulonephritis in 261 Greek patients with primary SS who had a mean follow-up of 3.6 years (870).

Clinical renal manifestations are heterogeneous and reflect the type of renal pathologic lesion (e.g., tubulointerstitial, glomerular, vascular) encountered on renal biopsy. Tubulointerstitial disease is by far the most common manifestation, glomerular disease is less common, and renal vasculitis is rare. The most common functional abnormality is distal (type I) RTA (in 15% to 73% of patients) (867,871,872). Distal RTA may be clinically silent and only revealed when an acid loading test unmasks impaired urinary acidification. When overt, distal RTA is characterized by hyperchloremic metabolic acidosis with an anion gap, hypokalemia, hypercalciuria, and reduced urinary titratable acidity that does not respond to exogenous acid load. Distal RTA is accompanied by impaired renal concentrating ability in up to 50% of patients, which can be documented by failure to respond to fluid deprivation or exogenous administration of vasopressin. Patients may present with isolated distal RTA years before other clinical features of SS become manifest (873). Because of hypercalciuria, up to 30% of patients may develop osteomalacia and nephrocalcinosis with an increased risk of nephrolithiasis (874). Distal RTA may produce profound hypokalemia and paralysis (875). Proximal tubular defects such as proximal (type II) RTA and Fanconi syndrome have been reported less frequently (876). Renal insufficiency is generally mild but may manifest as marked acute, subacute, or chronic renal failure (867). More commonly, patients with RTA or concentrating defects have a normal serum creatinine. Measures of tubular injury and dysfunction include increased urinary excretion of $\beta_2$-microglobulin and brush border enzymes. In a study of 78 patients with primary SS from Finland, factors predictive of development of distal RTA included higher levels of serum total gamma-globulin, total serum protein and serum $\beta_2$-microglobulin (877).

Whereas tubulointerstitial disease tends to be an early manifestation of SS, glomerulonephritis is usually a later development (878). Renal functional impairment, hematuria, proteinuria (with occasional nephrotic syndrome), cryoglobulinemia, and hypocomplementemia are more commonly identified in patients with glomerular disease.

Necrotizing arteritis of the kidney and other viscera has been reported, particularly with antibody to Ro/SSA (879). In patients with renal vasculitis, renal insufficiency, microhematuria, and hypertension may be present. Patients with vasculitis may have circulating terminal complement components C5b-9, suggesting a central role for complement activation (880). There are also multiple reports of anti-MPO ANCA-associated pauci-immune crescentic glomerulonephritis in patients with SS (881–885).

## Pathologic Findings

The most common renal biopsy finding is tubulointerstitial nephritis (867–869). This usually has the histologic features of a chronic but active process with patchy densely cellular infiltrates of lymphocytes, monocytes, and plasma cells, accompanied by varying degrees of tubulitis, tubular atrophy,

interstitial edema, and interstitial fibrosis (Fig. 14.77). In the Mayo Clinic series of 24 biopsies in primary SS, 71% showed tubulointerstitial nephritis and most (65%) of these were chronic (869). Immunophenotyping reveals that most of the interstitial cells are T cells of a helper/inducer (CD4) subset (886). Infiltrates rich in polymorphonuclear leukocytes (887) and granulomatous inflammation have rarely been described (869) Eosinophils are typically absent. Nephrocalcinosis may be seen. Nonspecific glomerular sclerosis, mesangial sclerosis, GBM thickening and wrinkling, and Bowman capsular thickening may be found in those examples with more chronic and severe tubulointerstitial damage (888). In some cases, the tubulointerstitial nephritis causes sudden renal enlargement, presenting as unilateral renomegaly (889), pseudolymphoma (890), or pseudotumor (891). Pseudolymphoma of the renal pelvis may produce hydronephrosis (892).

Immunofluorescence and electron microscopy typically reveal no immune deposits, although tubular basement membrane deposits of IgG and C3 have been reported in a few cases (893,894) (Fig. 14.78). IgG has been described in the cytoplasm of tubular epithelial cells and also in the cytoplasm of infiltrating interstitial plasma cells (895). The possibility that antitubular antibodies mediate the renal injury in some patients with SS is supported by the report of neonatal induction of renal tubular dysfunction by transplacentally acquired IgG from a mother with SS (896). Such antibodies may have specificity for the renal tubular plasma membranes (897). Circulating antibodies to carbonic anhydrase (862) and complete absence of H(+)-ATPase in the intercalated cells have also been described in some cases (898).

Less commonly, patients with SS have an immune complex–mediated glomerular disease, occurring as an isolated lesion or in association with tubulointerstitial nephritis (867–869,872). The most common patterns are membranoproliferative glomerulonephritis (with or without features of cryoglobulinemic glomerulonephritis) and membranous glomerulonephritis (867,870,899–903). Crescentic glomerulonephritis, coexistent either with membranous glomerulonephritis (904–906) or of the pauci-immune type (907) has also been described. Many of the cases of pauci-immune crescentic glomerulonephritis are associated with circulating anti-MPO ANCA (881–885,906). Several cases of IgA nephropathy have been described in patients with SS (908–912). In some cases, IgA nephropathy was present for several years prior to the diagnosis of SS (909,910) suggesting coexistent, unrelated diseases. On the other hand, at least two of these cases showed crescentic IgA nephropathy and were accompanied by signs of cutaneous vasculitis (911,912), suggesting a shared pathogenetic mechanism. Of note, cryoglobulins were not detected in either of these cases. Two cases were associated with low C4 levels and glomerular C4 deposits, and one case was associated with concurrent autoimmune hepatitis (911). There are also two reports of minimal change disease with mesangial IgA nephropathy (869,913) and one report of amicrobial pustulosis associated and SS and mild IgA nephropathy in a Japanese man (914). Of note, all but two of these cases occurred in East Asian patients, reflecting the higher prevalence of IgA nephropathy in these populations. There were no cases of IgA nephropathy in the largest renal biopsy series (24 cases) of primary SS from a predominantly Caucasian population followed at Mayo Clinic, United States (869), or in a series of 18 biopsies performed in patients from Greece (867).

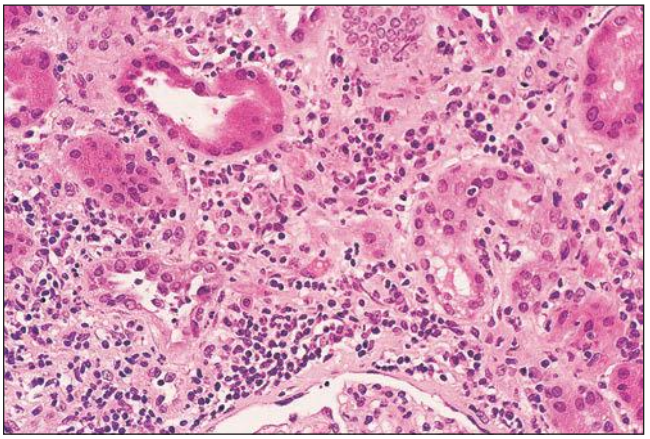


**FIGURE 14.77 Sjögren syndrome.** There is tubulointerstitial nephritis with interstitial inflammation by lymphocytes and plasma cells with mild focal tubulitis. (H&E, ×250.)

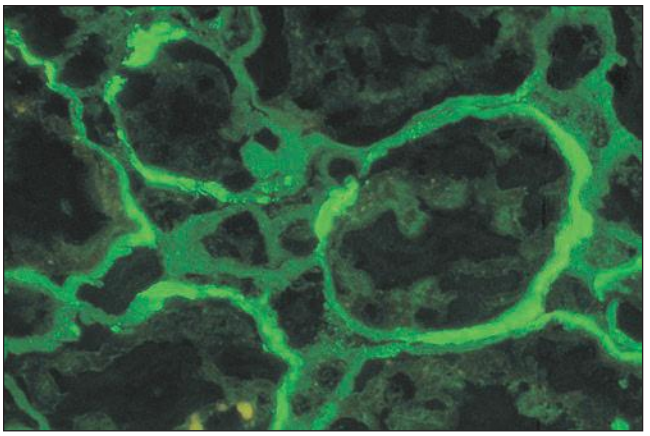


**FIGURE 14.78 Sjögren syndrome.** Immunofluorescence micrograph shows semilinear tubular basement membrane deposits of IgG. (×600.)

In secondary SS associated with SLE, all the patterns of lupus nephritis have been described, including mesangial proliferative, focal proliferative, diffuse proliferative or membranoproliferative, and membranous glomerulonephritis. Interestingly, renal disease appears to be *less* common in SLE patients with SS compared to those who do not have SS (915,916). In some cases, glomerulonephritis (documented on repeat renal biopsy) developed several years after isolated tubulointerstitial nephritis was identified on an initial renal biopsy (917). Immune deposits in mesangial, subendothelial, and subepithelial locations have been documented by immunofluorescence and electron microscopy, corresponding to the histologic pattern of glomerular injury. Dominant staining for IgG is typically seen, but IgM tends to predominate in the cases of membranoproliferative glomerulonephritis associated with cryoglobulinemia. In cases of combined glomerulonephritis and tubulointerstitial nephritis, immune deposits are typically found in both the glomerular and tubulointerstitial compartments.

There are rare case reports of other glomerular diseases in patients with primary SS, including type III membranoproliferative glomerulonephritis (903), membranoproliferative glomerulonephritis with podocytic infolding (918), membranous glomerulonephritis associated with EBV-positive diffuse large B-cell lymphoma (919), FSGS, minimal change disease (869), and secondary amyloidosis (920).

### Clinical Course, Prognosis, Therapy, and Clinicopathologic Correlations

Patients with mild renal involvement due to tubulointerstitial nephritis may not require specific therapy for their renal disease. Those patients with severe RTA and serum bicarbonate concentrations of less than 20 mM complicated by osteomalacia or nephrocalcinosis may respond to low-dose steroid therapy (921). Attention to adequate fluid intake is required to avert episodes of volume depletion from diabetes insipidus. High-dose oral or intravenous steroid therapy, with or without a cytotoxic agent, is reserved for cases of tubulointerstitial nephritis with evidence of renal insufficiency or a rapidly progressive course (922,923) and is efficacious in the majority of cases (868). Patients with lupus-like glomerulonephritis should be treated in an appropriate manner for the class of lupus nephritis identified by renal biopsy. The subgroup of patients manifesting renal vasculitis or pauci-immune crescentic glomerulonephritis may benefit from aggressive immunosuppressive or cytotoxic therapy in a similar regimen to that used in microscopic polyangiitis (882).

## RHEUMATOID ARTHRITIS

### Introduction

RA is an autoimmune inflammatory disease of the joints that may be accompanied by extra-articular manifestations, including rheumatoid nodules, mononeuritis multiplex, pericarditis, pulmonary nodules, pulmonary interstitial fibrosis, episcleritis, and systemic vasculitis. Renal disease associated with RA falls into three major categories: secondary amyloidosis related to amyloid A protein (AA amyloidosis), complications of drug therapy, and renal disease related to RA itself or to overlap with other autoimmune conditions (924–926). In addition, coincidental renal disease unrelated to RA or its therapy may occur. Compared with other collagen vascular diseases, the incidence of renal disease directly attributable to RA is relatively low. Nonetheless, such renal complications may cause significant morbidity and mortality (927,928).

The etiology and pathogenesis of RA are unknown but both genetic and environmental factors, notably cigarette smoking, play a role (929). RF and anticitrullinated peptide antibodies (ACPA) are highly specific for RA, and ACPA are enriched in the synovial fluid, suggesting local production or entrapment at sites of synovial inflammation (930,931). Certain HLA haplotypes increase susceptibility to RA, consistent with oligogenic influences (932). In the majority of cases (approximately 80%) that have RF and ACPA, there is a strong association with certain HLA-DRB1 alleles that share a conserved amino acid sequence at positions 67–74 (the "shared epitope"). ACPA-negative RA, on the other hand, is associated with HLA-DR3 alleles and interferon regulatory factor gene variants (933).

Morphologically, the first detectable abnormality in RA is infiltration of the synovial membrane by macrophages, followed by influx of B cells, T cells, and neutrophils; proliferation of blood vessels; and progressive thickenings of the synovial membrane, forming pannus (934,935). There is extensive class II antigen expression by macrophages, type A synoviocytes, dendritic cells, lymphocytes, and endothelial cells, as well as up-regulation of integrins, creating an immunologically activated environment (936). Macrophages can mediate cartilage destruction directly through protease release or indirectly through release of IL-1, which stimulates chondrocytes to release lysosomal enzymes (937). Because erosive and destructive joint disease can occur early in the course of RA, early diagnosis and initiation of treatment with disease modifying antirheumatic drugs (DMARD) are needed to prevent chronic injury.

### Diagnosis of Rheumatoid Arthritis

The 1987 revised ACR criteria for the classification of RA include the following seven criteria: morning stiffness in and around the joints, arthritis of three or more joint areas (including PIP, metacarpophalangeal, wrist, elbow, knee, ankle, and metatarsophalangeal joints), arthritis of hand joints, symmetric arthritis, rheumatoid nodules, serum RF, and radiographic changes such as erosions or bony decalcification adjacent to involved joints. The first four features must be present for at least 6 weeks to confirm the diagnosis.

The 2010 ACR and EULAR criteria were designed to identify features that are present at earlier stages of the disease (i.e., less than 6 weeks duration) prior to onset of persistent and/or erosive disease, with the goal of identification of patients most likely to benefit from early intervention with DMARD (938). These criteria include synovitis in at least one joint, with no alternative diagnosis better explaining the synovitis, and a score of 6 or more (of a possible 10) from the individual scores in the following four domains: number and site of involved joints (range 0 to 5); serologic abnormality (positive RF and/or ACPA [range 0 to 3]); elevated acute phase response (range 0 to 1); and symptom duration (less than or more than or equal to 6 weeks; range 0 to 1) (938). In a recent meta-analysis, the ACR/EULAR criteria showed good sensitivity (73% to 80%) and specificity (61% to 74%) for classifying cases of early RA (939). Compared to the 1987 ACR criteria, the 2011 ACR/EULAR criteria are more sensitive but less specific for detection of RA (940).

### Renal Disease in Rheumatoid Arthritis

The historical spectrum of RA-associated renal disease reflects the tissues studied (e.g., biopsy vs. autopsy, with or without immunofluorescence and electron microscopy), prevailing therapies, availability of serologic tests (e.g., for ANCA), and changing disease definitions. The role of intercurrent or terminal events such as infection, rheumatic fever, or overlap with other collagen diseases cannot be ascertained from historical reports. In the modern era, the commonest renal biopsy findings in RA patients consist of mesangial proliferative glomerulonephritis, membranous glomerulonephritis, AA amyloidosis, focal proliferative glomerulonephritis, minimal change disease, and acute tubulointerstitial nephritis (926,941). It is highly likely that current guidelines (942) recommending earlier use of nonbiologic and biologic DMARD to control inflammation will lead to further evolution in the spectrum of RA-associated renal disease.

## Pathologic Findings

### Secondary (AA) Amyloidosis

Secondary amyloidosis, characterized by extracellular fibrillar deposits composed of SAA protein, is a complication of diverse chronic inflammatory and infectious disorders (see Chapter 22). RA continues to be the single most common cause of AA amyloidosis in North America and in Northern Europe (943–945). AA amyloidosis may affect multiple organs, including heart, gastrointestinal tract, and liver, but renal involvement is the most common manifestation (present in up to 95% of patients), and this tends to dominate the clinical course, with a significant negative impact on patient survival.

The incidence of AA amyloidosis in RA patients is unknown. The prevalence in cross-sectional biopsy studies ranges from 0% to 5% but has exceeded 50% in series where proteinuria was the major biopsy indication (946–950). Autopsy prevalence rates range from 6% to 61% (average 15%) (946). Importantly, studies with fat pad and gastrointestinal biopsies in RA patients have shown that AA amyloidosis may be clinically silent in up to 50% of cases (951,952). Clinically symptomatic amyloidosis is usually associated with a duration of RA exceeding 10 to 15 years (953,954). The incidence of RA-associated amyloidosis appears to be decreasing (955,956), possibly reflecting better control of inflammation. In a study of 1666 RA patients who died in Finland during 1989, the prevalence of amyloidosis was 5.8% (941). However, one Japanese study reported that the renal biopsy prevalence of AA amyloidosis in RA patients remained unchanged between 1979 to 1988 (18%) and 1989 to 1996 (19%) (957).

Clinical manifestations include proteinuria and the nephrotic syndrome. Renal insufficiency is also common, particularly in cases with more advanced disease. Amyloidosis may affect glomeruli, tubular basement membranes, interstitium, and blood vessels in any combination. In general, proteinuria correlates best with glomerular involvement, and patients with predominantly vascular amyloid deposits may have little or no proteinuria. Cases with prominent tubulointerstitial involvement, including the medullary interstitium and tubular basement membranes, may develop specific tubular defects such as RTA or impaired concentrating ability leading to nephrogenic diabetes insipidus (958). Morphometric studies have shown a correlation between the parenchymal area replaced by amyloid and the degree of renal functional impairment (959).

By light microscopy and electron microscopy, RA-associated AA amyloidosis is indistinguishable from other causes of AA amyloidosis. By definition, amyloid deposits demonstrate Congo red positivity. Glomerular amyloid typically causes acellular mesangial expansion, sometimes with nodular features, and thickening of GBMs. Amyloid deposits characteristically have a pale eosinophilic hyaline appearance, are weakly PAS positive, trichrome gray, and nonargyrophilic in routine stains (Fig. 14.79). Rare cases of crescentic glomerulonephritis associated with AA amyloidosis have been described (960). The amyloid deposits react for SAA protein by immunohistochemical or immunofluorescence techniques performed on formalin-fixed or frozen tissue. By immunofluorescence microscopy, AA amyloidosis either is entirely negative or demonstrates weak smudgy reactivity with antisera to immunoglobulins and albumin, due to nonspecific trapping of plasma proteins in the amyloid deposits. Aspirated abdominal fat or rectal biopsy have been used successfully to diagnose systemic amyloidosis, but the sensitivity of these techniques for diagnosing renal AA amyloidosis is less than that of renal biopsy (fat pad, 58%; rectal biopsy, 82%; renal biopsy, 100%) (943). Moreover, interpretation of AA immunostains in aspirated fat samples is complicated by background staining. The presence of AA amyloid in fat pad biopsies can be confirmed by Western blot (961) and quantitated by ELISA (962). More recently, laser microdissection and mass spectrometry mass have emerged as valuable tools for classifying amyloid deposits, including AA amyloid (963). Radiolabeled serum amyloid P component, a serum protein codeposited in amyloid deposits, has been used as an imaging technique to determine the distribution of systemic amyloid deposition (964).

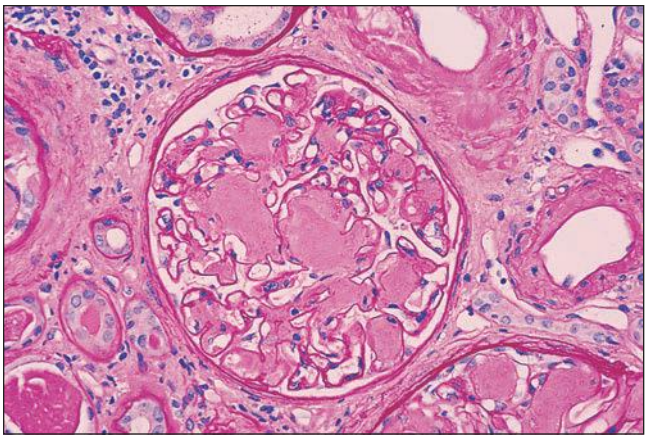


**FIGURE 14.79 Rheumatoid arthritis.** An elderly woman with a 15-year history of RA developed nephrotic syndrome. Renal biopsy revealed amyloidosis with large amorphous weakly PAS-positive deposits in the glomerular mesangium and in adjacent arterial walls.

The clinical outcome of RA-associated AA amyloidosis with renal disease is poor. In the study by Couverchel, the mean age of diagnosis of renal amyloidosis in a group of 34 patients with RA was 57 years, with a mean interval of 13.7 years from initial diagnosis of RA to the diagnosis of amyloidosis (954). Fifty percent of patients had renal insufficiency at the time of diagnosis of amyloidosis. During a 4-year follow-up period, 20 of the 34 patients died, and 95% of mortalities were related to renal failure in these patients, of whom 45% were on dialysis. Another study from the Netherlands (965) confirmed the bleak prognosis of RA patients with secondary amyloidosis and end-stage renal disease. The median patient survival of 13 RA patients with amyloidosis on dialysis was only 11 months, compared with 29 months for RA patients with end-stage renal disease due to other causes. Cardiovascular causes of death and problems with vascular access were especially common in the group with amyloidosis. Recurrence of AA amyloidosis in the renal allograft occurs in up to 14% of cases and is associated with significantly decreased patient survival, with deaths mainly due to infections and acute cardiovascular events (966).

There is no specific therapy for AA amyloidosis, but control of the underlying inflammation leads to better outcomes. Improved renal survival has been reported in patients treated with cyclophosphamide or with cyclophosphamide plus prednisolone, AZA, or the alkylating agent chlorambucil (954,967,968). Combined therapy with prednisolone and

methotrexate has also been successful in inducing a remission of nephrotic syndrome in some cases (969). More recently, the anti-TNF-α agents etanercept (970,971), infliximab (972), and tocilizumab (a humanized anti-IL-6 receptor antibody) (973) have shown efficacy in the treatment of RA-associated AA amyloidosis. Eprodisate, a small negatively charged, sulfonated molecule that interferes with interactions between amyloidogenic proteins and glycosaminoglycans, was shown to reduce progression in AA amyloidosis in a randomized, double-blind, placebo-controlled trial of 183 patients (974).

## Treatment-Related Renal Disease

The spectrum of RA-associated renal disease attributed to medications has changed over time, reflecting evolving standards of care. Thus, analgesic nephropathy and kidney disease related to gold salts are no longer commonly encountered and have mainly historical significance.

### Analgesic Nephropathy

Analgesic nephropathy, characterized by papillary necrosis and chronic tubulointerstitial nephritis, was featured in historical reports of RA-associated renal disease, but this complication has largely disappeared in the modern era. This likely reflects greater awareness of the nephrotoxic risk of analgesic agents, less frequent use of combination analgesic compounds, and removal of analgesic compounds containing phenacetin from the market. However, there are rare reports of renal papillary necrosis occurring in patients treated with the selective cyclooxygenase 2 (COX-2) inhibitor celecoxib (975).

### Nephrotoxicity of Nonsteroidal Anti-Inflammatory Agents

NSAIDs may cause acute tubulointerstitial nephritis, with or without associated minimal change disease (see Chapter 25). The combination of minimal change nephrotic syndrome and acute tubulointerstitial nephritis is specific to this class of drugs. Patients usually present after many months or years of drug exposure with acute onset of the nephrotic syndrome, pyuria and microscopic hematuria, and variable renal insufficiency. However, unlike other forms of drug-induced allergic tubulointerstitial nephritis, hypersensitivity features, including rash, fever, eosinophilia, and eosinophiluria, are usually lacking. Membranous glomerulonephritis is a less common complication of NSAID therapy (976). The membranous glomerulonephritis and proteinuria usually resolve following drug withdrawal (976). A similar spectrum of renal toxicities, including acute tubulointerstitial nephritis and membranous glomerulonephritis, can occur in patients treated with selective COX-2 inhibitors (977).

In some patients with RA, use of NSAIDs may precipitate episodes of acute renal failure due to inhibition of cyclooxygenase and reduced renal synthesis of vasodilatory prostaglandins. Patients at risk for this complication have underlying conditions that tend to jeopardize renal perfusion, such as volume depletion, congestive heart failure, ascites, or any form of intrinsic renal disease.

### Nephrotoxicity of Cyclosporine

Cyclosporine-induced nephrotoxicity includes vasoconstriction, causing a reversible functional reduction in the GFR, and intrinsic reversible or irreversible renal parenchymal disease, including tubular, interstitial, and vascular toxicities (978). As discussed in Chapter 29, these renal toxicities include isometric tubular vacuolation, interstitial fibrosis (which may be patchy [i.e., "stripe-like"] or diffuse), thrombotic microangiopathy, and hyaline arteriolopathy.

Coadministration of NSAIDs is a risk factor for cyclosporine toxicity (979). Cyclosporine therapy exacerbates the decrement in the GFR caused by treatment with sulindac and naprosyn (980).

A biopsy study of 41 patients with RA treated with cyclosporine for mean of 16 months at a maximum dose of 4.6 ± 1.2 mg/kg/d showed morphologic evidence of cyclosporine toxicity in four patients (10%), including moderate focal interstitial fibrosis and tubular atrophy, often accompanied by arteriolar lesions (981). Cyclosporine nephrotoxicity was most likely to occur in those receiving cyclosporine doses greater than 5 mg/kg/d and those with elevated initial serum creatinine levels at the outset of therapy. No patient younger than 50 years of age developed nephrotoxicity, suggesting an increased susceptibility to nephrotoxicity in older patients (981). Three (75%) of the four patients with biopsy-documented cyclosporine nephrotoxicity had received concurrent NSAID therapy, compared with 17 (46%) of 37 without NSAID therapy (981).

In a biopsy study of the Canadian Multicentre Rheumatology Group (982), 14 patients with severe RA and normal renal function after treatment with low-dose (less than 5 mg/kg/d) cyclosporine therapy for 6 months were subjected to surveillance renal biopsy. The renal biopsy findings were minimal and consisted of focal glomerular obsolescence (five cases), mild interstitial fibrosis (four cases), and intimal arterial thickening (nine cases). Only one biopsy was considered to demonstrate pathologic findings relatively specific for cyclosporine toxicity. Although no controls were studied, this study suggests that the incidence of irreversible renal toxicity due to low-dose cyclosporine in RA patients is relatively small. In a study of 22 RA patients receiving starting doses of cyclosporine less than 4 mg/kg/d and followed for up to 87 months, none developed cyclosporine nephrotoxicity (983), further underscoring the dose-dependent nature of the toxicity.

### Nephrotoxicity of Gold Salts and Penicillamine

The most common renal complication of treatment with gold salts and D-penicillamine is membranous glomerulonephritis. This is usually heralded clinically by the development of severe proteinuria or full nephrotic syndrome. Proteinuria complicates gold therapy in from 1% to 10% of treated patients (984–990) over a wide age range (2 to 73 years) with parenteral (990,991) or oral gold (986) administration. About 50% of patients develop proteinuria within the first 6 months of treatment and 85% by 24 months, with the peak onset between 3 and 6 months of the initiation of gold therapy (990–992). The severity of proteinuria ranged from 0.7 g to greater than 30 g daily according to one report (990), and in one third of cases, it was accompanied by the nephrotic syndrome. As previously noted, gold salts are no longer used routinely for the management of RA, and therefore, this complication is unlikely to be encountered in the modern era.

There appears to be no correlation between the total cumulative dose of gold received and the likelihood of developing proteinuria. Cumulative doses of gold before development of membranous glomerulonephritis have ranged from 10 to 6,000 mg (988,993–995). The outcome after discontinuation

of gold therapy varies. In one study (990), remission of proteinuria was achieved in 36% of patients by 6 months after discontinuing gold therapy, in 62% by 1 year, in 86% by 2 years, and in 100% by 3 years. However, according to some reports, proteinuria may persist in a minority of cases despite drug withdrawal (988,996–1000).

The subepithelial deposits in gold-associated membranous glomerulonephritis may be segmental in distribution. These deposits stain for IgG and C3, with occasional codeposits of IgM and IgA. In most cases, the glomerulus appears normal in cellularity, but mild mesangial hypercellularity may be seen. Most biopsies demonstrate early-stage membranous glomerulonephritis, either lacking spikes (stage 1) or exhibiting widespread spike formation (stage 2). Morphologically, gold-induced membranous glomerulonephritis is indistinguishable from idiopathic membranous glomerulonephritis. Unlike other secondary forms of membranous glomerulonephritis related to SLE or hepatitis B infection, no mesangial deposits are observed. A characteristic feature of gold-induced membranous glomerulonephritis is the ultrastructural demonstration of gold inclusions forming electron-dense filamentous strands within proximal tubular epithelial cells, glomerular epithelial cells, and mesangial cells (988,996,1000).

D-Penicillamine induces proteinuria in a larger percentage of treated patients (up to 30%) (946,1001) and is associated with a higher frequency of the nephrotic syndrome. The risk of developing proteinuria may (1001) or may not (1002,1003) be dose related. The onset of proteinuria is usually recognized within the first 6 months of therapy and is rarely observed after 1 year (1001,1004–1006). In most cases, proteinuria resolves spontaneously with discontinuation of the drug (1001). Morphologically, membranous glomerulonephritis associated with D-penicillamine is indistinguishable from primary membranous glomerulonephritis.

A less common complication of penicillamine therapy is the development of focal segmental necrotizing and crescentic glomerulonephritis (1007–1016). Many of these patients present with a pulmonary-renal syndrome of hemoptysis and rapidly progressive renal failure resembling Goodpasture syndrome. However, anti-GBM antibodies and linear GBM deposits have not been identified. Morphologically, there are scant or no glomerular immune deposits of IgG, IgM, IgA, and C3, consistent with pauci-immune crescentic glomerulonephritis. Of note, anti-MPO ANCA have been reported in many of these cases (1015,1016). One case displayed mixed features of ANCA-associated crescentic glomerulonephritis and membranous glomerulonephritis (1015). In most of these cases, it is impossible to determine whether the association of ANCA-positive crescentic glomerulonephritis is pathogenetically linked to penicillamine therapy or represents an autoimmune complication of RA itself.

Bucillamine, a cysteine derivative with close structural resemblance to D-penicillamine, has been widely prescribed in Japan for treatment of RA and is also associated with the development of proteinuria and membranous glomerulonephritis (1017,1018). At least 30 cases of bucillamine-associated nephrotic syndrome have been reported (1017–1020) with membranous glomerulonephritis in most biopsied cases, and single cases of minimal change disease and crescentic glomerulonephritis. By electron microscopy, electron-dense deposits were sometimes observed in the subepithelial and mesangial regions. Proteinuria usually resolves within 6 months after discontinuation of therapy.

The cause of membranous glomerulonephritis related to gold, penicillamine, and bucillamine therapy is uncertain. There is an increased association with HLA-DRw3 and HLA-B8 (1021–1025), suggesting predisposition to an autoimmune response. Because gold is known to be a tubular toxin in experimental animals, it has been proposed that release of renal tubular epithelial antigens might promote an autoantibody response to related podocyte antigens, similar to experimental models of Heymann nephritis. Gold-sulfur complexes have been demonstrated by x-ray microanalysis in proximal tubular epithelium but not in the glomerular capillary wall, supporting that the pathogenesis may involve renal tubular epithelial antigens, rather than gold acting as a hapten (1026). Although early studies of experimental gold nephropathy in guinea pigs and rats detected renal tubular epithelial antigens by indirect immunofluorescence in the glomerular immune complexes (1027) and the renal eluates (1028), such a mechanism has not been proved in humans.

### Nephrotoxicity of Biologic Agents

Anti-TNF-α agents used in the management of RA include etanercept, a soluble TNF-α receptor; infliximab, a chimeric monoclonal IgG1 anti-TNF-α antibody; and adalimumab, golimumab, and certolizumab, which are fully humanized monoclonal antibodies. Immune dysregulation caused by these agents is associated with the development of autoantibodies, including ANA, anti-dsDNA antibodies, anticardiolipin antibodies, and ANCA. In addition, there are several reports of new onset renal disease in RA patients while undergoing treatment with these agents (471,1029,1030). Renal manifestations include membranous glomerulonephritis, proliferative lupus-like nephritis (associated with positive lupus serologies and hypocomplementemia in most cases), pauci-immune necrotizing and crescentic glomerulonephritis (with or without ANCA seropositivity), anti-GBM disease, IgA nephropathy, and renal vasculitis (471,1029–1041). There is a single case report of systemic vasculitis and rapidly progressive glomerulonephritis associated with new development of anti-GBM antibodies and rising titer of MPO-ANCA in a patient receiving adalimumab for RA (1037). Following discontinuation of adalimumab and treatment with plasmapheresis, cyclophosphamide and steroids, both antibody titers declined rapidly but renal function did not recover (1037). In one case of membranous glomerulonephritis, the proteinuria resolved after cessation of anti-TNF-α treatment and relapsed after rechallenge (1042). In addition, several cases of IgA nephropathy (1038) and Henoch-Schonlein pupura nephritis (1039–1041) have also been reported in patients receiving anti-TNF-α agents (1038). The strong temporal association between the onset of new renal disease and anti-TNF-α therapy in these patients with longstanding RA and the improvement in clinical and laboratory abnormalities after drug withdrawal and initiation of immunosuppressive therapy in most cases strongly support a pathogenetic role for anti-TNF-α agents.

One case report describes new onset of a lupus-like focal proliferative glomerulonephritis (with "full-house" immunofluorescence staining for IgG, IgM, IgA, C3, C4, and C1q) during treatment of RA with tocilizumab, an anti-IL-6 monoclonal antibody (1043). On the other hand, tocilizumab has

shown efficacy in treating cases of RA-associated vasculitis, including two cases with necrotizing crescentic glomerulonephritis (1044,1045). Therefore, it appears that anti-IL-6 agents may precipitate or ameliorate some forms of RA-associated glomerulonephritis. There is also a single case report of mesangial proliferative IgA nephropathy developing after treatment of RA with abatacept, a recombinant human fusion protein that inhibits T-cell activation (1046). Following discontinuation of abatacept and treatment with corticosteroids, renal symptoms improved, suggesting a possible pathogenetic role.

#### Immune Complex Glomerulonephritis During Staphylococcal A Protein Immunoadsorption

Iglesias et al. reported a case of immune complex glomerulonephritis developing in a patient with RA while undergoing apheresis by immunoadsorption to staphylococcal A protein (1047). Renal biopsy showed diffuse endocapillary proliferative glomerulonephritis with protein thrombi. Immunofluorescence showed staining for IgG, IgM, IgA, C3, kappa, and lambda, with slightly stronger staining for IgA (2-3+), compared to IgG (2+) and IgM (1+). Electron microscopy revealed granular immune-type mesangial and subendothelial electron-dense deposits, without an organized substructure. Serum C4 level was decreased, but repeated cryoglobulin studies and anti-dsDNA serology were negative. The authors identified four additional cases, from the literature and from information supplied by the drug manufacturer, of immune complex glomerulonephritis developing in patients undergoing staphylococcal A protein immunoadsorption, including three cases with features of diffuse proliferative glomerulonephritis and one case of crescentic glomerulonephritis. The pathogenesis of immune complex glomerulonephritis in these cases is unknown, but 3 of the 5 patients had a history of leukocytoclastic vasculitis, suggesting that this was a significant risk factor for the development of glomerulonephritis.

## Rheumatoid Arthritis-Related Nephropathies

The existence of renal disease secondary to RA itself, independent of drug effects, has been a matter of considerable controversy. A diverse group of glomerular lesions that cannot be clearly linked to adverse complications of therapy have been reported, albeit rarely, in patients with RA (924,926,957,1048–1052). These include mesangial proliferative glomerulonephritis, membranous glomerulonephritis, and pauci-immune focal necrotizing and crescentic glomerulonephritis. There are also reports of FSGS, minimal change disease, diabetic nephropathy, fibrillary glomerulonephritis (442,1053), and IgA nephropathy (924,926), some of which may represent coexistent glomerular diseases not directly related to RA.

### Mesangial Proliferative Glomerulonephritis

Among 23 patients with mesangial proliferative glomerulonephritis followed by Korpela, presentations included isolated hematuria in 10, isolated proteinuria in 6, and combined hematuria and proteinuria in 7 (1050). The researchers determined that the mesangial lesion presenting with proteinuria was more likely to be related to antirheumatic therapy (six for gold, two for penicillamine, one for auranofin) and was often reversible on drug withdrawal. In contrast, the mesangial proliferative lesion presenting as isolated hematuria could not be linked to any particular therapy and was typically persistent after discontinuation of antirheumatic therapy. Renal function was normal in most of these patients (1050,1054).

By light microscopy, there is typically only mild mesangial hypercellularity and increased matrix without compromise of the glomerular capillary lumina. By immunofluorescence in 40 cases reported by Helin (926,1049), IgM was the sole immune reactant (12 specimens) or occurred with lesser amounts of C3 (4 specimens), IgA (6 specimens), C1q (1 specimen), IgG and C3 (1 specimen), or IgA and C3 (1 specimen). This form of "RA-associated IgM nephropathy" appears to be distinct from IgM nephropathy associated with idiopathic nephrotic syndrome, which falls within the minimal change disease/FSGS disease spectrum (1055). In a series of 158 Japanese patients with RA undergoing renal biopsy, mesangial proliferative glomerulonephritis was diagnosed in 54 patients, two thirds of whom had not received disease modifying drugs. Interestingly, 59% of these cases had IgA nephropathy, reflecting the frequency of this disease in the Japanese population (957). The ultrastructural findings and degree of foot process effacement have not been well documented. Small paramesangial electron-dense deposits may be seen. In one study, Korpela (948) observed that RA patients with mesangial glomerulonephritis were more likely to have high serum IgA and IgM concentrations compared with RA patients without nephropathy. An association between mesangial glomerulonephritis and high RF titers has also been described (1049,1056).

### Membranous Glomerulonephritis

Membranous glomerulonephritis may occur in some patients with RA who have never been treated with gold, penicillamine, or related therapeutic agents (957,1052,1057). In a series of four such patients reported by Honkanen, presentations included the nephrotic syndrome in two, persistent proteinuria and hematuria in one, and isolated hematuria in one (1057). One patient had received gold therapy 16 years before the development of proteinuria. The duration of RA before onset of renal disease varied from 3 to 22 years. Morphologically, three cases had exclusively subepithelial deposits and one had combined subepithelial and mesangial deposits. IgG and C3 were the sole or predominant immune reactants. Only one case had codeposition of IgM, IgA, and C1q.

Honkanen identified at least 18 other cases of membranous glomerulonephritis occurring in patients with RA that were unrelated to treatment (1057). Many of these patients were positive for RF. On follow-up, none developed evidence of SLE, suggesting that this lesion does not represent an overlap with lupus nephritis. Adu (924) reported five additional cases of membranous glomerulonephritis associated with RA. Only two of the five patients had received gold that was discontinued 13 and 18 years before the development of the nephrotic syndrome. They concluded that these cases could not be directly linked to therapy.

### Focal Necrotizing and Crescentic Glomerulonephritis

There have been numerous reports of pauci-immune focal segmental necrotizing and crescentic glomerulonephritis occurring in patients with RA who had not been exposed to gold, penicillamine, or anti-TNF-α agents (924,1058–1068). Many of these patients also had cutaneous or multisystem vasculitis.

Although ANCA serologies were not tested systematically in earlier reports, most such cases diagnosed in the modern era have been anti-MPO ANCA seropositive (1061,1063,1065–1068). In a series of 246 RA patients studied by Mustila et al., including 149 subjects who had renal disease, 52 patients (21%) had positive P-ANCA, and there was a highly significant association between the development of nephropathy and positive P-ANCA serology (1061). P-ANCA was not significantly associated with any particular pattern of renal disease but was positive in three of four (75%) cases with focal proliferative glomerulonephritis; however, the presence of crescents in these biopsies was not noted. In comparison to patients without RA, anti-MPO ANCA-associated crescentic glomerulonephritis in RA tends to occur at a younger age and demonstrates fewer extrarenal manifestations, a longer duration from onset of symptoms to renal biopsy confirmation, and more advanced glomerular scarring (834). These data suggest that focal segmental necrotizing and crescentic glomerulonephritis in RA patients is pathogenetically related to MPO-ANCA in most cases, consistent with the high prevalence of anti-MPO antibody in RA.

### Other Renal Lesions in RA

There are rare cases of rheumatoid vasculitis involving large renal arteries, some with associated aneurysm formation (950,1069). There is a single report of concurrent renal amyloidosis and crescentic glomerulonephritis with multisystem vasculitis (1070) and a few cases of fibrillary glomerulonephritis (442,1053). There are also case reports of membranoproliferative glomerulonephritis with monoclonal IgM deposits in the setting of coexistent Waldenström macroglobulinemia (1071) as well as renal thrombotic microangiopathy related to coexistent APL syndrome (1072).

## REFERENCES


1. Virchow R. Historical note on lupus [in German]. *Arch Pathol Anat* 1865;32:139.
2. Kaposi M. New reports on knowledge of lupus erythematosus [in German]. *Arch Dermatol Syph* 1872;4:36.
3. Cazenave A, Schedel HE. *Abrege pratique des maladies de la peau d'apres les auteurs les plus estimes, et surtout d'apres des documens puises dans les lecons cliniques de M. le Docteur Biett*. 3rd ed. Paris: Bechet, 1838.
4. Hutchinson J. On lupus and its treatment. *Br Med J* 1880;1:650.
5. Osler W. On the visceral complications of erythema exudativum multiforme. *Am J Med Sci* 1895;110:629.
6. Osler W. The visceral lesions of the erythema group. *Br J Dermatol* 1900;12:227.
7. Osler W. On the visceral manifestations of the erythema group of skin diseases. *Trans Assoc Am Physicians* 1903;18:599.
8. Libman E, Sacks B. A hitherto undescribed form of valvular and mural endocarditis. *Arch Intern Med* 1924;33:701.
9. Baehr G, Klemperer P, Schifrin A. A diffuse disease of the peripheral circulation (usually associated with lupus erythematosus and endocarditis). *Am J Med* 1952;13(5):591–596.
10. Hargraves MM, Richmond H, Morton R. Presentation of 2 bone marrow elements; "tart" cell and "L.E." cell. *Proc Staff Meet Mayo Clin* 1948;23:25.
11. Gross L. Cardiac lesions in Libman-Sacks disease with consideration of its relationship to acute diffuse lupus erythematosus. *Am J Pathol* 1940;16:375.
12. Coons A. Symposium on labeled antigens and antibodies. fluorescent antibodies as histochemical tools. *Fed Proc* 1951;10:558.
13. Friou GJ. The significance of the lupus globulin; nucleoprotein reaction. *Ann Intern Med* 1958;49(4):866–875.
14. Cameron JS. Lupus nephritis in childhood and adolescence. *Pediatr Nephrol* 1994;8(2):230–249.
15. Fish AJ, Blau EB, Westberg NG, et al. Systemic lupus erythematosus within the first two decades of life. *Am J Med* 1977;62(1):99–117.
16. Yang LY, Chen WP, Lin CY. Lupus nephritis in children—a review of 167 patients. *Pediatrics* 1994;94(3):335–340.
17. McCluskey R. *Lupus Nephritis*. East Norwalk, CT: Appleton-Century-Crofts, 1975.
18. Appel GB, Silva FG, Pirani CL, et al. Renal involvement in systemic lupus erythematosus (SLE): A study of 56 patients emphasizing histologic classification. *Medicine (Baltimore)* 1978;57(5):371–410.
19. Churg J, Sobin L. *Renal Disease: Classification and Atlas of Glomerular Diseases*. Tokyo, Japan: Igaku-Shoin, 1982.
20. Churg J, Bernstein J, Glassock R. *Renal Disease: Classification and Atlas of Glomerular Diseases*. 2nd ed. New York: Igaku-Shoin, 1995.
21. Weening JJ, D'Agati VD, Schwartz MM, et al. The classification of glomerulonephritis in systemic lupus erythematosus revisited. *J Am Soc Nephrol* 2004;15(2):241–250.
22. Weening JJ, D'Agati VD, Schwartz MM, et al. The classification of glomerulonephritis in systemic lupus erythematosus revisited. *Kidney Int* 2004;65(2):521–530.
23. Lewis EJ, Kawala K, Schwartz MM. Histologic features that correlate with the prognosis of patients with lupus nephritis. *Am J Kidney Dis* 1987;10(3):192–197.
24. Mittal B, Rennke H, Singh AK. The role of kidney biopsy in the management of lupus nephritis. *Curr Opin Nephrol Hypertens* 2005;14(1):1-8.
25. Kashgarian M. Lupus nephritis: Lessons from the path lab. *Kidney Int* 1994;45(3):928–938.
26. Neumann K, Wallace DJ, Azen C, et al. Lupus in the 1980s: III. Influence of clinical variables, biopsy, and treatment on the outcome in 150 patients with lupus nephritis seen at a single center. *Semin Arthritis Rheum* 1995;25(1):47–55.
27. Hahn BH, McMahon MA, Wilkinson A, et al. American College of Rheumatology guidelines for screening, treatment, and management of lupus nephritis. *Arthritis Care Res (Hoboken)* 2012;64(6):797–808.
28. Klemperer P, Pollack AD, Baehr G. Pathology of disseminated lupus erythematosus. *Arch Pathol Anat* 1941;32:569.
29. Dujovne I, Pollak VE, Pirani CL, et al. The distribution and character of glomerular deposits in systemic lupus erythematosus. *Kidney Int* 1972;2(1):33–50.
30. Kant KS, Pollak VE, Weiss MA, et al. Glomerular thrombosis in systemic lupus erythematosus: Prevalence and significance. *Medicine (Baltimore)* 1981;60(2):71–86.
31. Castiglione A, Bucci A, Fellin G, et al. The relationship of infiltrating renal leucocytes to disease activity in lupus and cryoglobulinaemic glomerulonephritis. *Nephron* 1988;50(1):14–23.
32. Alexopoulos E, Seron D, Hartley RB, et al. Lupus nephritis: Correlation of interstitial cells with glomerular function. *Kidney Int* 1990;37(1):100–109.
33. Magil AB, Puterman ML, Ballon HS, et al. Prognostic factors in diffuse proliferative lupus glomerulonephritis. *Kidney Int* 1988;34(4):511–517.
34. Hill GS, Delahousse M, Nochy D, et al. Predictive power of the second renal biopsy in lupus nephritis: Significance of macrophages. *Kidney Int* 2001;59(1):304–316.
35. Austin HA III, Muenz LR, Joyce KM, et al. Prognostic factors in lupus nephritis. Contribution of renal histologic data. *Am J Med* 1983;75(3):382–391.
36. Austin HA III, Muenz LR, Joyce KM, et al. Diffuse proliferative lupus nephritis: Identification of specific pathologic features affecting renal outcome. *Kidney Int* 1984;25(4):689–695.
37. Balow JE, Austin HA III. Renal disease in systemic lupus erythematosus. *Rheum Dis Clin North Am* 1988;14(1):117–133.
38. Makino H, Sugiyama H, Yamasaki Y, et al. Glomerular cell apoptosis in human lupus nephritis. *Virchows Arch* 2003;443(1):67–77.
39. Makino H, Hayashi Y, Yamasaki Y, et al. Clinical significance of necrosis in lupus nephritis. *Intern Med* 1994;33(8):461–465.
40. Yang XW, Tan Y, Yu F, et al. Combination of anti-C1q and anti-dsDNA antibodies is associated with higher renal disease activity and predicts renal prognosis of patients with lupus nephritis. *Nephrol Dial Transplant* 2012;27(9):3552–3559.

41. Silva F, ed. *The Nephropathies of Systemic Lupus Erythematosus*. New York: Churchill Livingstone, 1983.
42. Markowitz GS, D'Agati VD. The ISN/RPS 2003 classification of lupus nephritis: an assessment at 3 years. *Kidney Int* 2007;71(6):491–495.
43. Hodgin JB, Whelan J, Markowitz GS, et al. Giant cell tubulitis with immune complex deposits in a patient with lupus nephritis. *Am J Kidney Dis* 2009;53(3):513–517.
44. Pollack AD. Some observations on the pathology of systemic lupus erythematosus. *J Mt Sinai Hosp N Y* 1959;26(3):224–240.
45. Boucher A, Droz D, Adafer E, et al. Characterization of mononuclear cell subsets in renal cellular interstitial infiltrates. *Kidney Int* 1986;29(5):1043–1049.
46. D'Agati VD, Appel GB, Estes D, et al. Monoclonal antibody identification of infiltrating mononuclear leukocytes in lupus nephritis. *Kidney Int* 1986;30(4):573–581.
47. Jeruc J, Jurcic V, Vizjak A, et al. Tubulo-interstitial involvement in lupus nephritis with emphasis on pathogenesis. *Wien Klin Wochenschr* 2000;112(15–16):702–706.
48. Alexopoulos E, Seron D, Hartley RB, et al. Immune mechanisms in idiopathic membranous nephropathy: the role of the interstitial infiltrates. *Am J Kidney Dis* 1989;13(5):404–412.
49. Alexopoulos E, Seron D, Hartley RB, et al. The role of interstitial infiltrates in IgA nephropathy: a study with monoclonal antibodies. *Nephrol Dial Transplant* 1989;4(3):187–195.
50. Winchester R, Wiesendanger M, Zhang HZ, et al. Immunologic characteristics of intrarenal T cells: trafficking of expanded CD8+ T cell beta-chain clonotypes in progressive lupus nephritis. *Arthritis Rheum* 2012;64(5):1589–1600.
51. Chang A, Henderson SG, Brandt D, et al. In situ B cell-mediated immune responses and tubulointerstitial inflammation in human lupus nephritis. *J Immunol* 2011;186(3):1849–1860.
52. Espeli M, Bokers S, Giannico G, et al. Local renal autoantibody production in lupus nephritis. *J Am Soc Nephrol* 2011;22(2):296–305.
53. Crispin JC, Oukka M, Bayliss G, et al. Expanded double negative T cells in patients with system[ic lupus erythematosus produce IL-17 and infiltrate the kidneys. *J Immunol* 2008;181(12):8761–8766.
54. Hill GS, Delahousse M, Nochy D, et al. Proteinuria and tubulointerstitial lesions in lupus nephritis. *Kidney Int* 2001;60(5):1893–1903.
55. Zhang X, Nagaraja HN, Nadasdy T, et al. A composite urine biomarker reflects interstitial inflammation in lupus nephritis kidney biopsies. *Kidney Int* 2012;81(4):401–406.
56. Halloran PF, Urmson J, Ramassar V, et al. Increased class I and class II MHC products and mRNA in kidneys of MRL-lpr/lpr mice during autoimmune nephritis and inhibition by cyclosporine. *J Immunol* 1988;141(7):2303–2312.
57. Wuthrich RP, Yui MA, Mazoujian G, et al. Enhanced MHC class II expression in renal proximal tubules precedes loss of renal function in MRL/lpr mice with lupus nephritis. *Am J Pathol* 1989;134(1):45–51.
58. Mohan C, Shi Y, Laman JD, et al. Interaction between CD40 and its ligand gp39 in the development of murine lupus nephritis. *J Immunol* 1995;154(3):1470–1480.
59. Yellin MJ, D'Agati V, Parkinson G, et al. Immunohistologic analysis of renal CD40 and CD40L expression in lupus nephritis and other glomerulonephritides. *Arthritis Rheum* 1997;40(1):124–134.
60. Brentjens JR, Sepulveda M, Baliah T, et al. Interstitial immune complex nephritis in patients with systemic lupus erythematosus. *Kidney Int* 1975;7(5):342–350.
61. Lehman DH, Wilson CB, Dixon FJ. Extraglomerular immunoglobulin deposits in human nephritis. *Am J Med* 1975;58(6):765–796.
62. Satoskar AA, Brodsky SV, Nadasdy G, et al. Discrepancies in glomerular and tubulointerstitial/vascular immune complex IgG subclasses in lupus nephritis. *Lupus* 2011;20(13):1396–1403.
63. Asherson RA, Hughes GR, Derksen RH. Renal infarction associated with anti-phospholipid antibodies in systemic lupus erythematosus and "lupus-like" disease. *J Urol* 1988;140(5):1028.
64. Park MH, D'Agati V, Appel GB, et al. Tubulointerstitial disease in lupus nephritis: relationship to immune deposits, interstitial inflammation, glomerular changes, renal function, and prognosis. *Nephron* 1986;44(4):309–319.
65. Fujii K, Kobayashi Y. Quantitative analysis of interstitial alterations in lupus nephritis. *Virchows Arch A Pathol Anat Histopathol* 1988;414(1):45–52.
66. Schwartz MM, Fennell JS, Lewis EJ. Pathologic changes in the renal tubule in systemic lupus erythematosus. *Hum Pathol* 1982;13(6):534–547.
67. Hunter MG, Hurwitz S, Bellamy CO, et al. Quantitative morphometry of lupus nephritis: the significance of collagen, tubular space, and inflammatory infiltrate. *Kidney Int* 2005;67(1):94–102.
68. Howie AJ, Turhan N, Adu D. Powerful morphometric indicator of prognosis in lupus nephritis. *QJM* 2003;96(6):411–420.
69. Kozeny GA, Barr W, Bansal VK, et al. Occurrence of renal tubular dysfunction in lupus nephritis. *Arch Intern Med* 1987;147(5):891–895.
70. Yeung CK, Wong KL, Ng RP, et al. Tubular dysfunction in systemic lupus erythematosus. *Nephron* 1984;36(2):84–88.
71. Bastani B, Underhill D, Chu N, et al. Preservation of intercalated cell H(+)-ATPase in two patients with lupus nephritis and hyperkalemic distal renal tubular acidosis. *J Am Soc Nephrol* 1997;8(7):1109–1117.
72. Kozeny GA, Hurley RM, Fresco R, et al. Systemic lupus erythematosus presenting with hyporeninemic hypoaldosteronism in a 10–year-old girl. *Am J Nephrol* 1986;6(4):321–324.
73. Lim TK, Fong KY. Hyperkalemic distal renal tubular acidosis with hyporeninemic hypoaldosteronism in a patient with systemic lupus erythematosus—a case report. *Singapore Med J* 1987;28(6):560–561.
74. Case records of the Massachusetts General Hospital. *N Engl J Med* 1976;294:100.
75. Klahr SLR. Clinicopathologic Conference: interstitial nephritis in a patient with systemic lupus erythematosus. *Am J Med* 1980;69:775.
76. Cunningham E, Provost T, Brentjens J, et al. Acute renal failure secondary to interstitial lupus nephritis. *Arch Intern Med* 1978;138(10):1560–1561.
77. Gur H, Kopolovic Y, Gross DJ. Chronic predominant interstitial nephritis in a patient with systemic lupus erythematosus: a follow up of three years and review of the literature. *Ann Rheum Dis* 1987;46(8):617–623.
78. Mori Y, Kishimoto N, Yamahara H, et al. Predominant tubulointerstitial nephritis in a patient with systemic lupus nephritis. *Clin Exp Nephrol* 2005;9(1):79–84.
79. Tron F, Ganeval D, Droz D. Immunologically-mediated acute renal failure of nonglomerular origin in the course of systemic lupus erythematosus [SLE]. Report of two cases. *Am J Med* 1979;67(3):529–532.
80. Henry R, Williams AV, McFadden NR, et al. Histopathologic evaluation of lupus patients with transient renal failure. *Am J Kidney Dis* 1986;8(6):417–421.
81. Henry R, Williams AV, McFadden NR, et al. Histopathologic evaluation of lupus patients with transient renal failure. *Am J Kidney Dis* 1986;8:417.
82. Makker SP. Tubular basement membrane antibody-induced interstitial nephritis in systemic lupus erythematosus. *Am J Med* 1980;69(6):949–952.
83. Appel GB, Pirani CL, D'Agati V. Renal vascular complications of systemic lupus erythematosus. *J Am Soc Nephrol* 1994;4(8):1499–1515.
84. Banfi G, Bertani T, Boeri V, et al. Renal vascular lesions as a marker of poor prognosis in patients with lupus nephritis. Gruppo Italiano per lo Studio della Nefrite Lupica (GISNEL). *Am J Kidney Dis* 1991;18(2):240–248.
85. Grishman E, Venkataseshan VS. Vascular lesions in lupus nephritis. *Mod Pathol* 1988;1(3):235–241.
86. Grishman E, Gerber MA, Churg J. Patterns of renal injury in systemic lupus erythematosus: Light and immunofluorescence microscopic observations. *Am J Kidney Dis* 1982;2(1 Suppl 1):135–141.
87. Barber C, Herzenberg A, Aghdassi E, et al. Evaluation of clinical outcomes and renal vascular pathology among patients with lupus. *Clin J Am Soc Nephrol* 2012;7(5):757–764.
88. Descombes E, Droz D, Drouet L, et al. Renal vascular lesions in lupus nephritis. *Medicine (Baltimore)* 1997;76(5):355–368.
89. Wu LH, Yu F, Tan Y, et al. Inclusion of renal vascular lesions in the 2003 ISN/RPS system for classifying lupus nephritis improves renal outcome predictions. *Kidney Int* 2013;83:715–723.
90. Brentjens JR, Andres G. Immunopathogenesis of renal vasculitis. *Semin Nephrol.* 1985;5(1):3-14.

91. Pirani CL, Olesnicky L. Role of electron microscopy in the classification of lupus nephritis. *Am J Kidney Dis* 1982;2(1 Suppl 1):150–163.
92. Bhathena DB, Sobel BJ, Migdal SD. Noninflammatory renal microangiopathy of systemic lupus erythematosus ('lupus vasculitis'). *Am J Nephrol* 1981;1(3-4):144–159.
93. Baldwin DS, Gluck MC, Lowenstein J, et al. Lupus nephritis. Clinical course as related to morphologic forms and their transitions. *Am J Med* 1977;62(1):12–30.
94. Cohen D, Koopmans M, Kremer Hovinga IC, et al. Potential for glomerular C4d as an indicator of thrombotic microangiopathy in lupus nephritis. *Arthritis Rheum* 2008;58(8):2460–2469.
95. Gelfand J, Truong L, Stern L, et al. Thrombotic thrombocytopenic purpura syndrome in systemic lupus erythematosus: Treatment with plasma infusion. *Am J Kidney Dis* 1985;6(3):154–160.
96. Magil AB, McFadden D, Rae A. Lupus glomerulonephritis with thrombotic microangiopathy. *Hum Pathol* 1986;17(2):192–194.
97. Gatenby PA, Smith H, Kirwan P, et al. Systemic lupus erythematosus and thrombotic thrombocytopenic purpura. A case report and review of relationship. *J Rheumatol* 1981;8(3):504–508.
98. Cecere FA, Yoshinoya S, Pope RM. Fatal thrombotic thrombocytopenic purpura in a patient with systemic lupus erythematosus. Relationship to circulating immune complexes. *Arthritis Rheum* 1981;24(3):550–553.
99. Fox DA, Faix JD, Coblyn J, et al. Thrombotic thrombocytopenic purpura and systemic lupus erythematosus. *Ann Rheum Dis* 1986;45(4):319–322.
100. Tsai HM, Lian EC. Antibodies to von Willebrand factor-cleaving protease in acute thrombotic thrombocytopenic purpura. *N Engl J Med* 1998;339(22):1585–1594.
101. Song D, Wu LH, Wang FM, et al. The spectrum of renal thrombotic microangiopathy in lupus nephritis. *Arthritis Res Ther* 2013;15(1):R12.
102. Wang FM, Yu F, Tan Y, et al. Serum complement factor H is associated with clinical and pathological activities of patients with lupus nephritis. *Rheumatology (Oxford)* 2012;51(12):2269–2277.
103. Oen K, Petty RE, Schroeder ML, et al. Thrombotic thrombocytopenic purpura in a girl with systemic lupus erythematosus. *J Rheumatol* 1980;7(5):727–729.
104. Farrugia E, Torres VE, Gastineau D, et al. Lupus anticoagulant in systemic lupus erythematosus: a clinical and renal pathological study. *Am J Kidney Dis* 1992;20(5):463–471.
105. Frampton G, Hicks J, Cameron JS. Significance of anti-phospholipid antibodies in patients with lupus nephritis. *Kidney Int* 1991;39(6):1225–1231.
106. Kleinknecht D, Bobrie G, Meyer O, et al. Recurrent thrombosis and renal vascular disease in patients with a lupus anticoagulant. *Nephrol Dial Transplant* 1989;4(10):854–858.
107. Nochy D, Daugas E, Droz D, et al. The intrarenal vascular lesions associated with primary antiphospholipid syndrome. *J Am Soc Nephrol* 1999;10(3):507–518.
108. Perez RE, McClendon JR, Lie JT. Primary antiphospholipid syndrome with multiorgan arterial and venous thromboses. *J Rheumatol* 1992;19(8):1289–1292.
109. Nicholls K, Kincaid-Smith P. Antiphospholipid syndrome and renal thrombotic microangiopathy. *J Nephrol* 1995;8:123.
110. Espinosa G, Bucciarelli S, Cervera R, et al. Thrombotic microangiopathic haemolytic anaemia and antiphospholipid antibodies. *Ann Rheum Dis* 2004;63(6):730–736.
111. Escalante A, Brey RL, Mitchell BD Jr, et al. Accuracy of anticardiolipin antibodies in identifying a history of thrombosis among patients with systemic lupus erythematosus. *Am J Med* 1995;98(6):559–565.
112. Erkan D, Bateman H, Lockshin MD. Lupus anticoagulant-hypoprothrombinemia syndrome associated with systemic lupus erythematosus: Report of 2 cases and review of literature. *Lupus* 1999;8(7):560–564.
113. Love PE, Santoro SA. Antiphospholipid antibodies: Anticardiolipin and the lupus anticoagulant in SLE and non SLE disorders. *Ann Intern Med* 1990;12:682.
114. Harris EN, Asherson RA, Hughes GR. Antiphospholipid antibodies—autoantibodies with a difference. *Annu Rev Med* 1988;39:261–271.
115. Harris EN, Bos K. An acute disseminated coagulopathy-vasculopathy associated with the antiphospholipid syndrome. *Arch Intern Med* 1991;151(2):231–233.
116. McHugh NJ, Maymo J, Skinner RP, et al. Anticardiolipin antibodies, livedo reticularis, and major cerebrovascular and renal disease in systemic lupus erythematosus. *Ann Rheum Dis* 1988;47(2):110–115.
117. Levine JS, Branch DW, Rauch J. The antiphospholipid syndrome. *N Engl J Med* 2002;346(10):752–763.
118. Ames PR, Cianciaruso B, Bellizzi V, et al. Bilateral renal artery occlusion in a patient with primary antiphospholipid antibody syndrome: Thrombosis, vasculitis or both? *J Rheumatol* 1992;19(11):1802–1806.
119. Arnold MH, Schrieber L. Splenic and renal infarction in systemic lupus erythematosus: Association with anti-cardiolipin antibodies. *Clin Rheumatol* 1988;7(3):406–410.
120. Asherson RA, Lanham JG, Hull RG, et al. Renal vein thrombosis in systemic lupus erythematosus: association with the "lupus anticoagulant". *Clin Exp Rheumatol* 1984;2(1):75–79.
121. Asherson RA, Noble GE, Hughes GR. Hypertension, renal artery stenosis and the "primary" antiphospholipid syndrome. *J Rheumatol* 1991;18(9):1413–1415.
122. Asherson RA. The catastrophic antiphospholipid syndrome. *J Rheumatol* 1992;19(4):508–512.
123. Asherson RA, Buchanan N, Baguley E, et al. Postpartum bilateral renal vein thrombosis in the primary antiphospholipid syndrome. *J Rheumatol* 1993;20(5):874–876.
124. Glueck HI, Kant KS, Weiss MA, et al. Thrombosis in systemic lupus erythematosus. Relation to the presence of circulating anticoagulants. *Arch Intern Med* 1985;145(8):1389–1395.
125. Ostuni PA, Lazzarin P, Pengo V, et al. Renal artery thrombosis and hypertension in a 13 year old girl with antiphospholipid syndrome. *Ann Rheum Dis* 1990;49(3):184–187.
126. Sonpal GM, Sharma A, Miller A. Primary antiphospholipid antibody syndrome, renal infarction and hypertension. *J Rheumatol* 1993;20(7):1221–1223.
127. Daugas E, Nochy D, Huong du LT, et al. Antiphospholipid syndrome nephropathy in systemic lupus erythematosus. *J Am Soc Nephrol* 2002;13(1):42–52.
128. Cacoub P, Wechsler B, Piette JC, et al. Malignant hypertension in antiphospholipid syndrome without overt lupus nephritis. *Clin Exp Rheumatol* 1993;11(5):479–485.
129. D'Agati V, Kunis C, Williams G, et al. Anti-cardiolipin antibody and renal disease: A report three cases. *J Am Soc Nephrol* 1990;1(5):777–784.
130. Julkunen H, Kaaja R, Jouhikainen T, et al. Malignant hypertension and antiphospholipid antibodies as presenting features of SLE in a young woman using oral contraceptives. *Br J Rheumatol* 1991;30(6):471–472.
131. Leaker B, McGregor A, Griffiths M, et al. Insidious loss of renal function in patients with anticardiolipin antibodies and absence of overt nephritis. *Br J Rheumatol* 1991;30(6):422–425.
132. Perdiguero M, Boronat M, Marco P, et al. The role of antiphospholipid antibodies in lupus nephropathy. *Nephron* 1995;71(1):35–39.
133. Piette JC, Cacoub P, Wechsler B. Renal manifestations of the antiphospholipid syndrome. *Semin Arthritis Rheum* 1994;23(6):357–366.
134. Asherson RA, Cervera R, de Groot PG, et al. Catastrophic antiphospholipid syndrome: International consensus statement on classification criteria and treatment guidelines. *Lupus* 2003;12(7):530–534.
135. Kincaid-Smith P, Fairley KF, Kloss M. Lupus anticoagulant associated with renal thrombotic microangiopathy and pregnancy-related renal failure. *Q J Med* 1988;68(258):795–815.
136. Venkataseshan SB, Smith L, Needle S, et al. Renal disease in antiphospholipid antibody syndrome [Abstract]. *J Am Soc Nephrol* 1996;7:1343.
137. Moroni G, Ventura D, Riva P, et al. Antiphospholipid antibodies are associated with an increased risk for chronic renal insufficiency in patients with lupus nephritis. *Am J Kidney Dis* 2004;43(1):28–36.
138. Tektonidou MG, Sotsiou F, Nakopoulou L, et al. Antiphospholipid syndrome nephropathy in patients with systemic lupus erythematosus and antiphospholipid antibodies: Prevalence, clinical associations, and long-term outcome. *Arthritis Rheum* 2004;50(8):2569–2579.
139. Shoenfeld Y, Meroni PL, Toubi E. Antiphospholipid syndrome and systemic lupus erythematosus: are they separate entities or just clinical presentations on the same scale? *Curr Opin Rheumatol* 2009;21(5):495–500.

140. Alchi B, Griffiths M, Jayne D. What nephrologists need to know about antiphospholipid syndrome. *Nephrol Dial Transplant* 2010;25(10): 3147–3154.
141. Brandt JT, Triplett DA, Alving B, et al. Criteria for the diagnosis of lupus anticoagulants: an update. On behalf of the Subcommittee on Lupus Anticoagulant/Antiphospholipid Antibody of the Scientific and Standardisation Committee of the ISTH. *Thromb Haemost* 1995;74(4):1185–1190.
142. Rauch J, Tannenbaum M, Janoff AS. Distinguishing plasma lupus anticoagulants from anti-factor antibodies using hexagonal (II) phase phospholipids. *Thromb Haemost* 1989;62(3):892–896.
143. Bevers EM, Galli M. Cofactors involved in the antiphospholipid syndrome. *Lupus* 1992;1(2):51–53.
144. Galli M, Comfurius P, Maassen C, et al. Anticardiolipin antibodies (ACA) directed not to cardiolipin but to a plasma protein cofactor. *Lancet* 1990;335(8705):1544–1547.
145. McNeil HP, Simpson RJ, Chesterman CN, et al. Anti-phospholipid antibodies are directed against a complex antigen that includes a lipid-binding inhibitor of coagulation: Beta 2-glycoprotein I (apolipoprotein H). *Proc Natl Acad Sci U S A* 1990;87(11):4120–4124.
146. Horkko S, Miller E, Dudl E, et al. Antiphospholipid antibodies are directed against epitopes of oxidized phospholipids. Recognition of cardiolipin by monoclonal antibodies to epitopes of oxidized low density lipoprotein. *J Clin Invest* 1996;98(3):815–825.
147. Lim W, Crowther MA, Eikelboom JW. Management of antiphospholipid antibody syndrome: a systematic review. *JAMA* 2006;295(9):1050–1057.
148. Cameron JS, Turner DR, Ogg CS, et al. Systemic lupus with nephritis: a long-term study. *Q J Med* 1979;48(189):1-24.
149. Hill GS, Hinglais N, Tron F, et al. Systemic lupus erythematosus. Morphologic correlations with immunologic and clinical data at the time of biopsy. *Am J Med* 1978;64(1):61–79.
150. Sinniah R, Feng PH. Lupus nephritis: correlation between light, electron microscopic and immunofluorescent findings and renal function. *Clin Nephrol* 1976;6(2):340–351.
151. Orfila C, Rakotoarivony J, Manuel Y, et al. Immunofluorescence characterization of light chains in human nephropathies. *Virchows Arch A Pathol Anat Histopathol* 1988;412(6):591–594.
152. Jennette JC. The immunohistology of IgA nephropathy. *Am J Kidney Dis* 1988;12(5):348–352.
153. Williams DG, Peters DK, Fallows J, et al. Studies of serum complement in the hypocomplementaemic nephritides. *Clin Exp Immunol* 1974;18(3):391–405.
154. Rothfield N, Ross HA, Minta JO, et al. Glomerular and dermal deposition of properdin in systemic lupus erythematosus. *N Engl J Med* 1972;287(14):681–685.
155. Biesecker G, Katz S, Koffler D. Renal localization of the membrane attack complex in systemic lupus erythematosus nephritis. *J Exp Med* 1981;154(6):1779–1794.
156. Colasanti G, Morel-Maroger L, D'Amico G. Deposition of fibrin-stabilizing factor (F XIIIA and S), fibrinogen-related antigens, fibrinogen degradation products (FDPd and FDPe) and antihemolytic factor (F VIII) in renal disease: analysis of 161 cases by immunofluorescence microscopy. *Clin Nephrol* 1987;28(1):28–34.
157. McCutcheon J, Evans B, D'Cruz DP, et al. Fibrin deposition in SLE glomerulonephritis. *Lupus* 1993;2(2):99–103.
158. Jennette JC, Iskandar SS, Dalldorf FG. Pathologic differentiation between lupus and nonlupus membranous glomerulopathy. *Kidney Int* 1983;24(3):377–385.
159. Madaio MP, Yanase K. Cellular penetration and nuclear localization of anti-DNA antibodies: Mechanisms, consequences, implications and applications. *J Autoimmun* 1998;11(5):535–538.
160. Vlahakos DV, Foster MH, Adams S, et al. Anti-DNA antibodies form immune deposits at distinct glomerular and vascular sites. *Kidney Int* 1992;41(6):1690–1700.
161. Comerford FR, Cohen AS. The nephropathy of systemic lupus erythematosus: an assessment by clinical, light and electron microscopic criteria. *Medicine (Baltimore)* 1967;46:425.
162. Hayslett JP, Kashgarian M, Cook CD, et al. The effect of azathioprine on lupus glomerulonephritis. *Medicine (Baltimore)* 1972;51(5):393–412.
163. Grishman E, Porush JC, Rosen SM, et al. Lupus nephritis with organized deposits in the kidneys. *Lab Invest* 1967;16(5):717–725.
164. Su CF, Chen HH, Yeh JC, et al. Ultrastructural 'fingerprint' in cryoprecipitates and glomerular deposits: a clinicopathologic analysis of fingerprint deposits. *Nephron* 2002;90(1):37–42.
165. Kim YH, Choi YJ, Reiner L. Ultrastructural "fingerprint" in cryoprecipitate and glomerular deposits: a case report of systemic lupus erythematosus. *Hum Pathol* 1981;12(1):86–89.
166. Ogihara T, Saruta T, Saito I, et al. Finger print deposits of the kidney in pure monoclonal IgG kappa cryoglobulinemia. *Clin Nephrol* 1979;12(4):186–190.
167. Schwartz MM, Roberts JL, Lewis EJ. Subepithelial electron-dense deposits in proliferative glomerulonephritis of systemic lupus erythematosus. *Ultrastruct Pathol* 1982;3(2):105–118.
168. Weidner N, Lorentz WB Jr. Scanning electron microscopy of the acellular glomerular and tubular basement membrane in lupus nephritis. *Am J Clin Pathol* 1986;85(2):135–145.
169. Cohen AH, Zamboni L. Ultrastructural appearance and morphogenesis of renal glomerular hematoxylin bodies. *Am J Pathol* 1977;89(1):105–118.
170. Maldonado JE, Alarcon-Segovia D, Pease GL, et al. Electron microscopy of the lupus erythematosus cell. *Mayo Clin Proc* 1963;38: 219–224.
171. Grishman E, Churg J. Ultrastructure of hematoxylin bodies in systemic lupus erythematosus. *Arch Pathol Lab Med* 1979;103(11):573–576.
172. Bloodworth JM Jr, Shelp WD. Endothelial cytoplasmic inclusions. *Arch Pathol* 1970;90(3):252–258.
173. Dreyer EO, Muldiyarov PY, Nassonova VA, et al. Endothelial inclusions and "nuclear bodies" in systemic lupus erythematosus. *Ann Rheum Dis* 1973;32(5):444–449.
174. Goodman JR, Sylvester RA, Talal N, et al. Virus-like structures in lymphocytes of patients with systemic and discoid lupus erythematosus. *Ann Intern Med* 1973;79(3):396–402.
175. Haustein UF. Tubular structures in affected and normal skin in chronic discoid and systemic lupus erythematosus: Electron microscopic studies. *Br J Dermatol* 1973;89(1):1-13.
176. Gyorkey F, Min KW, Sincovics JG, et al. Systemic lupus erythematosus and myxovirus. *N Engl J Med* 1969;280(6):333.
177. Gyorkey F, Sinkovics JG. Microtubules of systemic lupus erythematosus. *Lancet* 1971;1(7690):131–132.
178. Schaff Z, Barry DW, Grimley PM. Cytochemistry of tubuloreticular structures in lymphocytes from patients with systemic lupus erythematosus and in cultured human lymphoid cells: Comparison to a paramyxovirus. *Lab Invest* 1973;29(6):577–586.
179. Rich SA. Human lupus inclusions and interferon. *Science* 1981; 213(4509):772–775.
180. Hurd ER, Eigenbrodt E, Worthen H, et al. Glomerular cytoplasmic tubular structures in renal biopsies of patients with systemic lupus erythematosus and other diseases. *Arthritis Rheum* 1971;14(4):539–550.
181. D'Agati V, Appel GB. HIV infection and the kidney. *J Am Soc Nephrol* 1997;8(1):138–152.
182. Kallen RJ, Lee SK, Aronson AJ, et al. Idiopathic membranous glomerulopathy preceding the emergence of systemic lupus erythematosus in two children. *J Pediatr* 1977;90(1):72–76.
183. Libit SA, Burke B, Michael AF, et al. Extramembranous glomerulonephritis in childhood: relationship to systemic lupus erythematosus. *J Pediatr* 1976;88(3):394–402.
184. The Southwest Pediatric Nephrology Study Group. Comparison of idiopathic and systemic lupus erythematosus-associated membranous glomerulonephropathy in children. *Am J Kidney Dis 1986*;7(2): 115–124.
185. Pollak VE, Pirani CL, Schwartz FD. The natural history of the renal manifestations of systemic lupus erythematosus. *J Lab Clin Med* 1964;63:537–550.
186. Baldwin DS, Lowenstein J, Rothfield NF, et al. The clinical course of the proliferative and membranous forms of lupus nephritis. *Ann Intern Med* 1970;73(6):929–942.
187. Schwartz MM, Lan SP, Bonsib SM, et al. Clinical outcome of three discrete histologic patterns of injury in severe lupus glomerulonephritis. *Am J Kidney Dis* 1989;13(4):273–283.

188. Adu D, Williams DG, Taube D, et al. Late onset systemic lupus erythematosus and lupus-like disease in patients with apparent idiopathic glomerulonephritis. *Q J Med* 1983;52(208):471–487.
189. Ginzler EM, Bollet AJ, Friedman EA. The natural history and response to therapy of lupus nephritis. *Annu Rev Med* 1980;31:463–487.
190. Tam LS, Li EK, Lai FM, et al. Mesangial lupus nephritis in Chinese is associated with a high rate of transformation to higher grade nephritis. *Lupus* 2003;12(9):665–671.
191. Ginzler EM, Nicastri AD, Chen CK, et al. Progression of mesangial and focal to diffuse lupus nephritis. *N Engl J Med* 1974;291(14):693–696.
192. Zimmerman SW, Jenkins PG, Shelf WD, et al. Progression from minimal or focal to diffuse proliferative lupus nephritis. *Lab Invest* 1975;32(5):665–672.
193. Seshan SV, Jennette JC. Renal disease in systemic lupus erythematosus with emphasis on classification of lupus glomerulonephritis: Advances and implications. *Arch Pathol Lab Med* 2009;133(2):233–248.
194. Morel-Maroger L, Mery JP, Richet G. Lupus nephritis: immunofluorescence study of 54 cases. *Perspect Nephrol Hypertens* 1973;1:1183–1186.
195. Mahajan SK, Ordonez NG, Spargo BH, et al. Changing histopathology patterns in lupus nephropathy. *Clin Nephrol* 1978;10(1):1-8.
196. Akhtar M, al-Dalaan A, el-Ramahi KM. Pauci-immune necrotizing lupus nephritis: report of two cases. *Am J Kidney Dis* 1994;23(2):320–325.
197. Ferrario F, Napodano P, Giordano A, et al. Peculiar type of focal and segmental lupus glomerulitis: glomerulonephritis or vasculitis? *Contrib Nephrol* 1992;99:86–93.
198. Marshall S, Dressler R, D'Agati V. Membranous lupus nephritis with antineutrophil cytoplasmic antibody-associated segmental necrotizing and crescentic glomerulonephritis. *Am J Kidney Dis* 1997;29(1):119–124.
199. Schwartz MM, Roberts JL, Lewis EJ. Necrotizing glomerulitis of systemic lupus erythematosus. *Hum Pathol* 1983;14(2):158–167.
200. Magil AB, Ballon HS, Rae A. Focal proliferative lupus nephritis. A clinicopathologic study using the W.H.O. classification. *Am J Med* 1982;72(4):620–630.
201. Cameron JJ. The course of lupus nephritis. In: Kincaid Smith PM, Th Becker EL, eds. *Glomerulonephritis: Morphology, Natural History and Treatment*. New York: Wiley; 1973:1187.
202. Nanra RS, Kincaid-Smith P. Lupus nephritis: clinical course in relation to treatment. In: Kincaid Smith P, el-Khatib MT, Becker EL, eds. *Glomerulonephritis: Morphology, Natural History and Treatment*. New York: Wiley; 1973:1193.
203. Striker GK, Quadracci LJ, Scribner BH. The course of lupus nephritis: relationship to the renal histologic findings. In: Kincaid Smith PM, Mathew TH, Becker EL, eds. *Glomerulonephritis: Morphology, Natural History and Treatment*. New York: Wiley; 1973:1141.
204. Appel GB, Cohen DJ, Pirani CL, et al. Long-term follow-up of patients with lupus nephritis. A study based on the classification of the World Health Organization. *Am J Med* 1987;83(5):877–885.
205. Schwartz MM, Kawala KS, Corwin HL, et al. The prognosis of segmental glomerulonephritis in systemic lupus erythematosus. *Kidney Int* 1987;32(2):274–279.
206. Hecht B, Siegel N, Adler M, et al. Prognostic indices in lupus nephritis. *Medicine (Baltimore)* 1976;55(2):163–181.
207. Morel-Maroger L, Mery JP, Delrieu F, et al. [Immuno-histochemical study of 29 renal biopsies made during disseminated erythematous lupus]. *J Urol Nephrol (Paris). 1971*;77(4):367–379.
208. Grishman E, Porush JG, Lee SL, et al. Renal biopsies in lupus nephritis. Correlation of electron microscopic findings with clinical course. *Nephron* 1973;10(1):25–36.
209. Tateno S, Kobayashi Y, Shigematsu H, et al. Study of lupus nephritis: Its classification and the significance of subendothelial deposits. *Q J Med* 1983;52(207):311–331.
210. Korbet SM, Lewis EJ, Schwartz MM, et al. Factors predictive of outcome in severe lupus nephritis. Lupus Nephritis Collaborative Study Group. *Am J Kidney Dis* 2000;35(5):904–914.
211. Najafi CC, Korbet SM, Lewis EJ, et al. Significance of histologic patterns of glomerular injury upon long-term prognosis in severe lupus glomerulonephritis. *Kidney Int* 2001;59(6):2156–2163.
212. Markowitz GS, D'Agati VD. Classification of lupus nephritis. *Curr Opin Nephrol Hypertens* 2009;18(3):220–225.
213. Mittal B, Hurwitz S, Rennke H, et al. New subcategories of class IV lupus nephritis: are there clinical, histologic, and outcome differences? *Am J Kidney Dis* 2004;44(6):1050–1059.
214. Hill GS, Delahousse M, Nochy D, et al. Class IV-S versus class IV-G lupus nephritis: clinical and morphologic differences suggesting different pathogenesis. *Kidney Int* 2005;68(5):2288–2297.
215. Yokoyama H, Wada T, Hara A, et al. The outcome and a new ISN/RPS 2003 classification of lupus nephritis in Japanese. *Kidney Int* 2004;66(6):2382–2388.
216. Haring CM, Rietveld A, van den Brand JA, et al. Segmental and global subclasses of class IV lupus nephritis have similar renal outcomes. *J Am Soc Nephrol* 2012;23(1):149–154.
217. Schwartz MM, Korbet SM, Lewis EJ. The prognosis and pathogenesis of severe lupus glomerulonephritis. *Nephrol Dial Transplant* 2008;23(4):1298–1306.
218. Grootscholten C, Bajema IM, Florquin S, et al. Interobserver agreement of scoring of histopathological characteristics and classification of lupus nephritis. *Nephrol Dial Transplant* 2008;23(1):223–230.
219. Hiramatsu N, Kuroiwa T, Ikeuchi H, et al. Revised classification of lupus nephritis is valuable in predicting renal outcome with an indication of the proportion of glomeruli affected by chronic lesions. *Rheumatology (Oxford)* 2008;47(5):702–707.
220. Yu F, Tan Y, Wu LH, et al. Class IV-G and IV-S lupus nephritis in Chinese patients: a large cohort study from a single center. *Lupus* 2009;18(12):1073–1081.
221. Kojo S, Sada KE, Kobayashi M, et al. Clinical usefulness of a prognostic score in histological analysis of renal biopsy in patients with lupus nephritis. *J Rheumatol* 2009;36(10):2218–2223.
222. Eiser AR, Katz SM, Swartz C. Clinically occult diffuse proliferative lupus nephritis. An age-related phenomenon. *Arch Intern Med* 1979;139(9):1022–1025.
223. Font J, Torras A, Cervera R, et al. Silent renal disease in systemic lupus erythematosus. *Clin Nephrol* 1987;27(6):283–288.
224. Leehey DJ, Katz AI, Azaran AH, et al. Silent diffuse lupus nephritis: Long-term follow-up. *Am J Kidney Dis* 1982;2(1 Suppl 1):188–196.
225. Mahajan SK, Ordonez NG, Feitelson PJ, et al. Lupus nephropathy without clinical renal involvement. *Medicine (Baltimore)* 1977;56(6):493–501.
226. Wada Y, Ito S, Ueno M, et al. Renal outcome and predictors of clinical renal involvement in patients with silent lupus nephritis. *Nephron Clin Pract* 2004;98(4):c105–c111.
227. Schwartz MM, Kawala K, Roberts JL, et al. Clinical and pathological features of membranous glomerulonephritis of systemic lupus erythematosus. *Am J Nephrol* 1984;4(5):301–311.
228. Giannico G, Fogo AB. Lupus nephritis: Is the kidney biopsy currently necessary in the management of lupus nephritis? *Clin J Am Soc Nephrol* 2013;8(1):138–145.
229. Fukatsu A, Matsuo S, Killen PD, et al. The glomerular distribution of type IV collagen and laminin in human membranous glomerulonephritis. *Hum Pathol* 1988;19(1):64–68.
230. Donadio JV Jr, Burgess JH, Holley KE. Membranous lupus nephropathy: a clinicopathologic study. *Medicine (Baltimore)* 1977;56(6):527–536.
231. Honig C, Mouradian JA, Montoliu J, et al. Mesangial electron-dense deposits in membranous nephropathy. *Lab Invest* 1980;42(4):427–432.
232. Sato M, Kogure T, Kanemitsu M. A case of systemic lupus erythematosus showing invagination of the podocyte into the glomerular basement membrane: An electron microscopic observation of a repeated-renal biopsy. *Clin Exp Nephrol* 2008;12(6):455–461.
233. Fujigaki Y, Muranaka Y, Sakakima M, et al. Analysis of intra-GBM microstructures in a SLE case with glomerulopathy associated with podocytic infolding. *Clin Exp Nephrol* 2008;12(6):432–439.
234. Markowitz GS, Stokes MB, Kambham N, et al. Renal pathology. *NephSAP* 2011;10(4):369–375.
235. Pasquali S, Banfi G, Zucchelli A, et al. Lupus membranous nephropathy: long-term outcome. *Clin Nephrol* 1993;39(4):175–182.
236. Sloan RP, Schwartz MM, Korbet SM, et al. Long-term outcome in systemic lupus erythematosus membranous glomerulonephritis.

Lupus Nephritis Collaborative Study Group. *J Am Soc Nephrol* 1996;7(2):299–305.
237. Gonzalez Dettoni HT, Tron F. Membranous glomerulopathy in systemic lupus erythematosus. In: Hamburger J, Crosnier J, Maxwell MH, eds. *Advances in Nephrology*. Chicago: Year Book Medical; 1984:347.
238. Shearn MA, Hopper J Jr, Biava CG. Membranous lupus nephropathy initially seen as idiopathic membranous nephropathy. Possible diagnostic value of tubular reticular structures. *Arch Intern Med* 1980;140(11):1521–1523.
239. Qin W, Beck LH Jr, Zeng C, et al. Anti-phospholipase A2 receptor antibody in membranous nephropathy. *J Am Soc Nephrol* 2011;22(6):1137–1143.
240. Hoxha E, Kneissler U, Stege G, et al. Enhanced expression of the M-type phospholipase A2 receptor in glomeruli correlates with serum receptor antibodies in primary membranous nephropathy. *Kidney Int* 2012;82(7):797–804.
241. Larsen CP, Messias NC, Silva FG, et al. Determination of primary versus secondary membranous glomerulopathy utilizing phospholipase A2 receptor staining in renal biopsies. *Mod Pathol* 2013;26(5):709–715.
242. Appel GB, Williams GS, Meltzer JI, et al. Renal vein thrombosis, nephrotic syndrome, and systemic lupus erythematosus: an association in four cases. *Ann Intern Med* 1976;85(3):310–317.
243. Mintz G, Acevedo-Vazquez E, Gutierrez-Espinosa G, et al. Renal vein thrombosis and inferior vena cava thrombosis in systemic lupus erythematosus. Frequency and risk factors. *Arthritis Rheum* 1984;27(5):539–544.
244. Morel Maroger LM, Droz JP, Godin D, et al. The course of lupus nephritis: contribution of serial renal biopsies. In: Hamburger J, Crosnier J, Maxwell MH, eds. *Advances in Nephrology*. Chicago: Year Book Medical; 1976:79.
245. Lu J, Tam LS, Lai FM, et al. Repeat renal biopsy in lupus nephritis: a change in histological pattern is common. *Am J Nephrol* 2011;34(3):220–225.
246. Esdaile JM, Joseph L, MacKenzie T, et al. The pathogenesis and prognosis of lupus nephritis: information from repeat renal biopsy. *Semin Arthritis Rheum* 1993;23(2):135–148.
247. Boelaert JMM, Méry JP. Renal insufficiency in lupus nephritis. In: Hamburger J, Crosnier J, Mazwell MH, eds. *Advances in Nephrology*. Chicago: Year Book Medical; 1974:249.
248. Dillard MG, Dujovne I, Pollak VE, et al. The effect of treatment with prednisone and nitrogen mustard on the renal lesions and life span of patients with lupus glomerulonephritis. *Nephron* 1973;10(5):273–291.
249. Drinkard JP, Stanley TM, Dornfeld L, et al. Azathioprine and prednisone in the treatment of adults with lupus nephritis. Clinical, histological, and immunological changes with therapy. *Medicine (Baltimore)* 1970;49(5):411–432.
250. Pollak VP, Pirani CL, eds. *Pathology of the Kidney in Systemic Lupus Erythematosus: Serial Renal Biopsy Studies and the Effects of Therapy on Kidney Lesions*. 2nd ed. Los Angeles: University of Southern California; 1974.
251. Austin HA III, Boumpas DT, Vaughan EM, et al. Predicting renal outcomes in severe lupus nephritis: contributions of clinical and histologic data. *Kidney Int* 1994;45(2):544–550.
252. Hill GS, Delahousse M, Nochy D, et al. A new morphologic index for the evaluation of renal biopsies in lupus nephritis. *Kidney Int* 2000;58(3):1160–1173.
253. Baumal R, Farine M, Poucell S. Clinical significance of renal biopsies showing mixed mesangial and global proliferative lupus nephritis. *Am J Kidney Dis* 1987;10(3):236–240.
254. Rush PJ, Baumal R, Shore A, et al. Correlation of renal histology with outcome in children with lupus nephritis. *Kidney Int* 1986;29(5):1066–1071.
255. Wallace DJ, Goldfinger D, Savage G, et al. Predictive value of clinical, laboratory, pathologic, and treatment variables in steroid/immunosuppressive resistant lupus nephritis. *J Clin Apher* 1988;4(1):30–34.
256. Schwartz MM, Bernstein J, Hill GS, et al. Predictive value of renal pathology in diffuse proliferative lupus glomerulonephritis. Lupus Nephritis Collaborative Study Group. *Kidney Int* 1989;36(5):891–896.
257. Schwartz MM, Lan SP, Bernstein J, et al. Role of pathology indices in the management of severe lupus glomerulonephritis. Lupus Nephritis Collaborative Study Group. *Kidney Int* 1992;42(3):743–748.
258. Schwartz MM, Lan SP, Bernstein J, et al. Irreproducibility of the activity and chronicity indices limits their utility in the management of lupus nephritis. Lupus Nephritis Collaborative Study Group. *Am J Kidney Dis* 1993;21(4):374–377.
259. Wernick RM, Smith DL, Houghton DC, et al. Reliability of histologic scoring for lupus nephritis: a community-based evaluation. *Ann Intern Med* 1993;119(8):805–811.
260. Furness PN, Taub N. Interobserver reproducibility and application of the ISN/RPS classification of lupus nephritis-a UK-wide study. *Am J Surg Pathol* 2006;30(8):1030–1035.
261. Harvey AM, Shulman LE, Tumulty PA, et al. Systemic lupus erythematosus: review of the literature and clinical analysis of 138 cases. *Medicine (Baltimore)* 1954;33(4):291–437.
262. Pistiner M, Wallace DJ, Nessim S, et al. Lupus erythematosus in the 1980s: a survey of 570 patients. *Semin Arthritis Rheum* 1991;21(1):55–64.
263. Worrall JG, Snaith ML, Batchelor JR, et al. SLE: a rheumatological view. Analysis of the clinical features, serology and immunogenetics of 100 SLE patients during long-term follow-up. *Q J Med* 1990;74(275):319–330.
264. Tax WJ, Kramers C, van Bruggen MC, et al. Apoptosis, nucleosomes, and nephritis in systemic lupus erythematosus. *Kidney Int* 1995;48(3):666–673.
265. Furukawa F, Kashihara-Sawami M, Lyons MB, et al. Binding of antibodies to the ENAs SS-A/Ro and SS-B/La is induced on the surface of human keratinocytes by ultraviolet light (UVL): implications for the pathogenesis of photosensitive cutaneous lupus. *J Invest Dermatol* 1990;94(1):77–85.
266. Grootscholten C, van Bruggen MC, van der Pijl JW, et al. Deposition of nucleosomal antigens (histones and DNA) in the epidermal basement membrane in human lupus nephritis. *Arthritis Rheum* 2003;48(5):1355–1362.
267. Grigor R, Edmonds J, Lewkonia R, et al. Systemic lupus erythematosus. A prospective analysis. *Ann Rheum Dis* 1978;37(2):121–128.
268. Inoue T, Kanayama Y, Ohe A, et al. Immunopathologic studies of pneumonitis in systemic lupus erythematosus. *Ann Intern Med* 1979;91(1):30–34.
269. Doherty NE, Siegel RJ. Cardiovascular manifestations of systemic lupus erythematosus. *Am Heart J* 1985;110(6):1257–1265.
270. Dubois EL, Tuffanelli DL. Clinical manifestations of systemic lupus erythematosus. Computer analysis of 520 cases. *JAMA* 1964;190:104–111.
271. Estes D, Christian CL. The natural history of systemic lupus erythematosus by prospective analysis. *Medicine (Baltimore)* 1971;50(2):85–95.
272. Bluestein HG. Neurocytotoxic antibodies in serum of patients with systemic lupus erythematosus. *Proc Natl Acad Sci U S A* 1978;75(8):3965–3969.
273. Tan EM, Cohen AS, Fries JF, et al. The 1982 revised criteria for the classification of systemic lupus erythematosus. *Arthritis Rheum* 1982;25(11):1271–1277.
274. Hochberg MC. Updating the American College of Rheumatology revised criteria for the classification of systemic lupus erythematosus. *Arthritis Rheum* 1997;40(9):1725.
275. Levin RE, Weinstein A, Peterson M, et al. A comparison of the sensitivity of the 1971 and 1982 American Rheumatism Association criteria for the classification of systemic lupus erythematosus. *Arthritis Rheum* 1984;27(5):530–538.
276. Bomback AS, Appel GB. Updates on the treatment of lupus nephritis. *J Am Soc Nephrol* 2010;21(12):2028–2035.
277. Borchers AT, Leibushor N, Naguwa SM, et al. Lupus nephritis: A critical review. *Autoimmun Rev* 2012;12(2):174–194.
278. Fanouriakis A, Krasoudaki E, Tzanakakis M, et al. Recent progress in the treatment of lupus nephritis. *Mod Rheumatol* 2012;22(6):803–813.
279. Lightstone L. Lupus nephritis: where are we now? *Curr Opin Rheumatol* 2010;22(3):252–256.
280. Balow JE, Austin HA III, Muenz LR, et al. Effect of treatment on the evolution of renal abnormalities in lupus nephritis. *N Engl J Med* 1984;311(8):491–495.
281. Gourley MF, Austin HA III, Scott D, et al. Methylprednisolone and cyclophosphamide, alone or in combination, in patients with

lupus nephritis. A randomized, controlled trial. *Ann Intern Med* 1996;125(7):549–557.
282. Boumpas DT, Austin HA III, Vaughn EM, et al. Controlled trial of pulse methylprednisolone versus two regimens of pulse cyclophosphamide in severe lupus nephritis. *Lancet* 1992;340(8822):741–745.
283. Illei GG, Austin HA, Crane M, et al. Combination therapy with pulse cyclophosphamide plus pulse methylprednisolone improves long-term renal outcome without adding toxicity in patients with lupus nephritis. *Ann Intern Med* 2001;135(4):248–257.
284. Dooley MA, Jayne D, Ginzler EM, et al. Mycophenolate versus azathioprine as maintenance therapy for lupus nephritis. *N Engl J Med* 2011;365(20):1886–1895.
285. Houssiau FA, D'Cruz D, Sangle S, et al. Azathioprine versus mycophenolate mofetil for long-term immunosuppression in lupus nephritis: results from the MAINTAIN Nephritis Trial. *Ann Rheum Dis* 2010;69(12):2083–2089.
286. Mok CC, Ho CT, Chan KW, et al. Outcome and prognostic indicators of diffuse proliferative lupus glomerulonephritis treated with sequential oral cyclophosphamide and azathioprine. *Arthritis Rheum* 2002;46(4):1003–1013.
287. Contreras G, Pardo V, Leclercq B, et al. Sequential therapies for proliferative lupus nephritis. *N Engl J Med* 2004;350(10):971–980.
288. Houssiau FA, Vasconcelos C, D'Cruz D, et al. Immunosuppressive therapy in lupus nephritis: the Euro-Lupus Nephritis Trial, a randomized trial of low-dose versus high-dose intravenous cyclophosphamide. *Arthritis Rheum* 2002;46(8):2121–2131.
289. Appel GB, Contreras G, Dooley MA, et al. Mycophenolate mofetil versus cyclophosphamide for induction treatment of lupus nephritis. *J Am Soc Nephrol* 2009;20(5):1103–1112.
290. Chan TM, Tse KC, Tang CS, et al. Long-term study of mycophenolate mofetil as continuous induction and maintenance treatment for diffuse proliferative lupus nephritis. *J Am Soc Nephrol* 2005;16(4):1076–1084.
291. Ginzler EM, Dooley MA, Aranow C, et al. Mycophenolate mofetil or intravenous cyclophosphamide for lupus nephritis. *N Engl J Med* 2005;353(21):2219–2228.
292. Touma Z, Gladman DD, Urowitz MB, et al. Mycophenolate mofetil for induction treatment of lupus nephritis: a systematic review and metaanalysis. *J Rheumatol* 2011;38(1):69–78.
293. Henderson LK, Masson P, Craig JC, et al. Induction and maintenance treatment of proliferative lupus nephritis: a meta-analysis of randomized controlled trials. *Am J Kidney Dis* 2013;61(1):74–87.
294. Chen W, Liu Q, Tang X, et al. Outcomes of maintenance therapy with tacrolimus versus azathioprine for active lupus nephritis: a multicenter randomized clinical trial. *Lupus* 2012;21(9):944–952.
295. Chen W, Tang X, Liu Q, et al. Short-term outcomes of induction therapy with tacrolimus versus cyclophosphamide for active lupus nephritis: a multicenter randomized clinical trial. *Am J Kidney Dis* 2011;57(2):235–244.
296. Moroni G, Doria A, Mosca M, et al. A randomized pilot trial comparing cyclosporine and azathioprine for maintenance therapy in diffuse lupus nephritis over four years. *Clin J Am Soc Nephrol* 2006;1(5):925–932.
297. Bertsias G, Ioannidis JP, Boletis J, et al. EULAR recommendations for the management of systemic lupus erythematosus. Report of a Task Force of the EULAR Standing Committee for International Clinical Studies Including Therapeutics. *Ann Rheum Dis* 2008;67(2):195–205.
298. Bertsias GK, Tektonidou M, Amoura Z, et al. Joint European League Against Rheumatism and European Renal Association-European Dialysis and Transplant Association (EULAR/ERA-EDTA) recommendations for the management of adult and paediatric lupus nephritis. *Ann Rheum Dis* 2012;71(11):1771–1782.
299. Duran-Barragan S, McGwin G Jr, Vila LM, et al. Angiotensin-converting enzyme inhibitors delay the occurrence of renal involvement and are associated with a decreased risk of disease activity in patients with systemic lupus erythematosus—results from LUMINA (LIX): A multiethnic US cohort. *Rheumatology (Oxford)* 2008;47(7):1093–1096.
300. Gordon C, Jayne D, Pusey C, et al. European consensus statement on the terminology used in the management of lupus glomerulonephritis. *Lupus* 2009;18(3):257–263.
301. Renal Disease Subcommittee of the American College of Rheumatology Ad Hoc Committee on Systemic Lupus Erythematosus Response Criteria. The American College of Rheumatology response criteria for proliferative and membranous renal disease in systemic lupus erythematosus clinical trials. *Arthritis Rheum* 2006;54(2):421–432.
302. Austin HA III, Klippel JH, Balow JE, et al. Therapy of lupus nephritis: Controlled trial of prednisone and cytotoxic drugs. *N Engl J Med* 1986;314(10):614–619.
303. Houssiau FA, Vasconcelos C, D'Cruz D, et al. The 10-year follow-up data of the Euro-Lupus Nephritis Trial comparing low-dose and high-dose intravenous cyclophosphamide. *Ann Rheum Dis* 2010;69(1):61–64.
304. Chan TM, Li FK, Tang CS, et al. Efficacy of mycophenolate mofetil in patients with diffuse proliferative lupus nephritis. Hong Kong-Guangzhou Nephrology Study Group. *N Engl J Med* 2000;343(16):1156–1162.
305. Weng MY, Weng CT, Liu MF. The efficacy of low-dose mycophenolate mofetil for treatment of lupus nephritis in Taiwanese patients with systemic lupus erythematosus. *Clin Rheumatol* 2010;29(7):771–775.
306. Punyawudho B, Lertdumrongluk P, Somparn P, et al. Population pharmacokinetics of mycophenolate mofetil in Thai lupus nephritis patients. *Int J Clin Pharmacol Ther* 2012;50(4):272–280.
307. Grootscholten C, Ligtenberg G, Hagen EC, et al. Azathioprine/methylprednisolone versus cyclophosphamide in proliferative lupus nephritis. A randomized controlled trial. *Kidney Int* 2006;70(4):732–742.
308. Grootscholten C, Bajema IM, Florquin S, et al. Treatment with cyclophosphamide delays the progression of chronic lesions more effectively than does treatment with azathioprine plus methylprednisolone in patients with proliferative lupus nephritis. *Arthritis Rheum* 2007;56(3):924–937.
309. Arends S, Grootscholten C, Derksen RH, et al. Long-term follow-up of a randomised controlled trial of azathioprine/methylprednisolone versus cyclophosphamide in patients with proliferative lupus nephritis. *Ann Rheum Dis* 2012;71(6):966–973.
310. Takahashi S, Hiromura K, Sakurai N, et al. Efficacy and safety of tacrolimus for induction therapy in patients with active lupus nephritis. *Mod Rheumatol* 2011;21(3):282–289.
311. Miyasaka N, Kawai S, Hashimoto H. Efficacy and safety of tacrolimus for lupus nephritis: a placebo-controlled double-blind multicenter study. *Mod Rheumatol* 2009;19(6):606–615.
312. Mok CC, Tong KH, To CH, et al. Tacrolimus for induction therapy of diffuse proliferative lupus nephritis: an open-labeled pilot study. *Kidney Int* 2005;68(2):813–817.
313. Li X, Ren H, Zhang Q, et al. Mycophenolate mofetil or tacrolimus compared with intravenous cyclophosphamide in the induction treatment for active lupus nephritis. *Nephrol Dial Transplant* 2012;27(4):1467–1472.
314. Fu LW, Yang LY, Chen WP, et al. Clinical efficacy of cyclosporin a neoral in the treatment of paediatric lupus nephritis with heavy proteinuria. *Br J Rheumatol* 1998;37(2):217–221.
315. Rihova Z, Vankova Z, Maixnerova D, et al. Treatment of lupus nephritis with cyclosporine: an outcome analysis. *Kidney Blood Press Res* 2007;30(2):124–128.
316. Zavada J, Pesickova S, Rysava R, et al. Cyclosporine A or intravenous cyclophosphamide for lupus nephritis: the Cyclofa-Lune study. *Lupus* 2010;19(11):1281–1289.
317. Bao H, Liu ZH, Xie HL, et al. Successful treatment of class V + IV lupus nephritis with multitarget therapy. *J Am Soc Nephrol* 2008;19(10):2001–2010.
318. Valeri A, Radhakrishnan J, Estes D, et al. Intravenous pulse cyclophosphamide treatment of severe lupus nephritis: a prospective five-year study. *Clin Nephrol* 1994;42(2):71–78.
319. Stoenoiu MS, Aydin S, Tektonidou M, et al. Repeat kidney biopsies fail to detect differences between azathioprine and mycophenolate mofetil maintenance therapy for lupus nephritis: Data from the MAINTAIN Nephritis Trial. *Nephrol Dial Transplant* 2012;27(5):1924–1930.
320. Appel GB, Valeri A. The course and treatment of lupus nephritis. *Annu Rev Med* 1994;45:525–537.
321. Radhakrishnan J, Moutzouris DA, Ginzler EM, et al. Mycophenolate mofetil and intravenous cyclophosphamide are similar as induction therapy for class V lupus nephritis. *Kidney Int* 2010;77(2):152–160.
322. Spetie DN, Tang Y, Rovin BH, et al. Mycophenolate therapy of SLE membranous nephropathy. *Kidney Int* 2004;66(6):2411–2415.

323. Mok CC, Ying KY, Lau CS, et al. Treatment of pure membranous lupus nephropathy with prednisone and azathioprine: An open-label trial. *Am J Kidney Dis* 2004;43(2):269–276.
324. Moroni G, Maccario M, Banfi G, et al. Treatment of membranous lupus nephritis. *Am J Kidney Dis* 1998;31(4):681–686.
325. Radhakrishnan J, Kunis CL, D'Agati V, et al. Cyclosporine treatment of lupus membranous nephropathy. *Clin Nephrol* 1994;42(3):147–154.
326. Yap DY, Yu X, Chen XM, et al. Pilot 24 month study to compare mycophenolate mofetil and tacrolimus in the treatment of membranous lupus nephritis with nephrotic syndrome. *Nephrology (Carlton)* 2012;17(4):352–357.
327. Austin HA III, Illei GG, Braun MJ, et al. Randomized, controlled trial of prednisone, cyclophosphamide, and cyclosporine in lupus membranous nephropathy. *J Am Soc Nephrol* 2009;20(4):901–911.
328. Jonsdottir T, Sundelin B, Welin Henriksson E, et al. Rituximab-treated membranous lupus nephritis: clinical outcome and effects on electron dense deposits. *Ann Rheum Dis* 2011;70(6):1172–1173.
329. Lu TY, Ng KP, Cambridge G, et al. A retrospective seven-year analysis of the use of B cell depletion therapy in systemic lupus erythematosus at University College London Hospital: the first fifty patients. *Arthritis Rheum* 2009;61(4):482–487.
330. Melander C, Sallee M, Trolliet P, et al. Rituximab in severe lupus nephritis: early B-cell depletion affects long-term renal outcome. *Clin J Am Soc Nephrol* 2009;4(3):579–587.
331. Ramos-Casals M, Diaz-Lagares C, Soto-Cardenas MJ, et al. Rituximab therapy in lupus nephritis: current clinical evidence. *Clin Rev Allergy Immunol* 2011;40(3):159–169.
332. Diaz-Lagares C, Croca S, Sangle S, et al. Efficacy of rituximab in 164 patients with biopsy-proven lupus nephritis: pooled data from European cohorts. *Autoimmun Rev* 2012;11(5):357–364.
333. Merrill JT, Neuwelt CM, Wallace DJ, et al. Efficacy and safety of rituximab in moderately-to-severely active systemic lupus erythematosus: the randomized, double-blind, phase II/III systemic lupus erythematosus evaluation of rituximab trial. *Arthritis Rheum* 2010;62(1): 222–233.
334. Rovin BH, Furie R, Latinis K, et al. Efficacy and safety of rituximab in patients with active proliferative lupus nephritis: the Lupus Nephritis Assessment with Rituximab study. *Arthritis Rheum* 2012;64(4):1215–1226.
335. Hahn BH. Belimumab for systemic lupus erythematosus. *N Engl J Med* 2013;368(16):1528–1535.
336. Dooley MA, Houssiau F, Aranow C, et al. Effect of belimumab treatment on renal outcomes: results from the phase 3 belimumab clinical trials in patients with SLE. *Lupus* 2013;22(1):63–72.
337. Finck BK, Linsley PS, Wofsy D. Treatment of murine lupus with CTLA4Ig. *Science* 1994;265(5176):1225–1227.
338. Wofsy D, Hillson JL, Diamond B. Abatacept for lupus nephritis: alternative definitions of complete response support conflicting conclusions. *Arthritis Rheum* 2012;64(11):3660–3665.
339. Wang HY, Cui TG, Hou FF, et al. Induction treatment of proliferative lupus nephritis with leflunomide combined with prednisone: a prospective multi-centre observational study. *Lupus* 2008;17(7):638–644.
340. Flanc RS, Roberts MA, Strippoli GF, et al. Treatment of diffuse proliferative lupus nephritis: a meta-analysis of randomized controlled trials. *Am J Kidney Dis* 2004;43(2):197–208.
341. Aringer M, Smolen JS, Graninger WB. Severe infections in plasmapheresis-treated systemic lupus erythematosus. *Arthritis Rheum* 1998;41(3):414–420.
342. Nasr SH, D'Agati VD, Park HR, et al. Necrotizing and crescentic lupus nephritis with antineutrophil cytoplasmic antibody seropositivity. *Clin J Am Soc Nephrol* 2008;3(3):682–690.
343. Toubi E, Kessel A, Shoenfeld Y. High-dose intravenous immunoglobulins: an option in the treatment of systemic lupus erythematosus. *Hum Immunol* 2005;66(4):395–402.
344. Lin CY, Hsu HC, Chiang H. Improvement of histological and immunological change in steroid and immunosuppressive drug-resistant lupus nephritis by high-dose intravenous gamma globulin. *Nephron* 1989;53(4):303–310.
345. Levy Y, Sherer Y, George J, et al. Intravenous immunoglobulin treatment of lupus nephritis. *Semin Arthritis Rheum* 2000;29(5):321–327.
346. Jordan SC. Intravenous gamma-globulin therapy in systemic lupus erythematosus and immune complex disease. *Clin Immunol Immunopathol* 1989;53(2 Pt 2):S164–S169.
347. Alchi B, Jayne D, Labopin M, et al. Autologous haematopoietic stem cell transplantation for systemic lupus erythematosus: Data from the European Group for Blood and Marrow Transplantation registry. *Lupus* 2013;22(3):245–253.
348. Aringer M, Graninger WB, Steiner G, et al. Safety and efficacy of tumor necrosis factor alpha blockade in systemic lupus erythematosus: An open-label study. *Arthritis Rheum* 2004;50(10):3161–3169.
349. Aringer M, Burkhardt H, Burmester GR, et al. Current state of evidence on 'off-label' therapeutic options for systemic lupus erythematosus, including biological immunosuppressive agents, in Germany, Austria and Switzerland—a consensus report. *Lupus* 2012;21(4):386–401.
350. Barrat FJ, Meeker T, Gregorio J, et al. Nucleic acids of mammalian origin can act as endogenous ligands for Toll-like receptors and may promote systemic lupus erythematosus. *J Exp Med* 2005;202(8):1131–1139.
351. Alarcon-Segovia D, Tumlin JA, Furie RA, et al. LJP 394 for the prevention of renal flare in patients with systemic lupus erythematosus: results from a randomized, double-blind, placebo-controlled study. *Arthritis Rheum* 2003;48(2):442–454.
352. Furie RA, Cash JM, Cronin ME, et al. Treatment of systemic lupus erythematosus with LJP 394. *J Rheumatol* 2001;28(2):257–265.
353. Cardiel MH, Tumlin JA, Furie RA, et al. Abetimus sodium for renal flare in systemic lupus erythematosus: results of a randomized, controlled phase III trial. *Arthritis Rheum* 2008;58(8):2470–2480.
354. Mysler EF SA, Guzman R, Bijl M, et al. Efficacy and safety of ocrelizumab, a humanized antiCD20 antibody, in patients with active proliferative lupus nephritis (LN): results from the randomized, double-blind phase III BELONG Study (Abstract). *Arthritis Rheum* 2010;62(Suppl 10):1455.
355. Boumpas DT, Furie R, Manzi S, et al. A short course of BG9588 (anti-CD40 ligand antibody) improves serologic activity and decreases hematuria in patients with proliferative lupus glomerulonephritis. *Arthritis Rheum* 2003;48(3):719–727.
356. Davis JC Jr, Totoritis MC, Rosenberg J, et al. Phase I clinical trial of a monoclonal antibody against CD40–ligand (IDEC-131) in patients with systemic lupus erythematosus. *J Rheumatol* 2001;28(1):95–101.
357. Chagnac A, Kiberd BA, Farinas MC, et al. Outcome of the acute glomerular injury in proliferative lupus nephritis. *J Clin Invest* 1989;84(3):922–930.
358. Moroni G, Banfi G, Ponticelli C. Clinical status of patients after 10 years of lupus nephritis. *Q J Med* 1992;84(305):681–689.
359. Yap DY, Tang CS, Ma MK, et al. Survival analysis and causes of mortality in patients with lupus nephritis. *Nephrol Dial Transplant* 2012;27(8):3248–3254.
360. Muehrcke RC, Kark RM, Pirani CL, et al. Lupus nephritis: A clinical and pathologic study based on renal biopsies. *Medicine (Baltimore)* 1957;36(1):1-145.
361. Ponticelli C, Zucchelli P, Moroni G, et al. Long-term prognosis of diffuse lupus nephritis. *Clin Nephrol* 1987;28(6):263–271.
362. Cameron JS. Lupus nephritis: an historical perspective 1968–1998. *J Nephrol* 1999;12(Suppl 2):S29–S41.
363. Cameron JS. Lupus nephritis. *J Am Soc Nephrol* 1999;10(2):413–424.
364. Fiehn C, Hajjar Y, Mueller K, et al. Improved clinical outcome of lupus nephritis during the past decade: importance of early diagnosis and treatment. *Ann Rheum Dis* 2003;62(5):435–439.
365. Houssiau FA. Management of lupus nephritis: an update. *J Am Soc Nephrol* 2004;15(10):2694–2704.
366. Bogdanovic R, Nikolic V, Pasic S, et al. Lupus nephritis in childhood: a review of 53 patients followed at a single center. *Pediatr Nephrol* 2004;19(1):36–44.
367. Costenbader KH, Desai A, Alarcon GS, et al. Trends in the incidence, demographics, and outcomes of end-stage renal disease due to lupus nephritis in the US from 1995 to 2006. *Arthritis Rheum* 2011;63(6):1681–1688.
368. Mok CC, Ying KY, Ng WL, et al. Long-term outcome of diffuse proliferative lupus glomerulonephritis treated with cyclophosphamide. *Am J Med 2006*;119(4):355 e25–355 e33.
369. Illei GG, Takada K, Parkin D, et al. Renal flares are common in patients with severe proliferative lupus nephritis treated with pulse

immunosuppressive therapy: long-term followup of a cohort of 145 patients participating in randomized controlled studies. *Arthritis Rheum* 2002;46(4):995–1002.
370. Moroni G, Quaglini S, Maccario M, et al. "Nephritic flares" are predictors of bad long-term renal outcome in lupus nephritis. *Kidney Int* 1996;50(6):2047–2053.
371. Chen YE, Korbet SM, Katz RS, et al. Value of a complete or partial remission in severe lupus nephritis. *Clin J Am Soc Nephrol* 2008;3(1):46–53.
372. Houssiau FA, Vasconcelos C, D'Cruz D, et al. Early response to immunosuppressive therapy predicts good renal outcome in lupus nephritis: lessons from long-term followup of patients in the Euro-Lupus Nephritis Trial. *Arthritis Rheum* 2004;50(12):3934–3940.
373. Adler SG, Johnson K, Louie JS, et al. Lupus membranous glomerulonephritis: different prognostic subgroups obscured by imprecise histologic classifications. *Mod Pathol* 1990;3(2):186–191.
374. Bakir AA, Levy PS, Dunea G. The prognosis of lupus nephritis in African-Americans: a retrospective analysis. *Am J Kidney Dis* 1994;24(2):159–171.
375. Barr RG, Seliger S, Appel GB, et al. Prognosis in proliferative lupus nephritis: the role of socio-economic status and race/ethnicity. *Nephrol Dial Transplant* 2003;18(10):2039–2046.
376. Dooley MA, Hogan S, Jennette C, et al. Cyclophosphamide therapy for lupus nephritis: poor renal survival in black Americans. Glomerular Disease Collaborative Network. *Kidney Int* 1997;51(4):1188–1195.
377. Sun HO, Hu WX, Xie HL, et al. Long-term outcome of Chinese patients with membranous lupus nephropathy. *Lupus* 2008;17(1):56–61.
378. Mok CC, Ying KY, Yim CW, et al. Very long-term outcome of pure lupus membranous nephropathy treated with glucocorticoid and azathioprine. *Lupus* 2009;18(12):1091–1095.
379. Austin HA III, Boumpas DT, Vaughan EM, et al. High-risk features of lupus nephritis: importance of race and clinical and histological factors in 166 patients. *Nephrol Dial Transplant* 1995;10(9):1620–1628.
380. Contreras G, Pardo V, Cely C, et al. Factors associated with poor outcomes in patients with lupus nephritis. *Lupus* 2005;14(11):890–895.
381. Iseki K, Miyasato F, Oura T, et al. An epidemiologic analysis of end-stage lupus nephritis. *Am J Kidney Dis* 1994;23(4):547–554.
382. Donadio JV Jr, Hart GM, Bergstralh EJ, et al. Prognostic determinants in lupus nephritis: a long-term clinicopathologic study. *Lupus* 1995;4(2):109–115.
383. Fraenkel L, MacKenzie T, Joseph L, et al. Response to treatment as a predictor of longterm outcome in patients with lupus nephritis. *J Rheumatol* 1994;21(11):2052–2057.
384. Esdaile JM, Joseph L, MacKenzie T, et al. The benefit of early treatment with immunosuppressive agents in lupus nephritis. *J Rheumatol* 1994;21(11):2046–2051.
385. Goulet JR, MacKenzie T, Levinton C, et al. The longterm prognosis of lupus nephritis: the impact of disease activity. *J Rheumatol* 1993;20(1):59–65.
386. Wu LH, Yu F, Tan Y, et al. Inclusion of renal vascular lesions in the 2003 ISN/RPS system for classifying lupus nephritis improves renal outcome predictions. *Kidney Int* 2013;83(4):715–723.
387. Croca SC, Rodrigues T, Isenberg DA. Assessment of a lupus nephritis cohort over a 30–year period. *Rheumatology (Oxford)* 2011;50(8):1424–1430.
388. Ward MM. Changes in the incidence of end-stage renal disease due to lupus nephritis, 1982–1995. *Arch Intern Med* 2000;160(20):3136–3140.
389. Ward MM. Changes in the incidence of endstage renal disease due to lupus nephritis in the United States, 1996–2004. *J Rheumatol* 2009;36(1):63–67.
390. Cheigh JS, Stenzel KH. End-stage renal disease in systemic lupus erythematosus. *Am J Kidney Dis* 1993;21(1):2-8.
391. Raskova J, Morrison AB. A decrease in cell-mediated immunity in uremia associated with an increase in activity of suppressor cells. *Am J Pathol* 1976;84(1):1-10.
392. Stone JH. End-stage renal disease in lupus: disease activity, dialysis, and the outcome of transplantation. *Lupus* 1998;7(9):654–659.
393. Tang H, Chelamcharla M, Baird BC, et al. Factors affecting kidney-transplant outcome in recipients with lupus nephritis. *Clin Transplant* 2008;22(3):263–272.
394. Bartosh SM, Fine RN, Sullivan EK. Outcome after transplantation of young patients with systemic lupus erythematosus: a report of the North American pediatric renal transplant cooperative study. *Transplantation* 2001;72(5):973–978.
395. Roth D, Milgrom M, Esquenazi V, et al. Renal transplantation in systemic lupus erythematosus: One center's experience. *Am J Nephrol* 1987;7(5):367–374.
396. Bumgardner GL, Mauer SM, Payne W, et al. Single-center 1-15–year results of renal transplantation in patients with systemic lupus erythematosus. *Transplantation* 1988;46(5):703–709.
397. Naveed A, Nilubol C, Melancon JK, et al. Preemptive kidney transplantation in systemic lupus erythematosus. *Transplant Proc* 2011;43(10):3713–3714.
398. Stone JH, Amend WJ, Criswell LA. Antiphospholipid antibody syndrome in renal transplantation: occurrence of clinical events in 96 consecutive patients with systemic lupus erythematosus. *Am J Kidney Dis* 1999;34(6):1040–1047.
399. Lochhead KM, Pirsch JD, D'Alessandro AM, et al. Risk factors for renal allograft loss in patients with systemic lupus erythematosus. *Kidney Int* 1996;49(2):512–517.
400. Ward MM. Outcomes of renal transplantation among patients with end-stage renal disease caused by lupus nephritis. *Kidney Int* 2000;57(5):2136–2143.
401. Bunnapradist S, Chung P, Peng A, et al. Outcomes of renal transplantation for recipients with lupus nephritis: analysis of the Organ Procurement and Transplantation Network database. *Transplantation* 2006;82(5):612–618.
402. Stone JH, Millward CL, Olson JL, et al. Frequency of recurrent lupus nephritis among ninety-seven renal transplant patients during the cyclosporine era. *Arthritis Rheum* 1998;41(4):678–686.
403. Gipson DS, Ferris ME, Dooley MA, et al. Renal transplantation in children with lupus nephritis. *Am J Kidney Dis* 2003;41(2):455–463.
404. Contreras G, Mattiazzi A, Schultz DR, et al. Kidney transplantation outcomes in African-, Hispanic- and Caucasian-Americans with lupus. *Lupus* 2012;21(1):3-12.
405. Burgos PI, Perkins EL, Pons-Estel GJ, et al. Risk factors and impact of recurrent lupus nephritis in patients with systemic lupus erythematosus undergoing renal transplantation: data from a single US institution. *Arthritis Rheum* 2009;60(9):2757–2766.
406. Nee R, Jindal RM, Little D, et al. Racial differences and income disparities are associated with poor outcomes in kidney transplant recipients with lupus nephritis. *Transplantation* 2013;95:1471–1478.
407. Mojcik CF, Klippel JH. End-stage renal disease and systemic lupus erythematosus. *Am J Med* 1996;101(1):100–107.
408. Ramos EL. Recurrent diseases in the renal allograft. *J Am Soc Nephrol* 1991;2(2):109–121.
409. Azevedo LS, Romao JE Jr, Malheiros D, et al. Renal transplantation in systemic lupus erythematosus. A case control study of 45 patients. *Nephrol Dial Transplant* 1998;13(11):2894–2898.
410. Goral S, Ynares C, Shappell SB, et al. Recurrent lupus nephritis in renal transplant recipients revisited: it is not rare. *Transplantation* 2003;75(5):651–656.
411. Yu TM, Wen MC, Li CY, et al. Impact of recurrent lupus nephritis on lupus kidney transplantation: a 20–year single center experience. *Clin Rheumatol* 2012;31(4):705–710.
412. Norby GE, Strom EH, Midtvedt K, et al. Recurrent lupus nephritis after kidney transplantation: a surveillance biopsy study. *Ann Rheum Dis* 2010;69(8):1484–1487.
413. Contreras G, Mattiazzi A, Guerra G, et al. Recurrence of lupus nephritis after kidney transplantation. *J Am Soc Nephrol* 2010;21(7):1200–1207.
414. Isaac J, Shihab FS. Recurrent lupus nephritis in the second allograft of a patient with systemic lupus erythematosus. *Nephron* 2002;92(4):947–949.
415. Kiser RL, Thomas DB, Andreoni K, et al. Preexisting crescentic glomerulonephritis in the renal allograft. *Am J Kidney Dis* 2003;42(5):E20–E26.
416. Radhakrishnan J, Williams GS, Appel GB, et al. Renal transplantation in anticardiolipin antibody-positive lupus erythematosus patients. *Am J Kidney Dis* 1994;23(2):286–289.
417. Brunet P, Aillaud MF, San Marco M, et al. Antiphospholipids in hemodialysis patients: relationship between lupus anticoagulant and thrombosis. *Kidney Int* 1995;48(3):794–800.

418. Garcia-Martin F, De Arriba G, Carrascosa T, et al. Anticardiolipin antibodies and lupus anticoagulant in end-stage renal disease. *Nephrol Dial Transplant* 1991;6(8):543–547.
419. Prakash R, Miller CC III, Suki WN. Anticardiolipin antibody in patients on maintenance hemodialysis and its association with recurrent arteriovenous graft thrombosis. *Am J Kidney Dis* 1995;26(2):347–352.
420. Zabaleta-Lanz M, Vargas-Arenas RE, Tapanes F, et al. Silent nephritis in systemic lupus erythematosus. *Lupus* 2003;12(1):26–30.
421. Zabaleta-Lanz ME, Munoz LE, Tapanes FJ, et al. Further description of early clinically silent lupus nephritis. *Lupus* 2006;15(12):845–851.
422. Gonzalez-Crespo MR, Lopez-Fernandez JI, Usera G, et al. Outcome of silent lupus nephritis. *Semin Arthritis Rheum* 1996;26(1):468–476.
423. Fessel WJ. ANA-negative systemic lupus erythematosus. *Am J Med* 1978;64(1):80–86.
424. Maddison PJ, Provost TT, Reichlin M. Serological findings in patients with "ANA-negative" systemic lupus erythematosus. *Medicine (Baltimore)* 1981;60(2):87–94.
425. Huerta A, Bomback AS, Liakopoulos V, et al. Renal-limited 'lupus-like' nephritis. *Nephrol Dial Transplant* 2012;27(6):2337–2342.
426. Hertig A, Droz D, Lesavre P, et al. SLE and idiopathic nephrotic syndrome: Coincidence or not? *Am J Kidney Dis* 2002;40(6):1179–1184.
427. Kraft SW, Schwartz MM, Korbet SM, et al. Glomerular podocytopathy in patients with systemic lupus erythematosus. *J Am Soc Nephrol* 2005;16(1):175–179.
428. Trivedi S, Zeier M, Reiser J. Role of podocytes in lupus nephritis. *Nephrol Dial Transplant* 2009;24(12):3607–3612.
429. Dube GK, Markowitz GS, Radhakrishnan J, et al. Minimal change disease in systemic lupus erythematosus. *Clin Nephrol* 2002;57(2):120–126.
430. Salvatore SP, Barisoni LM, Herzenberg AM, et al. Collapsing glomerulopathy in 19 patients with systemic lupus erythematosus or lupus-like disease. *Clin J Am Soc Nephrol* 2012;7(6):914–925.
431. Larsen CP, Beggs ML, Saeed M, et al. Apolipoprotein l1 risk variants associate with systemic lupus erythematosus-associated collapsing glomerulopathy. *J Am Soc Nephrol* 2013;24(5):722–725.
432. Gupta R, Sharma A, Bhowmik D, et al. Collapsing glomerulopathy occurring in HIV-negative patients with systemic lupus erythematosus: report of three cases and brief review of the literature. *Lupus* 2011;20(8):866–870.
433. Amoura Z, Georgin-Lavialle S, Haroche J, et al. Collapsing glomerulopathy in systemic autoimmune disorders: a case occurring in the course of full blown systemic lupus erythematosus. *Ann Rheum Dis* 2006;65(2):277–278.
434. Melo NC, Malheiros DM, Barros RT, et al. Collapsing glomerulopathy associated with proliferative lupus nephritis: reversible acute kidney injury. *Lupus* 2011;20(1):98–101.
435. Arbel O, Pizov G, Ben-Yehuda A, et al. Hyperacute renal failure as the initial presentation of systemic lupus erythematosus. *Lupus* 2005;14(4):331–333.
436. Marques LP, Pacheco GG, Rioja LS, et al. Can systemic lupus erythematosus be the cause of collapsing glomerulopathy? *Lupus* 2005;14(10):853–855.
437. Tungekar MF, Waller S, Clothier JC. Collapsing glomerulopathy in a girl with systemic lupus erythematosus. *Pediatr Nephrol* 2011; 26(5):809–813.
438. Pettersson T, Tornroth T, Totterman KJ, et al. AA amyloidosis in systemic lupus erythematosus. *J Rheumatol* 1987;14(4):835–838.
439. Singh NP, Prakash A, Sridhara G, et al. Renal and systemic amyloidosis in systemic lupus erythematosus. *Ren Fail* 2003;25(4):671–675.
440. Queffeulou G, Berenbaum F, Michel C, et al. AA amyloidosis in systemic lupus erythematosus: an unusual complication. *Nephrol Dial Transplant* 1998;13(7):1846–1848.
441. Nasr SH, Valeri AM, Cornell LD, et al. Fibrillary glomerulonephritis: a report of 66 cases from a single institution. *Clin J Am Soc Nephrol* 2011;6(4):775–784.
442. Rosenstock JL, Markowitz GS, Valeri AM, et al. Fibrillary and immunotactoid glomerulonephritis: distinct entities with different clinical and pathologic features. *Kidney Int* 2003;63(4):1450–1461.
443. Menon S, Zeng X, Valentini R. Fibrillary glomerulonephritis and renal failure in a child with systemic lupus erythematosus. *Pediatr Nephrol* 2009;24(8):1577–1581.
444. Magasic MV, Venkataseshan VS, Vitting KE. Concurrent renal sarcoidosis and lupus nephritis. *Nephron* 1993;64(3):496–497.
445. Baranowska-Daca E, Choi YJ, Barrios R, et al. Nonlupus nephritides in patients with systemic lupus erythematosus: a comprehensive clinicopathologic study and review of the literature. *Hum Pathol* 2001;32(10):1125–1135.
446. Vasoo S. Drug-induced lupus: an update. *Lupus* 2006;15(11):757–761.
447. Borchers AT, Keen CL, Gershwin ME. Drug-induced lupus. *Ann N Y Acad Sci* 2007;1108:166–182.
448. Hoffman B. Sensitivity of sulfadiazine resembling acute disseminated lupus erythematosus. *Arch Dermatol Syph* 1945;51:190.
449. Petri M, Allbritton J. Antibiotic allergy in systemic lupus erythematosus: A case-control study. *J Rheumatol* 1992;19(2):265–269.
450. Capponi A, De Simone C, Guerriero C, et al. Ro/SSA-positive cutaneous lupus erythematosus induced by carbamazepine. *Arch Dermatol* 2005;141(1):103–104.
451. Jolles S, Sewell WA, Leighton C. Drug-induced aseptic meningitis: Diagnosis and management. *Drug Saf* 2000;22(3):215–226.
452. Buyon JP, Petri MA, Kim MY, et al. The effect of combined estrogen and progesterone hormone replacement therapy on disease activity in systemic lupus erythematosus: a randomized trial. *Ann Intern Med* 2005;142(12 Pt 1):953–962.
453. Bernier MO, Mikaeloff Y, Hudson M, et al. Combined oral contraceptive use and the risk of systemic lupus erythematosus. *Arthritis Rheum* 2009;61(4):476–481.
454. Schoonen WM, Thomas SL, Somers EC, et al. Do selected drugs increase the risk of lupus? A matched case-control study. *Br J Clin Pharmacol* 2010;70(4):588–596.
455. Gota C, Calabrese L. Induction of clinical autoimmune disease by therapeutic interferon-alpha. *Autoimmunity* 2003;36(8):511–518.
456. Costa MF, Said NR, Zimmermann B. Drug-induced lupus due to anti-tumor necrosis factor alpha agents. *Semin Arthritis Rheum* 2008;37(6):381–387.
457. Rubin RL, Nusinow SR, Johnson AD, et al. Serologic changes during induction of lupus-like disease by procainamide. *Am J Med* 1986;80(5):999–1002.
458. Cameron HA, Ramsay LE. The lupus syndrome induced by hydralazine: a common complication with low dose treatment. *Br Med J (Clin Res Ed)* 1984;289(6442):410–412.
459. Lee SL, Chase PH. Drug-induced systemic lupus erythematosus: a critical review. *Semin Arthritis Rheum* 1975;5(1):83–103.
460. Mansilla-Tinoco R, Harland SJ, Ryan PJ, et al. Hydralazine, antinuclear antibodies, and the lupus syndrome. *Br Med J (Clin Res Ed)* 1982;284(6320):936–939.
461. Ramsay LE, Silas J, Freestone S. Hydralazine antinuclear antibodies and the lupus syndrome. *Br Med J (Clin Res Ed)* 1982;284(6330): 1711.
462. Sonnhag C, Karlsson E, Hed J. Procainamide-induced lupus erythematosus-like syndrome in relation to acetylator phenotype and plasma levels of procainamide. *Acta Med Scand* 1979;206(4):245–251.
463. Woosley RL, Drayer DE, Reidenberg MM, et al. Effect of acetylator phenotype on the rate at which procainamide induces antinuclear antibodies and the lupus syndrome. *N Engl J Med* 1978;298(21):1157–1159.
464. Speirs C, Fielder AH, Chapel H, et al. Complement system protein C4 and susceptibility to hydralazine-induced systemic lupus erythematosus. *Lancet* 1989;1(8644):922–924.
465. Yung RL, Richardson BC. Drug-induced lupus. *Rheum Dis Clin North Am* 1994;20(1):61–86.
466. De Bandt M, Sibilia J, Le Loet X, et al. Systemic lupus erythematosus induced by anti-tumour necrosis factor alpha therapy: a French national survey. *Arthritis Res Ther* 2005;7(3):R545–R551.
467. Kalkner KM, Ronnblom L, Karlsson Parra AK, et al. Antibodies against double-stranded DNA and development of polymyositis during treatment with interferon. *QJM* 1998;91(6):393–399.
468. Donnelly S, Levison DA, Doyle DV. Systemic lupus erythematosus-like syndrome with focal proliferative glomerulonephritis during D-penicillamine therapy. *Br J Rheumatol* 1993;32(3):251–253.
469. Bienaime F, Clerbaux G, Plaisier E, et al. D-Penicillamine-induced ANCA-associated crescentic glomerulonephritis in Wilson disease. *Am J Kidney Dis* 2007;50(5):821–825.

470. Ihle BU, Whitworth JA, Dowling JP, et al. Hydralazine and lupus nephritis. *Clin Nephrol* 1984;22(5):230–238.
471. Stokes MB, Foster K, Markowitz GS, et al. Development of glomerulonephritis during anti-TNF-alpha therapy for rheumatoid arthritis. *Nephrol Dial Transplant* 2005;20(7):1400–1406.
472. Prasad GV, Bastacky S, Johnson JP. Propylthiouracil-induced diffuse proliferative lupus nephritis: review of immunological complications. *J Am Soc Nephrol* 1997;8(7):1205–1210.
473. Jacobs C, Seigle RL, D'Agati V, et al. Epidemic propylthiouracil (PTU) induced ANCA positive (myeloperoxidase 2) vasculitis [Abstract]. *Arthritis Rheum* 1994(37):S267.
474. Vogt BA, Kim Y, Jennette JC, et al. Antineutrophil cytoplasmic autoantibody-positive crescentic glomerulonephritis as a complication of treatment with propylthiouracil in children. *J Pediatr* 1994;124(6):986–988.
475. Cornacchia E, Golbus J, Maybaum J, et al. Hydralazine and procainamide inhibit T cell DNA methylation and induce autoreactivity. *J Immunol* 1988;140(7):2197–2200.
476. Jiang X, Khursigara G, Rubin RL. Transformation of lupus-inducing drugs to cytotoxic products by activated neutrophils. *Science* 1994;266(5186):810–813.
477. Chang BG, Markowitz GS, Seshan SV, et al. Renal manifestations of concurrent systemic lupus erythematosus and HIV infection. *Am J Kidney Dis* 1999;33(3):441–449.
478. Faubert PF, Porush JG, Venkataseshan VS, et al. Lupus like syndromes. In: Grishman E, Churg J, Needle MA, et al., eds. *The Kidney in Collagen-Vascular Diseases.* New York: Raven Press, 1993.
479. Palacios R, Santos J, Valdivielso P, et al. Human immunodeficiency virus infection and systemic lupus erythematosus. An unusual case and a review of the literature. *Lupus* 2002;11(1):60–63.
480. Kopelman RG, Zolla-Pazner S. Association of human immunodeficiency virus infection and autoimmune phenomena. *Am J Med* 1988;84(1):82–88.
481. Calza L, Manfredi R, Colangeli V, et al. Systemic and discoid lupus erythematosus in HIV-infected patients treated with highly active antiretroviral therapy. *Int J STD AIDS* 2003;14(5):356–359.
482. Colon M, Martinez DE. Clinical remission of systemic lupus erythematosus after human immunodeficiency virus infection. *P R Health Sci J* 2007;26(1):79–81.
483. Fox RA, Isenberg DA. Human immunodeficiency virus infection in systemic lupus erythematosus. *Arthritis Rheum* 1997;40(6):1168–1172.
484. Strauss J, Abitbol C, Zilleruelo G, et al. Renal disease in children with the acquired immunodeficiency syndrome. *N Engl J Med* 1989;321(10):625–630.
485. D'Agati V, Seigle R. Coexistence of AIDS and lupus nephritis: a case report. *Am J Nephrol* 1990;10(3):243–247.
486. Contreras G, Green DF, Pardo V, et al. Systemic lupus erythematosus in two adults with human immunodeficiency virus infection. *Am J Kidney Dis* 1996;28(2):292–295.
487. Casanova S, Mazzucco G, Barbiano di Belgiojoso G, et al. Pattern of glomerular involvement in human immunodeficiency virus-infected patients: an Italian study. *Am J Kidney Dis* 1995;26(3):446–453.
488. Haas M, Kaul S, Eustace JA. HIV-associated immune complex glomerulonephritis with "lupus-like features: a clinicopathologic study of 14 cases. *Kidney Int* 2005;67(4):1381–1390.
489. Nochy D, Glotz D, Dosquet P, et al. Renal disease associated with HIV infection: a multicentric study of 60 patients from Paris hospitals. *Nephrol Dial Transplant* 1993;8(1):11–19.
490. Tabechian D, Pattanaik D, Suresh U, et al. Lupus-like nephritis in an HIV-positive patient: report of a case and review of the literature. *Clin Nephrol* 2003;60(3):187–194.
491. Crispin JC, Liossis SN, Kis-Toth K, et al. Pathogenesis of human systemic lupus erythematosus: recent advances. *Trends Mol Med* 2010;16(2):47–57.
492. Liu Z, Davidson A. Taming lupus-a new understanding of pathogenesis is leading to clinical advances. *Nat Med* 2012;18(6):871–882.
493. Perry D, Sang A, Yin Y, et al. Murine models of systemic lupus erythematosus. *J Biomed Biotechnol* 2011;2011:271694.
494. Morel L. Genetics of SLE: evidence from mouse models. *Nat Rev Rheumatol* 2010;6(6):348–357.
495. Fismen S, Mortensen ES, Rekvig OP. Nuclease deficiencies promote end-stage lupus nephritis but not nephritogenic autoimmunity in (NZB x NZW) F1 mice. *Immunol Cell Biol* 2011;89(1):90–99.
496. Andrews BS, Eisenberg RA, Theofilopoulos AN, et al. Spontaneous murine lupus-like syndromes. Clinical and immunopathological manifestations in several strains. *J Exp Med* 1978;148(5):1198–1215.
497. Burnet FM, Holmes MC. The natural history of the NZB/NZW F1 hybrid mouse: a laboratory model of systemic lupus erythematosus. *Australas Ann Med* 1965;14(3):185–191.
498. Waters ST, Fu SM, Gaskin F, et al. NZM2328: a new mouse model of systemic lupus erythematosus with unique genetic susceptibility loci. *Clin Immunol* 2001;100(3):372–383.
499. Dixon FJ, Oldstone MB, Tonietti G. Pathogenesis of immune complex glomerulonephritis of New Zealand mice. *J Exp Med* 1971;134(3 Pt 2): 65s-71s.
500. Ebling F, Hahn BH. Restricted subpopulations of DNA antibodies in kidneys of mice with systemic lupus. Comparison of antibodies in serum and renal eluates. *Arthritis Rheum* 1980;23(4):392–403.
501. Lambert PH, Dixon FJ. Pathogenesis of the glomerulonephritis of NZB/W mice. *J Exp Med* 1968;127(3):507–522.
502. Stetler DA, Cavallo T. Anti-RNA polymerase I antibodies: Potential role in the induction and progression of murine lupus nephritis. *J Immunol* 1987;138(7):2119–2123.
503. Cavallo T, Graves K, Granholm NA, et al. Association of glycoprotein gp70 with progression or attenuation of murine lupus nephritis. *J Clin Lab Immunol* 1985;18(2):63–67.
504. Maruyama N, Furukawa F, Nakai Y, et al. Genetic studies of autoimmunity in New Zealand mice. IV. Contribution of NZB and NZW genes to the spontaneous occurrence of retroviral gp70 immune complexes in (NZB X NZW)F1 hybrid and the correlation to renal disease. *J Immunol* 1983;130(2):740–746.
505. Tsao BP, Ebling FM, Roman C, et al. Structural characteristics of the variable regions of immunoglobulin genes encoding a pathogenic autoantibody in murine lupus. *J Clin Invest* 1990;85(2):530–540.
506. Roubinian J, Talal N, Siiteri PK, et al. Sex hormone modulation of autoimmunity in NZB/NZW mice. *Arthritis Rheum* 1979;22(11):1162–1169.
507. Roubinian JR, Papoian R, Talal N. Androgenic hormones modulate autoantibody responses and improve survival in murine lupus. *J Clin Invest* 1977;59(6):1066–1070.
508. Roubinian JR, Talal N, Greenspan JS, et al. Effect of castration and sex hormone treatment on survival, anti-nucleic acid antibodies, and glomerulonephritis in NZB/NZW F1 mice. *J Exp Med* 1978;147(6):1568–1583.
509. Morel L, Croker BP, Blenman KR, et al. Genetic reconstitution of systemic lupus erythematosus immunopathology with polycongenic murine strains. *Proc Natl Acad Sci U S A* 2000;97(12):6670–6675.
510. Lee YH, Ji JD, Song GG. Fcgamma receptor IIB and IIIB polymorphisms and susceptibility to systemic lupus erythematosus and lupus nephritis: a meta-analysis. *Lupus* 2009;18(8):727–734.
511. Douglas KB, Windels DC, Zhao J, et al. Complement receptor 2 polymorphisms associated with systemic lupus erythematosus modulate alternative splicing. *Genes Immun* 2009;10(5):457–469.
512. Cunninghame Graham DS, Vyse TJ, Fortin PR, et al. Association of LY9 in UK and Canadian SLE families. *Genes Immun* 2008;9(2):93–102.
513. Liu K, Li QZ, Delgado-Vega AM, et al. Kallikrein genes are associated with lupus and glomerular basement membrane-specific antibody-induced nephritis in mice and humans. *J Clin Invest* 2009;119(4):911–923.
514. Singh RR, Saxena V, Zang S, et al. Differential contribution of IL-4 and STAT6 vs STAT4 to the development of lupus nephritis. *J Immunol* 2003;170(9):4818–4825.
515. Datta SK, Manny N, Andrzejewski C, et al. Genetic studies of autoimmunity and retrovirus expression in crosses of New Zealand black mice I. Xenotropic virus. *J Exp Med* 1978;147(3):854–871.
516. Gavalchin J, Datta SK. The NZB X SWR model of lupus nephritis. II. Autoantibodies deposited in renal lesions show a distinctive and restricted idiotypic diversity. *J Immunol* 1987;138(1):138–148.
517. Gavalchin J, Seder RA, Datta SK. The NZB X SWR model of lupus nephritis. I. Cross-reactive idiotypes of monoclonal anti-DNA antibodies

in relation to antigenic specificity, charge, and allotype. Identification of interconnected idiotype families inherited from the normal SWR and the autoimmune NZB parents. *J Immunol* 1987;138(1):128–137.
518. Ebling FM, Hahn BH. Pathogenic subsets of antibodies to DNA. *Int Rev Immunol* 1989;5(1):79–95.
519. Gauthier VJ, Mannik M. A small proportion of cationic antibodies in immune complexes is sufficient to mediate their deposition in glomeruli. *J Immunol* 1990;145(10):3348–3352.
520. Murphy ER, Roths JB. New inbred strains. *Mouse News Letter* 1978;58:51.
521. Pisitkun P, Deane JA, Difilippantonio MJ, et al. Autoreactive B cell responses to RNA-related antigens due to TLR7 gene duplication. *Science* 2006;312(5780):1669–1672.
522. Shen N, Fu Q, Deng Y, et al. Sex-specific association of X-linked Toll-like receptor 7 (TLR7) with male systemic lupus erythematosus. *Proc Natl Acad Sci U S A* 2010;107(36):15838–15843.
523. Watanabe-Fukunaga R, Brannan CI, Copeland NG, et al. Lymphoproliferation disorder in mice explained by defects in Fas antigen that mediates apoptosis. *Nature* 1992;356(6367):314–317.
524. Kumar KR, Li L, Yan M, et al. Regulation of B cell tolerance by the lupus susceptibility gene Ly108. *Science* 2006;312(5780):1665–1669.
525. Haraldsson MK, Louis-Dit-Sully CA, Lawson BR, et al. The lupus-related Lmb3 locus contains a disease-suppressing Coronin-1A gene mutation. *Immunity* 2008;28(1):40–51.
526. Mackay F, Woodcock SA, Lawton P, et al. Mice transgenic for BAFF develop lymphocytic disorders along with autoimmune manifestations. *J Exp Med* 1999;190(11):1697–1710.
527. Jorgensen TN, Gubbels MR, Kotzin BL. Links between type I interferons and the genetic basis of disease in mouse lupus. *Autoimmunity* 2003;36(8):491–502.
528. Jorgensen TN, Gubbels MR, Kotzin BL. New insights into disease pathogenesis from mouse lupus genetics. *Curr Opin Immunol* 2004;16(6):787–793.
529. Shim GJ, Kis LL, Warner M, et al. Autoimmune glomerulonephritis with spontaneous formation of splenic germinal centers in mice lacking the estrogen receptor alpha gene. *Proc Natl Acad Sci U S A* 2004;101(6):1720–1724.
530. Wakeland EK, Liu K, Graham RR, et al. Delineating the genetic basis of systemic lupus erythematosus. *Immunity* 2001;15(3):397–408.
531. Gateva V, Sandling JK, Hom G, et al. A large-scale replication study identifies TNIP1, PRDM1, JAZF1, UHRF1BP1 and IL10 as risk loci for systemic lupus erythematosus. *Nat Genet* 2009;41(11):1228–1233.
532. Moser KL, Kelly JA, Lessard CJ, et al. Recent insights into the genetic basis of systemic lupus erythematosus. *Genes Immun* 2009;10(5):373–379.
533. Cervino AC, Tsinoremas NF, Hoffman RW. A genome-wide study of lupus: Preliminary analysis and data release. *Ann N Y Acad Sci* 2007;1110:131–139.
534. Graham RR, Cotsapas C, Davies L, et al. Genetic variants near TNFAIP3 on 6q23 are associated with systemic lupus erythematosus. *Nat Genet* 2008;40(9):1059–1061.
535. Harley JB, Alarcon-Riquelme ME, Criswell LA, et al. Genome-wide association scan in women with systemic lupus erythematosus identifies susceptibility variants in ITGAM, PXK, KIAA1542 and other loci. *Nat Genet* 2008;40(2):204–210.
536. Hom G, Graham RR, Modrek B, et al. Association of systemic lupus erythematosus with C8orf13–BLK and ITGAM-ITGAX. *N Engl J Med* 2008;358(9):900–909.
537. Kozyrev SV, Abelson AK, Wojcik J, et al. Functional variants in the B-cell gene BANK1 are associated with systemic lupus erythematosus. *Nat Genet* 2008;40(2):211–216.
538. Jacob CO, Zhu J, Armstrong DL, et al. Identification of IRAK1 as a risk gene with critical role in the pathogenesis of systemic lupus erythematosus. *Proc Natl Acad Sci U S A* 2009;106(15):6256–6261.
539. Sawalha AH, Webb R, Han S, et al. Common variants within MECP2 confer risk of systemic lupus erythematosus. *PLoS One* 2008;3(3):e1727.
540. Han JW, Zheng HF, Cui Y, et al. Genome-wide association study in a Chinese Han population identifies nine new susceptibility loci for systemic lupus erythematosus. *Nat Genet* 2009;41(11):1234–1237.
541. Ramos PS, Williams AH, Ziegler JT, et al. Genetic analyses of interferon pathway-related genes reveal multiple new loci associated with systemic lupus erythematosus. *Arthritis Rheum* 2011;63(7):2049–2057.
542. Cunninghame Graham DS, Morris DL, Bhangale TR, et al. Association of NCF2, IKZF1, IRF8, IFIH1, and TYK2 with systemic lupus erythematosus. *PLoS Genet* 2011;7(10):e1002341.
543. Jarvinen TM, Hellquist A, Zucchelli M, et al. Replication of GWAS-identified systemic lupus erythematosus susceptibility genes affirms B-cell receptor pathway signalling and strengthens the role of IRF5 in disease susceptibility in a Northern European population. *Rheumatology (Oxford)* 2012;51(1):87–92.
544. Alarcon-Segovia D, Alarcon-Riquelme ME, Cardiel MH, et al. Familial aggregation of systemic lupus erythematosus, rheumatoid arthritis, and other autoimmune diseases in 1,177 lupus patients from the GLADEL cohort. *Arthritis Rheum* 2005;52(4):1138–1147.
545. Lu Q, Wu A, Tesmer L, et al. Demethylation of CD40LG on the inactive X in T cells from women with lupus. *J Immunol* 2007;179(9):6352–6358.
546. Scofield RH, Bruner GR, Namjou B, et al. Klinefelter's syndrome (47,XXY) in male systemic lupus erythematosus patients: support for the notion of a gene-dose effect from the X chromosome. *Arthritis Rheum* 2008;58(8):2511–2517.
547. Feldman CH, Hiraki LT, Liu J, et al. Epidemiology and sociodemographics of systemic lupus erythematosus and lupus nephritis among US adults with Medicaid coverage, 2000–2004. *Arthritis Rheum* 2013;65(3):753–763.
548. Tan TC, Fang H, Magder LS, et al. Differences between male and female systemic lupus erythematosus in a multiethnic population. *J Rheumatol* 2012;39(4):759–769.
549. Lu LJ, Wallace DJ, Ishimori ML, et al. Review: male systemic lupus erythematosus: A review of sex disparities in this disease. *Lupus* 2010;19(2):119–129.
550. McCarty DJ, Manzi S, Medsger TA Jr, et al. Incidence of systemic lupus erythematosus. Race and gender differences. *Arthritis Rheum* 1995;38(9):1260–1270.
551. Lawrence RC, Helmick CG, Arnett FC, et al. Estimates of the prevalence of arthritis and selected musculoskeletal disorders in the United States. *Arthritis Rheum* 1998;41(5):778–799.
552. Chakravarty EF, Bush TM, Manzi S, et al. Prevalence of adult systemic lupus erythematosus in California and Pennsylvania in 2000: estimates obtained using hospitalization data. *Arthritis Rheum* 2007;56(6):2092–2094.
553. Pons-Estel GJ, Alarcon GS, Scofield L, et al. Understanding the epidemiology and progression of systemic lupus erythematosus. *Semin Arthritis Rheum* 2010;39(4):257–268.
554. Johnson AE, Gordon C, Hobbs FD, et al. Undiagnosed systemic lupus erythematosus in the community. *Lancet* 1996;347(8998):367–369.
555. Johnson AE, Gordon C, Palmer RG, et al. The prevalence and incidence of systemic lupus erythematosus in Birmingham, England. Relationship to ethnicity and country of birth. *Arthritis Rheum* 1995;38(4):551–558.
556. Symmons DP. Frequency of lupus in people of African origin. *Lupus* 1995;4(3):176–178.
557. Chiu YM, Lai CH. Nationwide population-based epidemiologic study of systemic lupus erythematosus in Taiwan. *Lupus* 2010;19(10):1250–1255.
558. Serdula MK, Rhoads GG. Frequency of systemic lupus erythematosus in different ethnic groups in Hawaii. *Arthritis Rheum* 1979;22(4):328–333.
559. Ramos PS, Oates JC, Kamen DL, et al. Variable association of reactive intermediate genes with systemic lupus erythematosus in populations with different African ancestry. *J Rheumatol* 2013;40(6):842–849.
560. Salmon JE, Millard S, Schachter LA, et al. Fc gamma RIIA alleles are heritable risk factors for lupus nephritis in African Americans. *J Clin Invest* 1996;97(5):1348–1354.
561. Marchini M, Antonioli R, Lleo A, et al. HLA class II antigens associated with lupus nephritis in Italian SLE patients. *Hum Immunol* 2003;64(4):462–468.
562. Taylor KE, Remmers EF, Lee AT, et al. Specificity of the STAT4 genetic association for severe disease manifestations of systemic lupus erythematosus. *PLoS Genet* 2008;4(5):e1000084.

563. Sigurdsson S, Nordmark G, Garnier S, et al. A risk haplotype of STAT4 for systemic lupus erythematosus is over-expressed, correlates with anti-dsDNA and shows additive effects with two risk alleles of IRF5. *Hum Mol Genet* 2008;17(18):2868–2876.
564. Kawasaki A, Ito I, Hikami K, et al. Role of STAT4 polymorphisms in systemic lupus erythematosus in a Japanese population: a case-control association study of the STAT1-STAT4 region. *Arthritis Res Ther* 2008;10(5):R113.
565. Li P, Cao C, Luan H, et al. Association of genetic variations in the STAT4 and IRF7/KIAA1542 regions with systemic lupus erythematosus in a Northern Han Chinese population. *Hum Immunol* 2011;72(3):249–255.
566. Edberg JC, Wu J, Langefeld CD, et al. Genetic variation in the CRP promoter: association with systemic lupus erythematosus. *Hum Mol Genet* 2008;17(8):1147–1155.
567. Jonsen A, Gunnarsson I, Gullstrand B, et al. Association between SLE nephritis and polymorphic variants of the CRP and FcgammaRIIIa genes. *Rheumatology (Oxford)* 2007;46(9):1417–1421.
568. Li LH, Yuan H, Pan HF, et al. Role of the Fcgamma receptor IIIA-V/F158 polymorphism in susceptibility to systemic lupus erythematosus and lupus nephritis: a meta-analysis. *Scand J Rheumatol* 2010;39(2):148–154.
569. Duits AJ, Bootsma H, Derksen RH, et al. Skewed distribution of IgG Fc receptor IIa (CD32) polymorphism is associated with renal disease in systemic lupus erythematosus patients. *Arthritis Rheum* 1995;38(12):1832–1836.
570. Gelmetti AP, Freitas AC, Woronik V, et al. Polymorphism of the FcgammaRIIalpha IgG receptor in patients with lupus nephritis and glomerulopathy. *J Rheumatol* 2006;33(3):523–530.
571. Kim-Howard X, Maiti AK, Anaya JM, et al. ITGAM coding variant (rs1143679) influences the risk of renal disease, discoid rash and immunological manifestations in patients with systemic lupus erythematosus with European ancestry. *Ann Rheum Dis* 2010;69(7):1329–1332.
572. Tan W, Sunahori K, Zhao J, et al. Association of PPP2CA polymorphisms with systemic lupus erythematosus susceptibility in multiple ethnic groups. *Arthritis Rheum* 2011;63(9):2755–2763.
573. Lin CP, Adrianto I, Lessard CJ, et al. Role of MYH9 and APOL1 in African and non-African populations with lupus nephritis. *Genes Immun* 2012;13(3):232–238.
574. Zhou XJ, Lu XL, Nath SK, et al. Gene-gene interaction of BLK, TNFSF4, TRAF1, TNFAIP3, and REL in systemic lupus erythematosus. *Arthritis Rheum* 2012;64(1):222–231.
575. Fan W, Liang D, Tang Y, et al. Identification of microRNA-31 as a novel regulator contributing to impaired interleukin-2 production in T cells from patients with systemic lupus erythematosus. *Arthritis Rheum* 2012;64(11):3715–3725.
576. Deng Y, Zhao J, Sakurai D, et al. MicroRNA-3148 modulates allelic expression of toll-like receptor 7 variant associated with systemic lupus erythematosus. *PLoS Genet* 2013;9(2):e1003336.
577. Bennett L, Palucka AK, Arce E, et al. Interferon and granulopoiesis signatures in systemic lupus erythematosus blood. *J Exp Med* 2003;197(6):711–723.
578. Lee-Kirsch MA, Gong M, Chowdhury D, et al. Mutations in the gene encoding the 3′-5′ DNA exonuclease TREX1 are associated with systemic lupus erythematosus. *Nat Genet* 2007;39(9):1065–1067.
579. Namjou B, Kothari PH, Kelly JA, et al. Evaluation of the TREX1 gene in a large multi-ancestral lupus cohort. *Genes Immun* 2011;12(4):270–279.
580. Yasutomo K, Horiuchi T, Kagami S, et al. Mutation of DNASE1 in people with systemic lupus erythematosus. *Nat Genet* 2001;28(4):313–314.
581. Arnett FC, Moulds JM. HLA class III molecules and autoimmune rheumatic diseases. *Clin Exp Rheumatol* 1991;9(3):289–296.
582. Fielder AH, Walport MJ, Batchelor JR, et al. Family study of the major histocompatibility complex in patients with systemic lupus erythematosus: Importance of null alleles of C4A and C4B in determining disease susceptibility. *Br Med J (Clin Res Ed)* 1983;286(6363):425–428.
583. Meyer O, Hauptmann G, Tappeiner G, et al. Genetic deficiency of C4, C2 or C1q and lupus syndromes. Association with anti-Ro (SS-A) antibodies. *Clin Exp Immunol* 1985;62(3):678–684.
584. Agnello V. Lupus diseases associated with hereditary and acquired deficiencies of complement. *Springer Semin Immunopathol* 1986;9(2-3):161–178.
585. Manderson AP, Botto M, Walport MJ. The role of complement in the development of systemic lupus erythematosus. *Annu Rev Immunol* 2004;22:431–456.
586. Sturfelt G, Truedsson L. Complement and its breakdown products in SLE. *Rheumatology (Oxford)* 2005;44(10):1227–1232.
587. Tsao BP. Update on human systemic lupus erythematosus genetics. *Curr Opin Rheumatol* 2004;16(5):513–521.
588. Botto M, Dell'Agnola C, Bygrave AE, et al. Homozygous C1q deficiency causes glomerulonephritis associated with multiple apoptotic bodies. *Nat Genet* 1998;19(1):56–59.
589. Mitchell DA, Pickering MC, Warren J, et al. C1q deficiency and autoimmunity: the effects of genetic background on disease expression. *J Immunol* 2002;168(5):2538–2543.
590. Peterson KS, Huang JF, Zhu J, et al. Characterization of heterogeneity in the molecular pathogenesis of lupus nephritis from transcriptional profiles of laser-captured glomeruli. *J Clin Invest* 2004;113(12):1722–1733.
591. Teramoto K, Negoro N, Kitamoto K, et al. Microarray analysis of glomerular gene expression in murine lupus nephritis. *J Pharmacol Sci* 2008;106(1):56–67.
592. James JA, Harley JB, Scofield RH. Epstein-Barr virus and systemic lupus erythematosus. *Curr Opin Rheumatol* 2006;18(5):462–467.
593. McClain MT, Heinlen LD, Dennis GJ, et al. Early events in lupus humoral autoimmunity suggest initiation through molecular mimicry. *Nat Med* 2005;11(1):85–89.
594. James JA, Kaufman KM, Farris AD, et al. An increased prevalence of Epstein-Barr virus infection in young patients suggests a possible etiology for systemic lupus erythematosus. *J Clin Invest* 1997;100(12):3019–3026.
595. Yung R, Powers D, Johnson K, et al. Mechanisms of drug-induced lupus. II. T cells overexpressing lymphocyte function-associated antigen 1 become autoreactive and cause a lupuslike disease in syngeneic mice. *J Clin Invest* 1996;97(12):2866–2871.
596. Finckh A, Cooper GS, Chibnik LB, et al. Occupational silica and solvent exposures and risk of systemic lupus erythematosus in urban women. *Arthritis Rheum* 2006;54(11):3648–3654.
597. Parks CG, Cooper GS, Nylander-French LA, et al. Occupational exposure to crystalline silica and risk of systemic lupus erythematosus: a population-based, case-control study in the southeastern United States. *Arthritis Rheum* 2002;46(7):1840–1850.
598. Costenbader KH, Kim DJ, Peerzada J, et al. Cigarette smoking and the risk of systemic lupus erythematosus: a meta-analysis. *Arthritis Rheum* 2004;50(3):849–857.
599. Formica MK, Palmer JR, Rosenberg L, et al. Smoking, alcohol consumption, and risk of systemic lupus erythematosus in the Black Women's Health Study. *J Rheumatol* 2003;30(6):1222–1226.
600. Sanchez-Guerrero J, Karlson EW, Colditz GA, et al. Hair dye use and the risk of developing systemic lupus erythematosus. *Arthritis Rheum* 1996;39(4):657–662.
601. Packham DK, Lam SS, Nicholls K, et al. Lupus nephritis and pregnancy. *Q J Med* 1992;83(300):315–324.
602. Jungers P, Nahoul K, Pelissier C, et al. Low plasma androgens in women with active or quiescent systemic lupus erythematosus. *Arthritis Rheum* 1982;25(4):454–457.
603. Lahita RG, Bradlow HL, Ginzler E, et al. Low plasma androgens in women with systemic lupus erythematosus. *Arthritis Rheum* 1987;30(3):241–248.
604. Lavalle C, Gonzalez-Barcena D, Graef A, et al. Gonadotropins pituitary secretion in systemic lupus erythematosus. *Clin Exp Rheumatol* 1984;2(2):163–165.
605. Mackworth-Young CG, Parke AL, Morley KD, et al. Sex hormones in male patients with systemic lupus erythematosus: a comparison with other disease groups. *Eur J Rheumatol Inflamm* 1983;6(3):228–232.
606. Lahita R, ed. *Sex and Age in Systemic Lupus Erythematosus*. New York: Wiley, 1984.
607. Schwartzman-Morris J, Putterman C. Gender differences in the pathogenesis and outcome of lupus and of lupus nephritis. *Clin Dev Immunol* 2012;2012:604892.
608. Hughes T, Adler A, Merrill JT, et al. Analysis of autosomal genes reveals gene-sex interactions and higher total genetic risk in men with systemic lupus erythematosus. *Ann Rheum Dis* 2012;71(5):694–699.

609. Cunningham M, Gilkeson G. Estrogen receptors in immunity and autoimmunity. *Clin Rev Allergy Immunol* 2011;40(1):66–73.
610. Berden JH. Lupus nephritis: consequence of disturbed removal of apoptotic cells? *Neth J Med* 2003;61(8):233–238.
611. Migliorini A, Anders HJ. A novel pathogenetic concept-antiviral immunity in lupus nephritis. *Nat Rev Nephrol* 2012;8(3):183–189.
612. Abdulahad DA, Westra J, Bijzet J, et al. High mobility group box 1 (HMGB1) and anti-HMGB1 antibodies and their relation to disease characteristics in systemic lupus erythematosus. *Arthritis Res Ther* 2011;13(3):R71.
613. Harley JB, James JA. Autoepitopes in lupus. *J Lab Clin Med* 1995;126(6):509–516.
614. McCurdy DK, Bick M, Gatti RA, et al. Autoantibodies in systemic lupus erythematosus. *Dis Markers* 1992;10(1):37–49.
615. Spronk PE, Limburg PC, Kallenberg CG. Serological markers of disease activity in systemic lupus erythematosus. *Lupus* 1995;4(2):86–94.
616. Reeves WH, Satoh M, Wang J, et al. Systemic lupus erythematosus. Antibodies to DNA, DNA-binding proteins, and histones. *Rheum Dis Clin North Am* 1994;20(1):1-28.
617. Kumar S, Bunting KA, Kalsi J, et al. Lupus autoantibodies to native DNA preferentially bind DNA presented on PolIV. *Immunology* 2005;114(3):418–427.
618. Seret G, Le Meur Y, Renaudineau Y, et al. Mesangial cell-specific antibodies are central to the pathogenesis of lupus nephritis. *Clin Dev Immunol* 2012;2012:579670.
619. Walz LeBlanc BA, Gladman DD, Urowitz MB. Serologically active clinically quiescent systemic lupus erythematosus—predictors of clinical flares. *J Rheumatol* 1994;21(12):2239–2241.
620. Gladman DD, Hirani N, Ibanez D, et al. Clinically active serologically quiescent systemic lupus erythematosus. *J Rheumatol* 2003;30(9): 1960–1962.
621. Budhai L, Oh K, Davidson A. An in vitro assay for detection of glomerular binding IgG autoantibodies in patients with systemic lupus erythematosus. *J Clin Invest* 1996;98(7):1585–1593.
622. Li QZ, Xie C, Wu T, et al. Identification of autoantibody clusters that best predict lupus disease activity using glomerular proteome arrays. *J Clin Invest* 2005;115(12):3428–3439.
623. Sontheimer RD, McCauliffe DP, Zappi E, et al. Antinuclear antibodies: Clinical correlations and biologic significance. *Adv Dermatol* 1992;7: 3-52; discussion 3.
624. Tan EM, Feltkamp TE, Smolen JS, et al. Range of antinuclear antibodies in "healthy" individuals. *Arthritis Rheum* 1997;40(9):1601–1611.
625. Arbuckle MR, McClain MT, Rubertone MV, et al. Development of autoantibodies before the clinical onset of systemic lupus erythematosus. *N Engl J Med* 2003;349(16):1526–1533.
626. Tan EM, Kunkel HG. Characteristics of a soluble nuclear antigen precipitating with sera of patients with systemic lupus erythematosus. *J Immunol* 1966;96(3):464–471.
627. Homma M, Mimori T, Takeda Y, et al. Autoantibodies to the Sm antigen: Immunological approach to clinical aspects of systemic lupus erythematosus. *J Rheumatol Suppl* 1987;14(Suppl 13):188–193.
628. Craft J. Antibodies to snRNPs in systemic lupus erythematosus. *Rheum Dis Clin North Am* 1992;18(2):311–335.
629. Hinterberger M, Pettersson I, Steitz JA. Isolation of small nuclear ribonucleoproteins containing U1, U2, U4, U5, and U6 RNAs. *J Biol Chem* 1983;258(4):2604–2613.
630. Beaufils M, Kouki F, Mignon F, et al. Clinical significance of anti-Sm antibodies in systemic lupus erythematosus. *Am J Med* 1983;74(2):201–205.
631. Combe B, Rucheton M, Graafland H, et al. Clinical significance of anti-RNP and anti-Sm autoantibodies as determined by immunoblotting and immunoprecipitation in sera from patients with connective tissue diseases. *Clin Exp Immunol* 1989;75(1):18–24.
632. Reeves WH, Fisher DE, Lahita RG, et al. Autoimmune sera reactive with Sm antigen contain high levels of RNP-like antibodies. *J Clin Invest* 1985;75(2):580–587.
633. Sharp GC, Irvin WS, May CM, et al. Association of antibodies to ribonucleoprotein and Sm antigens with mixed connective-tissue disease, systematic lupus erythematosus and other rheumatic diseases. *N Engl J Med* 1976;295(21):1149–1154.
634. Bizzaro N, Villalta D, Giavarina D, et al. Are anti-nucleosome antibodies a better diagnostic marker than anti-dsDNA antibodies for systemic lupus erythematosus? A systematic review and a study of metanalysis. *Autoimmun Rev* 2012;12(2):97–106.
635. Anderson JR, Gray KG, Beck JS, et al. Precipitating auto-antibodies in the connective tissue diseases. *Ann Rheum Dis* 1962;21:360–369.
636. Tang X, Huang Y, Deng W, et al. Clinical and serologic correlations and autoantibody clusters in systemic lupus erythematosus: a retrospective review of 917 patients in South China. *Medicine (Baltimore)* 2010;89(1):62–67.
637. Blomberg S, Ronnblom L, Wallgren AC, et al. Anti-SSA/Ro antibody determination by enzyme-linked immunosorbent assay as a supplement to standard immunofluorescence in antinuclear antibody screening. *Scand J Immunol* 2000;51(6):612–617.
638. McCauliffe DP, Zappi E, Lieu TS, et al. A human Ro/SS-A autoantigen is the homologue of calreticulin and is highly homologous with onchocercal RAL-1 antigen and an aplysia "memory molecule". *J Clin Invest* 1990;86(1):332–335.
639. Kurilla MG, Keene JD. The leader RNA of vesicular stomatitis virus is bound by a cellular protein reactive with anti-La lupus antibodies. *Cell* 1983;34(3):837–845.
640. Reichlin M, Van Venrooij WJ. Autoantibodies to the URNP particles: relationship to clinical diagnosis and nephritis. *Clin Exp Immunol* 1991;83(2):286–290.
641. Tsacheva I, Radanova M, Todorova N, et al. Detection of autoantibodies against the globular domain of human C1q in the sera of systemic lupus erythematosus patients. *Mol Immunol* 2007;44(8):2147–2151.
642. Sinico RA, Radice A, Ikehata M, et al. Anti-C1q autoantibodies in lupus nephritis: prevalence and clinical significance. *Ann N Y Acad Sci* 2005;1050:193–200.
643. Coremans IE, Spronk PE, Bootsma H, et al. Changes in antibodies to C1q predict renal relapses in systemic lupus erythematosus. *Am J Kidney Dis* 1995;26(4):595–601.
644. Yin Y, Wu X, Shan G, et al. Diagnostic value of serum anti-C1q antibodies in patients with lupus nephritis: a meta-analysis. *Lupus* 2012;21(10):1088–1097.
645. Fremeaux-Bacchi V, Noel LH, Schifferli JA. No lupus nephritis in the absence of antiC1q autoantibodies? *Nephrol Dial Transplant* 2002;17(12):2041–2043.
646. Trendelenburg M, Marfurt J, Gerber I, et al. Lack of occurrence of severe lupus nephritis among anti-C1q autoantibody-negative patients. *Arthritis Rheum* 1999;42(1):187–188.
647. Radanova M, Vasilev V, Deliyska B, et al. Anti-C1q autoantibodies specific against the globular domain of the C1qB-chain from patient with lupus nephritis inhibit C1q binding to IgG and CRP. *Immunobiology* 2012;217(7):684–691.
648. Trouw LA, Groeneveld TW, Seelen MA, et al. Anti-C1q autoantibodies deposit in glomeruli but are only pathogenic in combination with glomerular C1q-containing immune complexes. *J Clin Invest.* 2004;114(5):679–688.
649. Miyazaki M, Endoh M, Suga T, et al. Rheumatoid factors and glomerulonephritis. *Clin Exp Immunol* 1990;81(2):250–255.
650. Garcia-Carrasco M, Ramos-Casals M, Cervera R, et al. Cryoglobulinemia in systemic lupus erythematosus: prevalence and clinical characteristics in a series of 122 patients. *Semin Arthritis Rheum* 2001;30(5):366–373.
651. Agnello V, Koffler D, Kunkel HG. Immune complex systems in the nephritis of systemic lupus erythematosus. *Kidney Int* 1973;3(2):90–99.
652. Sugisaki T, Takase S. Composition of immune deposits present in glomeruli of NZB/WF1 mice. *Clin Immunol Immunopathol* 1991;61(3):296–308.
653. Cines DB, Lyss AP, Reeber M, et al. Presence of complement-fixing anti-endothelial cell antibodies in systemic lupus erythematosus. *J Clin Invest* 1984;73(3):611–625.
654. D'Cruz DP, Houssiau FA, Ramirez G, et al. Antibodies to endothelial cells in systemic lupus erythematosus: a potential marker for nephritis and vasculitis. *Clin Exp Immunol* 1991;85(2):254–261.
655. van der Zee JM, Siegert CE, de Vreede TA, et al. Characterization of anti-endothelial cell antibodies in systemic lupus erythematosus (SLE). *Clin Exp Immunol* 1991;84(2):238–244.

656. Wang MX, Walker RG, Kincaid-Smith P. Clinicopathologic associations of anti-endothelial cell antibodies in immunoglobulin A nephropathy and lupus nephritis. *Am J Kidney Dis* 1993;22(3):378–386.
657. Matsuo S, Fukatsu A, Taub ML, et al. Glomerulonephritis induced in the rabbit by antiendothelial antibodies. *J Clin Invest* 1987;79(6): 1798–1811.
658. Perry GJ, Elston T, Khouri NA, et al. Antiendothelial cell antibodies in lupus: Correlations with renal injury and circulating markers of endothelial damage. *Q J Med* 1993;86(11):727–734.
659. Frampton G, Moriya S, Pearson JD, et al. Identification of candidate endothelial cell autoantigens in systemic lupus erythematosus using a molecular cloning strategy: a role for ribosomal P protein P0 as an endothelial cell autoantigen. *Rheumatology (Oxford)* 2000;39(10):1114–1120.
660. Fujii H, Nakatani K, Arita N, et al. Internalization of antibodies by endothelial cells via fibronectin implicating a novel mechanism in lupus nephritis. *Kidney Int* 2003;64(5):1662–1670.
661. Cambridge G, Wallace H, Bernstein RM, et al. Autoantibodies to myeloperoxidase in idiopathic and drug-induced systemic lupus erythematosus and vasculitis. *Br J Rheumatol* 1994;33(2):109–114.
662. Gallicchio MC, Savige JA. Detection of anti-myeloperoxidase and anti-elastase antibodies in vasculitides and infections. *Clin Exp Immunol* 1991;84(2):232–237.
663. Kuster S, Apenberg S, Andrassy K, et al. Antineutrophil cytoplasmic antibodies in systemic lupus erythematosus. *Contrib Nephrol* 1992;99:94–98.
664. Lee SS, Lawton JW, Chan CE, et al. Antilactoferrin antibody in systemic lupus erythematosus. *Br J Rheumatol* 1992;31(10):669–673.
665. Nassberger L, Sjoholm AG, Jonsson H, et al. Autoantibodies against neutrophil cytoplasm components in systemic lupus erythematosus and in hydralazine-induced lupus. *Clin Exp Immunol* 1990;81(3):380–383.
666. Mongan ES, Leddy JP, Atwater EC, et al. Direct antiglobulin (Coombs) reactions in patients with connective tissue diseases. *Arthritis Rheum* 1967;10(6):502–508.
667. Berchtold P, Harris JP, Tani P, et al. Autoantibodies to platelet glycoproteins in patients with disease-related immune thrombocytopenia. *Br J Haematol* 1989;73(3):365–368.
668. Messner RP, De Horatius R, Ferrone S. Lymphocytotoxic antibodies in systemic lupus erythematosus patients and their relatives: reactivity with the HLA antigenic molecular complex. *Arthritis Rheum* 1980;23(3):265–272.
669. Winfield JB, Mimura T. Pathogenetic significance of anti-lymphocyte autoantibodies in systemic lupus erythematosus. *Clin Immunol Immunopathol* 1992;63(1):13–16.
670. Kuroki A, Shibata T, Honda H, et al. Glomerular and serum IgG subclasses in diffuse proliferative lupus nephritis, membranous lupus nephritis, and idiopathic membranous nephropathy. *Intern Med* 2002;41(11):936–942.
671. Winkler TH, Henschel TA, Kalies I, et al. Constant isotype pattern of anti-dsDNA antibodies in patients with systemic lupus erythematosus. *Clin Exp Immunol* 1988;72(3):434–439.
672. Zuniga R, Markowitz GS, Arkachaisri T, et al. Identification of IgG subclasses and C-reactive protein in lupus nephritis: the relationship between the composition of immune deposits and FCgamma receptor type IIA alleles. *Arthritis Rheum* 2003;48(2):460–470.
673. Imai H, Hamai K, Komatsuda A, et al. IgG subclasses in patients with membranoproliferative glomerulonephritis, membranous nephropathy, and lupus nephritis. *Kidney Int* 1997;51(1):270–276.
674. Noel LH, Aucouturier P, Monteiro RC, et al. Glomerular and serum immunoglobulin G subclasses in membranous nephropathy and anti-glomerular basement membrane nephritis. *Clin Immunol Immunopathol* 1988;46(2):186–194.
675. Roberts JL, Wyatt RJ, Schwartz MM, et al. Differential characteristics of immune-bound antibodies in diffuse proliferative and membranous forms of lupus glomerulonephritis. *Clin Immunol Immunopathol* 1983;29(2):223–241.
676. Iskandar SS, Falk RJ, Jennette JC. Clinical and pathologic features of fibrillary glomerulonephritis. *Kidney Int* 1992;42(6):1401–1407.
677. Haas M. IgG subclass deposits in glomeruli of lupus and nonlupus membranous nephropathies. *Am J Kidney Dis* 1994;23(3):358–364.
678. Devey ME, Lee SR, Le Page S, et al. Serial studies of the IgG subclass and functional affinity of DNA antibodies in systemic lupus erythematosus. *J Autoimmun* 1988;1(5):483–494.
679. Pennebaker JB, Gilliam JN, Ziff M. Immunoglobulin classes of DNA binding activity in serum and skin in systemic lupus erythematosus. *J Clin Invest* 1977;60(6):1331–1338.
680. Bijl M, Dijstelbloem HM, Oost WW, et al. IgG subclass distribution of autoantibodies differs between renal and extra-renal relapses in patients with systemic lupus erythematosus. *Rheumatology (Oxford)* 2002;41(1):62–67.
681. Amoura Z, Koutouzov S, Chabre H, et al. Presence of antinucleosome autoantibodies in a restricted set of connective tissue diseases: antinucleosome antibodies of the IgG3 subclass are markers of renal pathogenicity in systemic lupus erythematosus. *Arthritis Rheum* 2000;43(1):76–84.
682. Takahashi S, Nose M, Sasaki J, et al. IgG3 production in MRL/lpr mice is responsible for development of lupus nephritis. *J Immunol* 1991;147(2):515–519.
683. Lemoine R, Berney T, Shibata T, et al. Induction of "wire-loop" lesions by murine monoclonal IgG3 cryoglobulins. *Kidney Int* 1992;41(1):65–72.
684. Asano Y, Nakamoto Y. Avidity of anti-native DNA antibody and glomerular immune complex localization in lupus nephritis. *Clin Nephrol* 1978;10(4):123–129.
685. Winfield JB, Faiferman I, Koffler D. Avidity of anti-DNA antibodies in serum and IgG glomerular eluates from patients with systemic lupus erythematosus. Association of high avidity antinative DNA antibody with glomerulonephritis. *J Clin Invest* 1977;59(1):90–96.
686. Dang H, Harbeck RJ. A comparison of anti-DNA antibodies from serum and kidney eluates of NZB x NZW F1 mice. *J Clin Lab Immunol* 1982;9(2):139–145.
687. Pankewycz OG, Migliorini P, Madaio MP. Polyreactive autoantibodies are nephritogenic in murine lupus nephritis. *J Immunol* 1987;139(10):3287–3294.
688. Gilkeson GS, Bernstein K, Pippen AM, et al. The influence of variable-region somatic mutations on the specificity and pathogenicity of murine monoclonal anti-DNA antibodies. *Clin Immunol Immunopathol* 1995;76(1 Pt 1):59–67.
689. Harada T, Suzuki N, Mizushima Y, et al. Usage of a novel class of germline Ig variable region gene for cationic anti-DNA autoantibodies in human lupus nephritis and its role for the development of the disease. *J Immunol* 1994;153(10):4806–4815.
690. Demaison C, Chastagner P, Theze J, et al. Somatic diversification in the heavy chain variable region genes expressed by human autoantibodies bearing a lupus-associated nephritogenic anti-DNA idiotype. *Proc Natl Acad Sci U S A* 1994;91(2):514–518.
691. Izui S, Lambert PH, Miescher PA. In vitro demonstration of a particular affinity of glomerular basement membrane and collagen for DNA A possible basis for a local formation of DNA-anti-DNA complexes in systemic lupus erythematosus. *J Exp Med* 1976;144(2):428–443.
692. Gay S, Losman MJ, Koopman WJ, et al. Interaction of DNA with connective tissue matrix proteins reveals preferential binding to type V collagen. *J Immunol* 1985;135(2):1097–1100.
693. Lake RA, Morgan A, Henderson B, et al. A key role for fibronectin in the sequential binding of native dsDNA and monoclonal anti-DNA antibodies to components of the extracellular matrix: its possible significance in glomerulonephritis. *Immunology* 1985;54(2):389–395.
694. Termaat RM, Assmann KJ, Dijkman HB, et al. Anti-DNA antibodies can bind to the glomerulus via two distinct mechanisms. *Kidney Int* 1992;42(6):1363–1371.
695. Malide D, Londono I, Russo P, et al. Ultrastructural localization of DNA in immune deposits of human lupus nephritis. *Am J Pathol* 1993;143(1):304–311.
696. Fournie GJ. Circulating DNA and lupus nephritis. *Kidney Int* 1988;33(2):487–497.
697. Barnes JL, Venkatachalam MA. Enhancement of glomerular immune complex deposition by a circulating polycation. *J Exp Med* 1984;160(1):286–293.
698. Brinkman K, Termaat R, Berden JH, et al. Anti-DNA antibodies and lupus nephritis: the complexity of crossreactivity. *Immunol Today* 1990;11(7):232–234.

699. Kornberg RD, Thomas JO. Chromatin structure; oligomers of the histones. *Science* 1974;184(139):865–868.
700. Schmiedeke TM, Stockl FW, Weber R, et al. Histones have high affinity for the glomerular basement membrane. Relevance for immune complex formation in lupus nephritis. *J Exp Med* 1989;169(6):1879–1894.
701. Termaat RM, Brinkman K, van Gompel F, et al. Cross-reactivity of monoclonal anti-DNA antibodies with heparan sulfate is mediated via bound DNA/histone complexes. *J Autoimmun* 1990;3(5):531–545.
702. Stockl F, Muller S, Batsford S, et al. A role for histones and ubiquitin in lupus nephritis? *Clin Nephrol* 1994;41(1):10–17.
703. Schmiedeke T, Stoeckl F, Muller S, et al. Glomerular immune deposits in murine lupus models may contain histones. *Clin Exp Immunol* 1992;90(3):453–458.
704. Amoura Z, Chabre H, Koutouzov S, et al. Nucleosome-restricted antibodies are detected before anti-dsDNA and/or antihistone antibodies in serum of MRL-Mp lpr/lpr and +/+ mice, and are present in kidney eluates of lupus mice with proteinuria. *Arthritis Rheum* 1994;37(11):1684–1688.
705. Andres GA, Accinni L, Beiser SM, et al. Localization of fluorescein-labeled antinucleoside antibodies in glomeruli of patients with active systemic lupus erythematosus nephritis. *J Clin Invest* 1970;49(11):2106–2118.
706. Koffler D, Agnello V, Kimkel HG. Polynucleotide immune complexes in serum and glomeruli of patients with systemic lupus erythematosus. *Am J Pathol* 1974;74(1):109–124.
707. Mohan C, Morel L, Yang P, et al. Genetic dissection of lupus pathogenesis: A recipe for nephrophilic autoantibodies. *J Clin Invest* 1999;103(12):1685–1695.
708. Sasaki T, Hatakeyama A, Shibata S, et al. Heterogeneity of immune complex-derived anti-DNA antibodies associated with lupus nephritis. *Kidney Int* 1991;39(4):746–753.
709. Termaat RM, Assmann KJ, van Son JP, et al. Antigen-specificity of antibodies bound to glomeruli of mice with systemic lupus erythematosus-like syndromes. *Lab Invest* 1993;68(2):164–173.
710. van Bruggen MC, Kramers C, Hylkema MN, et al. Significance of antinuclear and anti-extracellular matrix autoantibodies for albuminuria in murine lupus nephritis; a longitudinal study on plasma and glomerular eluates in MRL/l mice. *Clin Exp Immunol* 1996;105(1):132–139.
711. Xie C, Liang Z, Chang S, et al. Use of a novel elution regimen reveals the dominance of polyreactive antinuclear autoantibodies in lupus kidneys. *Arthritis Rheum* 2003;48(8):2343–2352.
712. Yoshida H, Yoshida M, Izui S, et al. Distinct clonotypes of anti-DNA antibodies in mice with lupus nephritis. *J Clin Invest* 1985;76(2):685–694.
713. Sabbaga J, Pankewycz OG, Lufft V, et al. Cross-reactivity distinguishes serum and nephritogenic anti-DNA antibodies in human lupus from their natural counterparts in normal serum. *J Autoimmun* 1990;3(2):215–235.
714. Mannik M, Merrill CE, Stamps LD, et al. Multiple autoantibodies form the glomerular immune deposits in patients with systemic lupus erythematosus. *J Rheumatol* 2003;30(7):1495–1504.
715. Jacob L, Viard JP, Allenet B, et al. A monoclonal anti-double-stranded DNA autoantibody binds to a 94–kDa cell-surface protein on various cell types via nucleosomes or a DNA-histone complex. *Proc Natl Acad Sci U S A* 1989;86(12):4669–4673.
716. Burlingame RW, Rubin RL, Balderas RS, et al. Genesis and evolution of antichromatin autoantibodies in murine lupus implicates T-dependent immunization with self antigen. *J Clin Invest* 1993;91(4):1687–1696.
717. Elouaai F, Lule J, Benoist H, et al. Autoimmunity to histones, ubiquitin, and ubiquitinated histone H2A in NZB x NZW and MRL-lpr/lpr mice. Anti-histone antibodies are concentrated in glomerular eluates of lupus mice. *Nephrol Dial Transplant* 1994;9(4):362–366.
718. Berden JH, van Bruggen MC. Nucleosomes and the pathogenesis of lupus nephritis. *Kidney Blood Press Res* 1997;20(3):198–200.
719. Berden JH, Grootscholten C, Jurgen WC, et al. Lupus nephritis: A nucleosome waste disposal defect? *J Nephrol 2002*;15(Suppl 6):S1–S10.
720. Kalaaji M, Fenton KA, Mortensen ES, et al. Glomerular apoptotic nucleosomes are central target structures for nephritogenic antibodies in human SLE nephritis. *Kidney Int* 2007;71(7):664–672.
721. Chen XM, Aozuka S, Kamata K, et al. Characterization of a polyreactive monoclonal antibody to dsDNA, F x 1A, and heparan sulfate generated from BALB/c mice immunized with rat renal homogenates. *Clin Immunol Immunopathol* 1989;51(3):326–337.
722. Faaber P, Rijke TP, van de Putte LB, et al. Cross-reactivity of human and murine anti-DNA antibodies with heparan sulfate. The major glycosaminoglycan in glomerular basement membranes. *J Clin Invest* 1986;77(6):1824–1830.
723. Krishnan MR, Wang C, Marion TN. Anti-DNA autoantibodies initiate experimental lupus nephritis by binding directly to the glomerular basement membrane in mice. *Kidney Int* 2012;82(2):184–192.
724. Lafer EM, Rauch J, Andrzejewski C Jr, et al. Polyspecific monoclonal lupus autoantibodies reactive with both polynucleotides and phospholipids. *J Exp Med* 1981;153(4):897–909.
725. Shoenfeld Y, Rauch J, Massicotte H, et al. Polyspecificity of monoclonal lupus autoantibodies produced by human-human hybridomas. *N Engl J Med* 1983;308(8):414–420.
726. Smeenk RJ, Lucassen WA, Swaak TJ. Is anticardiolipin activity a cross-reaction of anti-DNA or a separate entity? *Arthritis Rheum* 1987;30(6):607–617.
727. Bruijn JA, van Leer EH, Baelde HJ, et al. Characterization and in vivo transfer of nephritogenic autoantibodies directed against dipeptidyl peptidase IV and laminin in experimental lupus nephritis. *Lab Invest* 1990;63(3):350–359.
728. Sabbaga J, Line SR, Potocnjak P, et al. A murine nephritogenic monoclonal anti-DNA autoantibody binds directly to mouse laminin, the major non-collagenous protein component of the glomerular basement membrane. *Eur J Immunol* 1989;19(1):137–143.
729. Bruijn JA, Bergijk EC, de Heer E, et al. Induction and progression of experimental lupus nephritis: exploration of a pathogenetic pathway. *Kidney Int* 1992;41(1):5-13.
730. Rolink AG, Radaszkiewicz T, Melchers F. Monoclonal autoantibodies specific for kidney proximal tubular brush border from mice with experimentally induced chronic graft-versus-host disease. *Scand J Immunol* 1988;28(1):29–41.
731. Andre-Schwartz J, Datta SK, Shoenfeld Y, et al. Binding of cytoskeletal proteins by monoclonal anti-DNA lupus autoantibodies. *Clin Immunol Immunopathol* 1984;31(2):261–271.
732. Mostoslavsky G, Fischel R, Yachimovich N, et al. Lupus anti-DNA autoantibodies cross-react with a glomerular structural protein: a case for tissue injury by molecular mimicry. *Eur J Immunol* 2001;31(4):1221–1227.
733. Zhao Z, Weinstein E, Tuzova M, et al. Cross-reactivity of human lupus anti-DNA antibodies with alpha-actinin and nephritogenic potential. *Arthritis Rheum* 2005;52(2):522–530.
734. Frampton G, Hobby P, Morgan A, et al. A role for DNA in anti-DNA antibodies binding to endothelial cells. *J Autoimmun* 1991;4(3):463–478.
735. Jacob L, Lety MA, Louvard D, et al. Binding of a monoclonal anti-DNA autoantibody to identical protein(s) present at the surface of several human cell types involved in lupus pathogenesis. *J Clin Invest* 1985;75(1):315–317.
736. Jacob L, Lety MA, Monteiro RC, et al. Altered cell-surface protein(s), crossreactive with DNA, on spleen cells of autoimmune lupic mice. *Proc Natl Acad Sci U S A* 1987;84(5):1361–1363.
737. Suzuki N, Harada T, Mizushima Y, et al. Possible pathogenic role of cationic anti-DNA autoantibodies in the development of nephritis in patients with systemic lupus erythematosus. *J Immunol* 1993;151(2):1128–1136.
738. Suzuki N, Otuka I, Harada T, et al. Preferential adsorption of cationic anti-DNA antibodies with immobilized polyanionic compounds, dextran sulfate. *Autoimmunity* 1994;19(2):105–112.
739. Kramers C, Termaat RM, ter Borg EJ, et al. Higher anti-heparan sulphate reactivity during systemic lupus erythematosus (SLE) disease exacerbations with renal manifestations; a long term prospective analysis. *Clin Exp Immunol* 1993;93(1):34–38.
740. Lloyd W, Schur PH. Immune complexes, complement, and anti-DNA in exacerbations of systemic lupus erythematosus (SLE). *Medicine (Baltimore)* 1981;60(3):208–217.
741. Cameron J. The nephritis of systemic lupus erythematosus. In: Kincaid Smith PdA, AJF Atkins RC, eds. *Progress in Glomerulonephritis*. New York: Wiley, 1979:387.

742. Gunnarsson I, Sundelin B, Heimburger M, et al. Repeated renal biopsy in proliferative lupus nephritis—predictive role of serum C1q and albuminuria. *J Rheumatol* 2002;29(4):693–699.
743. Buyon JP, Tamerius J, Belmont HM, et al. Assessment of disease activity and impending flare in patients with systemic lupus erythematosus. Comparison of the use of complement split products and conventional measurements of complement. *Arthritis Rheum* 1992;35(9):1028–1037.
744. Manzi S, Rairie JE, Carpenter AB, et al. Sensitivity and specificity of plasma and urine complement split products as indicators of lupus disease activity. *Arthritis Rheum* 1996;39(7):1178–1188.
745. Shen Y, Chen XW, Sun CY, et al. Association between anti-beta2 glycoprotein I antibodies and renal glomerular C4d deposition in lupus nephritis patients with glomerular microthrombosis: a prospective study of 155 cases. *Lupus* 2010;19(10):1195–1203.
746. Blaese RM, Grayson J, Steinberg AD. Increased immunoglobulin-secreting cells in the blood of patients with active systemic lupus erythematosus. *Am J Med* 1980;69(3):345–350.
747. Ishigatsubo Y, Sakamoto H, Hagiwara E, et al. Quantitation of autoantibody-secreting B cells in systemic lupus erythematosus. *Autoimmunity* 1989;5(1-2):71–78.
748. Klinman DM, Steinberg AD. Systemic autoimmune disease arises from polyclonal B cell activation. *J Exp Med* 1987;165(6):1755–1760.
749. Klinman DM, Steinberg AD. Inquiry into murine and human lupus. *Immunol Rev* 1995;144:157–193.
750. Eisenberg RA, Dyer K, Craven SY, et al. Subclass restriction and polyclonality of the systemic lupus erythematosus marker antibody anti-Sm. *J Clin Invest* 1985;75(4):1270–1277.
751. Eisenberg RA, Craven SY, Warren RW, et al. Stochastic control of anti-Sm autoantibodies in MRL/Mp-lpr/lpr mice. *J Clin Invest* 1987;80(3):691–697.
752. Gharavi AE, Chu JL, Elkon KB. Autoantibodies to intracellular proteins in human systemic lupus erythematosus are not due to random polyclonal B cell activation. *Arthritis Rheum* 1988;31(11):1337–1345.
753. Schwartz RS, Stollar BD. Origins of anti-DNA autoantibodies. *J Clin Invest* 1985;75(2):321–327.
754. Strasser A, Whittingham S, Vaux DL, et al. Enforced BCL2 expression in B-lymphoid cells prolongs antibody responses and elicits autoimmune disease. *Proc Natl Acad Sci U S A* 1991;88(19):8661–8665.
755. Blank MC, Stefanescu RN, Masuda E, et al. Decreased transcription of the human FCGR2B gene mediated by the -343G/C promoter polymorphism and association with systemic lupus erythematosus. *Hum Genet* 2005;117(2-3):220–227.
756. Liossis SN, Solomou EE, Dimopoulos MA, et al. B-cell kinase lyn deficiency in patients with systemic lupus erythematosus. *J Investig Med* 2001;49(2):157–165.
757. Su W, Madaio MP. Recent advances in the pathogenesis of lupus nephritis: Autoantibodies and B cells. *Semin Nephrol* 2003;23(6):564–568.
758. Adams S, Leblanc P, Datta SK. Junctional region sequences of T-cell receptor beta-chain genes expressed by pathogenic anti-DNA autoantibody-inducing helper T cells from lupus mice: possible selection by cationic autoantigens. *Proc Natl Acad Sci U S A* 1991;88(24):11271–11275.
759. Desai-Mehta A, Mao C, Rajagopalan S, et al. Structure and specificity of T cell receptors expressed by potentially pathogenic anti-DNA autoantibody-inducing T cells in human lupus. *J Clin Invest* 1995;95(2):531–541.
760. Morimoto C, Reinherz EL, Schlossman SF, et al. Alterations in immunoregulatory T cell subsets in active systemic lupus erythematosus. *J Clin Invest* 1980;66(5):1171–1174.
761. Tsokos GC, Balow JE. Phenotypes of T lymphocytes in systemic lupus erythematosus: decreased cytotoxic/suppressor subpopulation is associated with deficient allogeneic cytotoxic responses rather than with concanavalin A-induced suppressor cells. *Clin Immunol Immunopathol* 1983;26(2):267–276.
762. Linker-Israeli M, Quismorio FP Jr, Horwitz DA. CD8+ lymphocytes from patients with systemic lupus erythematosus sustain, rather than suppress, spontaneous polyclonal IgG production and synergize with CD4+ cells to support autoantibody synthesis. *Arthritis Rheum* 1990;33(8):1216–1225.
763. Takahashi T, Gray JD, Horwitz DA. Human CD8+ lymphocytes stimulated in the absence of CD4+ cells enhance IgG production by antibody-secreting B cells. *Clin Immunol Immunopathol* 1991;58(3):352–365.
764. Raziuddin S, Nur MA, Alwabel AA. Selective loss of the CD4+ inducers of suppressor T cell subsets (2H4+) in active systemic lupus erythematosus. *J Rheumatol* 1989;16(10):1315–1319.
765. Apostolidis SA, Crispin JC, Tsokos GC. IL-17–producing T cells in lupus nephritis. *Lupus* 2011;20(2):120–124.
766. Kammer GM, Perl A, Richardson BC, et al. Abnormal T cell signal transduction in systemic lupus erythematosus. *Arthritis Rheum* 2002;46(5):1139–1154.
767. Takeuchi T, Tsuzaka K, Abe T, et al. T cell abnormalities in systemic lupus erythematosus. *Autoimmunity* 2005;38(5):339–346.
768. Gorman CL, Russell AI, Zhang Z, et al. Polymorphisms in the CD3Z gene influence TCRzeta expression in systemic lupus erythematosus patients and healthy controls. *J Immunol* 2008;180(2):1060–1070.
769. Warchol T, Piotrowski P, Lianeri M, et al. The CD3Z 844 T > A polymorphism within the 3′-UTR of CD3Z confers increased risk of incidence of systemic lupus erythematosus. *Tissue Antigens* 2009;74(1):68–72.
770. Li R, Yang W, Zhang J, et al. Association of CD247 with systemic lupus erythematosus in Asian populations. *Lupus* 2012;21(1):75–83.
771. Kammer GM, Khan IU, Malemud CJ. Deficient type I protein kinase A isozyme activity in systemic lupus erythematosus T lymphocytes. *J Clin Invest* 1994;94(1):422–430.
772. Zhang Y, Zhao M, Sawalha AH, et al. Impaired DNA methylation and its mechanisms in CD4(+)T cells of systemic lupus erythematosus. *J Autoimmun* 2013;41:92–99.
773. Anders HJ, Vielhauer V, Eis V, et al. Activation of toll-like receptor-9 induces progression of renal disease in MRL-Fas(lpr) mice. *FASEB J* 2004;18(3):534–536.
774. Yung RL, Richardson BC. Role of T cell DNA methylation in lupus syndromes. *Lupus* 1994;3(6):487–491.
775. Clynes R, Dumitru C, Ravetch JV. Uncoupling of immune complex formation and kidney damage in autoimmune glomerulonephritis. *Science* 1998;279(5353):1052–1054.
776. Rubin Kelley V, Bloom RD, Yui MA, et al. Pivotal role of colony stimulating factor-1 in lupus nephritis. *Kidney Int Suppl* 1994;45:S83–S85.
777. Bloom RD, Florquin S, Singer GG, et al. Colony stimulating factor-1 in the induction of lupus nephritis. *Kidney Int* 1993;43(5):1000–1009.
778. Noris M, Bernasconi S, Casiraghi F, et al. Monocyte chemoattractant protein-1 is excreted in excessive amounts in the urine of patients with lupus nephritis. *Lab Invest* 1995;73(6):804–809.
779. Moore KJ, Wada T, Barbee SD, et al. Gene transfer of RANTES elicits autoimmune renal injury in MRL-Fas(1pr) mice. *Kidney Int* 1998;53(6):1631–1641.
780. Wuthrich RP, Jevnikar AM, Takei F, et al. Intercellular adhesion molecule-1 (ICAM-1) expression is upregulated in autoimmune murine lupus nephritis. *Am J Pathol* 1990;136(2):441–450.
781. Wuthrich RP. Vascular cell adhesion molecule-1 (VCAM-1) expression in murine lupus nephritis. *Kidney Int* 1992;42(4):903–914.
782. Wuthrich RP, Fan X, Ritthaler T, et al. Enhanced osteopontin expression and macrophage infiltration in MRL-Fas(lpr) mice with lupus nephritis. *Autoimmunity* 1998;28(3):139–150.
783. Diaz Gallo C, Jevnikar AM, Brennan DC, et al. Autoreactive kidney-infiltrating T-cell clones in murine lupus nephritis. *Kidney Int* 1992;42(4):851–859.
784. Fuiano G, Sepe V, Magri P, et al. Intercellular adhesion molecule-1 as a marker of activity in lupus nephropathy. *Contrib Nephrol* 1992;99:131–134.
785. Yokoyama H, Takabatake T, Takaeda M, et al. Up-regulated MHC-class II expression and gamma-IFN and soluble IL-2R in lupus nephritis. *Kidney Int* 1992;42(3):755–763.
786. Lande R, Ganguly D, Facchinetti V, et al. Neutrophils activate plasmacytoid dendritic cells by releasing self-DNA-peptide complexes in systemic lupus erythematosus. *Sci Transl Med* 2011;3(73):73ra19.
787. Starke C, Frey S, Wellmann U, et al. High frequency of autoantibody-secreting cells and long-lived plasma cells within inflamed kidneys of NZB/WF1 lupus mice. *Eur J Immunol* 2011;41(7):2107–2112.
788. Dolff S, Abdulahad WH, van Dijk MC, et al. Urinary T cells in active lupus nephritis show an effector memory phenotype. *Ann Rheum Dis* 2010;69(11):2034–2041.

789. Saxena R, Mahajan T, Mohan C. Lupus nephritis: current update. *Arthritis Res Ther* 2011;13(5):240.
790. van Kooten C, Gerritsma JS, Paape ME, et al. Possible role for CD40–CD40L in the regulation of interstitial infiltration in the kidney. *Kidney Int* 1997;51(3):711–721.
791. Apostolidis SA, Lieberman LA, Kis-Toth K, et al. The dysregulation of cytokine networks in systemic lupus erythematosus. *J Interferon Cytokine Res* 2011;31(10):769–779.
792. Tsai CY, Wu TH, Sun KH, et al. Increased excretion of soluble interleukin 2 receptors and free light chain immunoglobulins in the urine of patients with active lupus nephritis. *Ann Rheum Dis* 1992;51(2):168–172.
793. Iwano M, Dohi K, Hirata E, et al. Urinary levels of IL-6 in patients with active lupus nephritis. *Clin Nephrol* 1993;40(1):16–21.
794. Schwartz N, Rubinstein T, Burkly LC, et al. Urinary TWEAK as a biomarker of lupus nephritis: a multicenter cohort study. *Arthritis Res Ther* 2009;11(5):R143.
795. Michaelson JS, Wisniacki N, Burkly LC, et al. Role of TWEAK in lupus nephritis: a bench-to-bedside review. *J Autoimmun* 2012; 39(3):130–142.
796. Marchesi SL, Aptekar RG, Steinberg AD, et al. Urinary fibrin split products in lupus nephritis. Correlation with other parameters of renal disease. *Arthritis Rheum* 1974;17(2):158–164.
797. Cronlund M, Hardin J, Burton J, et al. Fibrinopeptide A in plasma of normal subjects and patients with disseminated intravascular coagulation and systemic lupus erythematosus. *J Clin Invest* 1976;58(1): 142–151.
798. Sergent JS, Sherman RL, Al-Mondhiry H. Fibrinogen catabolism in systemic lupus erythematosus. *Arthritis Rheum* 1976;19(2):195–198.
799. Cole EH, Schulman J, Urowitz M, et al. Monocyte procoagulant activity in glomerulonephritis associated with systemic lupus erythematosus. *J Clin Invest* 1985;75(3):861–868.
800. Keeton M, Ahn C, Eguchi Y, et al. Expression of type 1 plasminogen activator inhibitor in renal tissue in murine lupus nephritis. *Kidney Int* 1995;47(1):148–157.
801. Moll S, Menoud PA, Fulpius T, et al. Induction of plasminogen activator inhibitor type 1 in murine lupus-like glomerulonephritis. *Kidney Int* 1995;48(5):1459–1468.
802. Kelley VE, Sneve S, Musinski S. Increased renal thromboxane production in murine lupus nephritis. *J Clin Invest* 1986;77(1):252–259.
803. Clark WF, Parbtani A, McDonald JW, et al. The effects of a thromboxane synthase inhibitor, a prostacyclin analog and PGE1 on the nephritis of the NZB/WF1 mouse. *Clin Nephrol* 1987;28(6):288–294.
804. Spurney RF, Fan PY, Ruiz P, et al. Thromboxane receptor blockade reduces renal injury in murine lupus nephritis. *Kidney Int* 1992;41(4):973–982.
805. Camussi G, Tetta C, Mazzucco G, et al. Platelet cationic proteins are present in glomeruli of lupus nephritis patients. *Kidney Int* 1986;30(4):555–565.
806. Sharp GC, Irvin WS, Tan EM, et al. Mixed connective tissue disease—an apparently distinct rheumatic disease syndrome associated with a specific antibody to an extractable nuclear antigen (ENA). *Am J Med* 1972;52(2):148–159.
807. Hoffman RW, Maldonado ME. Immune pathogenesis of mixed connective tissue disease: a short analytical review. *Clin Immunol* 2008;128(1):8-17.
808. van den Hoogen FH, Spronk PE, Boerbooms AM, et al. Long-term follow-up of 46 patients with anti-(U1)snRNP antibodies. *Br J Rheumatol* 1994;33(12):1117–1120.
809. Danieli MG, Fraticelli P, Salvi A, et al. Undifferentiated connective tissue disease: Natural history and evolution into definite CTD assessed in 84 patients initially diagnosed as early UCTD. *Clin Rheumatol* 1998;17(3):195–201.
810. Cappelli S, Bellando Randone S, Martinovic D, et al. "To be or not to be," ten years after: evidence for mixed connective tissue disease as a distinct entity. *Semin Arthritis Rheum* 2012;41(4):589–598.
811. Kaneoka H, Hsu KC, Takeda Y, et al. Molecular genetic analysis of HLA-DR and HLA-DQ genes among anti-U1-70–kd autoantibody positive connective tissue disease patients. *Arthritis Rheum* 1992;35(1):83–94.
812. Burdt MA, Hoffman RW, Deutscher SL, et al. Long-term outcome in mixed connective tissue disease: longitudinal clinical and serologic findings. *Arthritis Rheum* 1999;42(5):899–909.
813. Gendi NS, Welsh KI, Van Venrooij WJ, et al. HLA type as a predictor of mixed connective tissue disease differentiation. Ten-year clinical and immunogenetic followup of 46 patients. *Arthritis Rheum* 1995;38(2):259–266.
814. Ortega-Hernandez OD, Shoenfeld Y. Mixed connective tissue disease: An overview of clinical manifestations, diagnosis and treatment. *Best Pract Res Clin Rheumatol* 2012;26(1):61–72.
815. Venables PJ. Mixed connective tissue disease. *Lupus* 2006;15(3): 132–137.
816. Kotajima L, Aotsuka S, Sumiya M, et al. Clinical features of patients with juvenile onset mixed connective tissue disease: analysis of data collected in a nationwide collaborative study in Japan. *J Rheumatol* 1996;23(6):1088–1094.
817. Akmal M. Mixed connective tissue disease and renal disease. *Adv Exp Med Biol* 1989;252:53–58.
818. Kitridou RC, Akmal M, Turkel SB, et al. Renal involvement in mixed connective tissue disease: a longitudinal clinicopathologic study. *Semin Arthritis Rheum* 1986;16(2):135–145.
819. Nimelstein SH, Brody S, McShane D, et al. Mixed connective tissue disease: a subsequent evaluation of the original 25 patients. *Medicine (Baltimore)* 1980;59(4):239–248.
820. Lemmer JP, Curry NH, Mallory JH, et al. Clinical characteristics and course in patients with high titer anti-RNP antibodies. *J Rheumatol* 1982;9(4):536–542.
821. Alarcon-Segovia D, Cardiel MH. Comparison between 3 diagnostic criteria for mixed connective tissue disease. Study of 593 patients. *J Rheumatol* 1989;16(3):328–334.
822. Amigues JM, Cantagrel A, Abbal M, et al. Comparative study of 4 diagnosis criteria sets for mixed connective tissue disease in patients with anti-RNP antibodies. Autoimmunity Group of the Hospitals of Toulouse. *J Rheumatol* 1996;23(12):2055–2062.
823. Aringer M, Steiner G, Smolen JS. Does mixed connective tissue disease exist? Yes. *Rheum Dis Clin North Am* 2005;31(3):411–420.
824. Ito S, Nakamura T, Kurosawa R, et al. Glomerulonephritis in children with mixed connective tissue disease. *Clin Nephrol* 2006;66(3):160–165.
825. Kobayashi S, Nagase M, Kimura M, et al. Renal involvement in mixed connective tissue disease. Report of 5 cases. *Am J Nephrol* 1985;5(4):282–289.
826. Satoh K, Imai H, Yasuda T, et al. Sclerodermatous renal crisis in a patient with mixed connective tissue disease. *Am J Kidney Dis* 1994;24(2):215–218.
827. Germain MJ, Davidman M. Pulmonary hemorrhage and acute renal failure in a patient with mixed connective tissue disease. *Am J Kidney Dis* 1984;3(6):420–424.
828. Hamburger M, Hodes S, Barland P. The incidence and clinical significance of antibodies to extractable nuclear antigens. *Am J Med Sci* 1977;273(1):21–28.
829. Palferman TG, McIntosh CS, Kershaw M. Mixed connective tissue disease with associated glomerulonephritis and hypocomplementaemia. *Postgrad Med J* 1980;56(653):177–179.
830. Bennett R. Mixed connective tissue disease. In: Grishman EC, J Needle MA Venkataseshan VS, eds. *The Kidney in Collagen Vascular Diseases*. New York: Raven Press, 1993:167.
831. Sawai T, Murakami K, Kurasono Y. Morphometric analysis of the kidney lesions in mixed connective tissue disease (MCTD). *Tohoku J Exp Med* 1994;174(2):141–154.
832. Omokawa A, Komatsuda A, Nara M, et al. Distribution of glomerular IgG subclass deposits in patients with membranous nephropathy and anti-U1 ribonucleoprotein antibody. *Nephrol Dial Transplant* 2012;27(5):1937–1941.
833. Kitaura K, Miyagawa T, Asano K, et al. Mixed connective tissue disease associated with MPO-ANCA-positive polyangiitis. *Intern Med* 2006;45(20):1177–1182.
834. Kurita N, Mise N, Fujii A, et al. Myeloperoxidase-antineutrophil cytoplasmic antibody-associated crescentic glomerulonephritis with rheumatoid arthritis: A comparison of patients without rheumatoid arthritis. *Clin Exp Nephrol* 2010;14(4):325–332.
835. Murakami T, Endo S, Moriki T, et al. Mixed connective tissue disease developing into MPO-ANCA-positive polyangiitis. *Intern Med* 2011;50(6):591–595.

836. Hernandez-Molina G, Reyes E, Crispin JC. ANCA associated glomerulonephritis in a patient with mixed connective tissue disease. *Ann Rheum Dis* 2006;65(3):410–411.
837. Uzu T, Iwatani H, Ko M, et al. Minimal-change nephrotic syndrome associated with mixed connective-tissue disease. *Nephrol Dial Transplant* 2001;16(6):1299–1300.
838. Rifkin SI, Gutta H, Nair R, et al. Collapsing glomerulopathy in a patient with mixed connective tissue disease. *Clin Nephrol* 2011;75(Suppl 1): 32–36.
839. Kessler E, Halpern M, Chagnac A, et al. Unusual renal deposit in mixed connective tissue disease. *Arch Pathol Lab Med* 1992;116(3):261–264.
840. Singsen BH, Swanson VL, Bernstein BH, et al. A histologic evaluation of mixed connective tissue disease in childhood. *Am J Med* 1980;68(5):710–717.
841. Andersen GN, Vasko J. Scleroderma renal crisis and concurrent isolated pulmonary hypertension in mixed connective tissue disease and overlap syndrome: report of two cases. *Clin Rheumatol* 2002;21(2):164–169.
842. Yamaguchi T, Ohshima S, Tanaka T, et al. Renal crisis due to intimal hyperplasia in a patient with mixed connective tissue disease (MCTD) accompanied by pulmonary hypertension. *Intern Med* 2001;40(12):1250–1253.
843. Braun J, Sieper J, Schwarz A, et al. Widespread vasculopathy with HUS, perimyocarditis and cystic pancreatitis in a young woman with mixed connective tissue disease. Case report and review of the literature. *Rheumatol Int* 1993;13(1):31–36.
844. Perinbasekar S, Chawla K, Rosner F, et al. Complete recovery from renal infarcts in a patient with mixed connective tissue disease. *Am J Kidney Dis* 1995;26(4):649–653.
845. Manthorpe R, Elling H, van der Meulen JT, et al. Two fatal cases of mixed connective tissue disease. Description of case histories terminating as progressive systemic sclerosis. *Scand J Rheumatol* 1980;9(1):7-10.
846. Sjögren H. Zur Kenntnis der Karatoconjunctivitis Sicca. *Acta Ophthalmol* 1933;11:1.
847. Pillemer SR, Matteson EL, Jacobsson LT, et al. Incidence of physician-diagnosed primary Sjögren syndrome in residents of Olmsted County, Minnesota. *Mayo Clin Proc* 2001;76(6):593–599.
848. Alamanos Y, Tsifetaki N, Voulgari PV, et al. Epidemiology of primary Sjogren's syndrome in north-west Greece, 1982—2003. *Rheumatology (Oxford)* 2006;45(2):187–191.
849. Plesivcnik Novljan M, Rozman B, Hocevar A, et al. Incidence of primary Sjogren's syndrome in Slovenia. *Ann Rheum Dis* 2004;63(7):874–876.
850. Asmussen K, Andersen V, Bendixen G, et al. A new model for classification of disease manifestations in primary Sjogren's syndrome: evaluation in a retrospective long-term study. *J Intern Med* 1996;239(6):475–482.
851. Flescher E, Talal N. Do viruses contribute to the development of Sjogren's syndrome? *Am J Med* 1991;90(3):283–285.
852. Talal N. Immunologic and viral factors in Sjogren's syndrome. *Clin Expe Rheumatol* 1990;8(Suppl 5):23–26.
853. Becker JL, Miller F, Nuovo GJ, et al. Epstein-Barr virus infection of renal proximal tubule cells: possible role in chronic interstitial nephritis. *J Clin Invest* 1999;104(12):1673–1681.
854. Ramos-Casals M, Stone JH, Moutsopoulos HM. *Sjogren's Syndrome Diagnosis and Therapeutics*. London: Springer, 2012. Available from: http://www.columbia.edu/cgi-bin/cul/resolve?clio9393074
855. Hernandez-Molina G, Zamora-Legoff T, Romero-Diaz J, et al. Predicting Sjogren's syndrome in patients with recent-onset SLE. *Rheumatology (Oxford)* 2013;53:1438–1442.
856. Moutsopoulos HM, Talal N. New developments in Sjogren's syndrome. *Curr Opin Rheumatol* 1989;1(3):332–338.
857. Inaba H, Kawasaki H, Nakamura S, et al. Anaplastic large cell lymphoma associated with Sjogren's syndrome. *Leuk Lymphoma* 1998;32(1-2):183–188.
858. Kassan SS, Thomas TL, Moutsopoulos HM, et al. Increased risk of lymphoma in sicca syndrome. *Ann Intern Med* 1978;89(6):888–892.
859. Moutsopoulos HM. New insights into the physiopathology of primary Sjogren's syndrome. *Ann Med Interne (Paris)* 1990;141(1):53–56.
860. Talal N, Moutsopoulos HM, Kassan SS, eds. *Sjogren's Syndrome: Clinical and Immunological Aspects*. Berlin, Germany: Springer Verlag, 1987.
861. Ramos-Casals M, Nardi N, Brito-Zeron P, et al. Atypical autoantibodies in patients with primary Sjögren syndrome: clinical characteristics and follow-up of 82 cases. *Semin Arthritis Rheum* 2006;35(5):312–321.
862. Fox RI, Tornwall J, Maruyama T, et al. Evolving concepts of diagnosis, pathogenesis, and therapy of Sjogren's syndrome. *Curr Opin Rheumatol* 1998;10(5):446–456.
863. Vitali C, Bombardieri S, Jonsson R, et al. Classification criteria for Sjogren's syndrome: a revised version of the European criteria proposed by the American-European Consensus Group. *Ann Rheum Dis* 2002;61(6):554–558.
864. Shiboski SC, Shiboski CH, Criswell L, et al. American College of Rheumatology classification criteria for Sjogren's syndrome: a data-driven, expert consensus approach in the Sjogren's International Collaborative Clinical Alliance cohort. *Arthritis Care Res (Hoboken)* 2012;64(4):475–487.
865. Malladi AS, Sack KE, Shiboski SC, et al. Primary Sjogren's syndrome as a systemic disease: a study of participants enrolled in an international Sjogren's syndrome registry. *Arthritis Care Res (Hoboken)* 2012;64(6):911–918.
866. Garcia-Carrasco M, Ramos-Casals M, Rosas J, et al. Primary Sjögren syndrome: clinical and immunologic disease patterns in a cohort of 400 patients. *Medicine (Baltimore)* 2002;81(4):270–280.
867. Goules A, Masouridi S, Tzioufas AG, et al. Clinically significant and biopsy-documented renal involvement in primary Sjögren syndrome. *Medicine (Baltimore)* 2000;79(4):241–249.
868. Kaufman I, Schwartz D, Caspi D, et al. Sjogren's syndrome—not just Sicca: renal involvement in Sjogren's syndrome. *Scand J Rheumatol* 2008;37(3):213–218.
869. Maripuri S, Grande JP, Osborn TG, et al. Renal involvement in primary Sjogren's syndrome: A clinicopathologic study. *Clin J Am Soc Nephrol* 2009;4(9):1423–1431.
870. Skopouli FN, Dafni U, Ioannidis JP, et al. Clinical evolution, and morbidity and mortality of primary Sjogren's syndrome. *Semin Arthritis Rheum* 2000;29(5):296–304.
871. Aasarod K, Haga HJ, Berg KJ, et al. Renal involvement in primary Sjogren's syndrome. *QJM* 2000;93(5):297–304.
872. Bossini N, Savoldi S, Franceschini F, et al. Clinical and morphological features of kidney involvement in primary Sjogren's syndrome. *Nephrol Dial Transplant* 2001;16(12):2328–2336.
873. Koenig M, Hacini J, Thibaudin D, et al. [Distal renal tubular acidosis ten years before Sjogren's syndrome]. *Rev Med Interne* 2004;25(10):764–766.
874. Zanuto AC, Bueno T, Delfino VD, et al. Nephrocalcinosis in a patient with Sjogren's syndrome/systemic lupus erythematosus. *Rev Assoc Med Bras* 2012;58(3):279–280.
875. Ohtani H, Imai H, Kodama T, et al. Severe hypokalaemia and respiratory arrest due to renal tubular acidosis in a patient with Sjögren syndrome. *Nephrol Dial Transplant* 1999;14(9):2201–2203.
876. Bridoux F, Kyndt X, Abou-Ayache R, et al. Proximal tubular dysfunction in primary Sjogren's syndrome: a clinicopathological study of 2 cases. *Clin Nephrol* 2004;61(6):434–439.
877. Pertovaara M, Korpela M, Pasternack A. Factors predictive of renal involvement in patients with primary Sjogren's syndrome. *Clin Nephrol* 2001;56(1):10–18.
878. Skopouli FN. Kidney injury in Sjogren's syndrome. *Nephrol Dial Transplant* 2001;16(Suppl 6):63–64.
879. Molina R, Provost TT, Alexander EL. Two types of inflammatory vascular disease in Sjogren's syndrome. Differential association with seroreactivity to rheumatoid factor and antibodies to Ro (SS-A) and with hypocomplementemia. *Arthritis Rheum* 1985;28(11):1251–1258.
880. Alexander EL, Provost TT, Sanders ME, et al. Serum complement activation in central nervous system disease in Sjogren's syndrome. *Am J Med* 1988;85(4):513–518.
881. Akposso K, Martinant de Preneuf H, Larousserie F, et al. [Rapidly progressive acute renal failure. A rare complication of primary Sjögren syndrome]. *Presse Med* 2000;29(30):1647–1649.
882. Hernandez JL, Rodrigo E, De Francisco AL, et al. ANCA-associated pauci-immune crescentic glomerulonephritis complicating Sjogren's syndrome. *Nephrol Dial Transplant* 1996;11(11):2313–2315.
883. Kamachi M, Migita K, Tominaga M, et al. Sjogren's syndrome complicated by MPO-ANCA positive crescentic glomerulonephritis. *Nephrol Dial Transplant* 1999;14(4):1033–1034.
884. Wang WJ, Wu HS, Chu TS. Anti-neutrophil cytoplasmic antibody-associated Pauci-immune crescentic glomerulonephritis complicating Sjogren's syndrome. *J Formos Med Assoc* 2011;110(7):473–477.

885. Guillot X, Solau-Gervais E, Coulon A, et al. Sjogren's syndrome with ANCA-associated crescentic extramembranous glomerulonephritis. *Joint Bone Spine* 2009;76(2):188–189.
886. Rosenberg ME, Schendel PB, McCurdy FA, et al. Characterization of immune cells in kidneys from patients with Sjogren's syndrome. *Am J Kidney Dis* 1988;11(1):20–22.
887. Hu DC, Cathro HP, Okusa MD. Polymorphoneutrophilic infiltration in acute interstitial nephritis of Sjögren syndrome. *Am J Med Sci* 2004;327(5):278–280.
888. Enestrom S, Denneberg T, Eriksson P. Histopathology of renal biopsies with correlation to clinical findings in primary Sjogren's syndrome. *Clin Exp Rheumatol* 1995;13(6):697–703.
889. Manthorpe R. Sudden onset unilateral renomegaly as an initial manifestation of primary Sjogren's syndrome in a teenage girl. *Scand J Rheumatol* 1996;25(3):186–188.
890. Cacoub P, Ginsburg C, Tazi Z, et al. Sjogren's syndrome with acute renal failure caused by renal pseudolymphoma. *Am J Kidney Dis* 1996;28(5):762–766.
891. Oyen RH, Vandercruysse D, Baert A. Case report: renal involvement in primary Sjögren syndrome—a rare cause of renal pseudotumour. *Clin Radiol* 1995;50(4):271–273.
892. Teranishi J, Ogawa T, Saito K, et al. [Sjogren's syndrome with bilateral hydronephrosis caused by pseudolymphoma of bilateral renal pelves: a case report]. *Hinyokika Kiyo.* 2003;49(2):91–93.
893. Gentric A, Herve JP, Pennec YL, et al. Severe renal involvement in primary Sjogren's syndrome. *Adv Exp Med Biol* 1989;252:73–81.
894. Winer RL, Cohen AH, Sawhney AS, et al. Sjogren's syndrome with immune-complex tubulointerstitial renal disease. *Clin Immunol Immunopathol* 1977;8(3):494–503.
895. Pijpe J, Vissink A, Van der Wal JE, et al. Interstitial nephritis with infiltration of IgG-kappa positive plasma cells in a patient with Sjogren's syndrome. *Rheumatology (Oxford)* 2004;43(1):108–110.
896. Jordan SC, Sakai R, Tabak MA, et al. Induction of neonatal renal tubular dysfunction by transplacentally acquired IgG from a mother with Sjögren syndrome. *J Pediatr* 1985;107(4):566–569.
897. Yamada K, Tamura Y, Tomioka H, et al. Possible existence of anti-renal tubular plasma membrane autoantibody which blocked parathyroid hormone-induced phosphaturia in a patient with pseudohypoparathyroidism type II and Sjogren's syndrome. *J Clin Endocrinol Metab* 1984;58(2):339–343.
898. Joo KW, Jeon US, Han JS, et al. Absence of H(+)-ATPase in the intercalated cells of renal tissues in classic distal renal tubular acidosis. *Clin Nephrol* 1998;49(4):226–231.
899. Kau CK, Hu JC, Lu LY, et al. Primary Sjogren's syndrome complicated with cryoglobulinemic glomerulonephritis, myocarditis, and multiorgan involvement. *J Formos Med Assoc* 2004;103(9):707–710.
900. Khan MA, Akhtar M, Taher SM. Membranoproliferative glomerulonephritis in a patient with primary Sjogren's syndrome. Report of a case with review of the literature. *Am J Nephrol* 1988;8(3):235–239.
901. Moutsopoulos HM, Balow JE, Lawley TJ, et al. Immune complex glomerulonephritis in sicca syndrome. *Am J Med* 1978;64(6):955–960.
902. Schlesinger I, Carlson TS, Nelson D. Type III membranoproliferative glomerulonephritis in primary Sjogren's syndrome. *Conn Med* 1989;53(11):629–632.
903. Sun IO, Hong YA, Park HS, et al. Type III membranoproliferative glomerulonephritis in a patient with primary Sjogren's syndrome. *Clin Nephrol* 2013;79(2):171–174.
904. Dabadghao S, Aggarwal A, Arora P, et al. Glomerulonephritis leading to end stage renal disease in a patient with primary Sjögren syndrome. *Clin Exp Rheumatol* 1995;13(4):509–511.
905. Tatsumi H, Tateno S, Hiki Y, et al. Crescentic glomerulonephritis associated with membranous nephropathy in a case with primary Sjogren's syndrome. *Nephrol Dial Transplant* 1998;13(10):2624–2627.
906. Tatsumi H, Tateno S, Hiki Y, et al. Crescentic glomerulonephritis and primary Sjogren's syndrome. *Nephron* 2000;86(4):505–506.
907. Kagan M, Bervina N, Vorobyeva O, et al. Pauci-immune crescentic glomerulonephritis complicating Sjogren's syndrome in a 12–year-old girl. *Pediatr Nephrol* 2011;26(6):991–992.
908. Jung SK, Park KH, Yim HE, et al. Primary Sjogren's syndrome with mesangial proliferative glomerulonephritis and IgA deposits in a child. *Pediatr Nephrol* 2010;25(3):567–568.
909. Watanabe M, Fujimoto T, Iwano M, et al. [Report of a patient of primary Sjögren syndrome, IgA nephropathy and chronic idiopathic thrombocytopenic purpura]. *Nihon Rinsho Meneki Gakkai Kaishi* 2002;25(2):191–198.
910. Ito S, Kamei K, Ikoma M. Primary Sjögren syndrome that developed after IgA nephropathy. *Pediatr Nephrol* 2010;25(8):1579–1580.
911. Chen Y, Zhao X, Tang D, et al. IgA nephropathy in two patients with Sjogren's syndrome: one with concomitant autoimmune hepatitis. *Intern Med* 2010;49(1):37–43.
912. Tsai TC, Chen CY, Lin WT, et al. Sjogren's syndrome complicated with IgA nephropathy and leukocytoclastic vasculitis. *Ren Fail* 2008;30(7):755–758.
913. Mon C, Sanchez Hernandez R, Fernandez Reyes MJ, et al. [Minimal-change disease with mesangial IgA deposits associated with Sjogren syndrome]. *Nefrologia* 2002;22(4):386–389.
914. Natsuga K, Sawamura D, Homma E, et al. Amicrobial pustulosis associated with IgA nephropathy and Sjögren's syndrome. *J Am Acad Dermatol* 2007;57(3):523–526.
915. Baer AN, Maynard JW, Shaikh F, et al. Secondary Sjogren's syndrome in systemic lupus erythematosus defines a distinct disease subset. *J Rheumatol* 2010;37(6):1143–1149.
916. Manoussakis MN, Georgopoulou C, Zintzaras E, et al. Sjogren's syndrome associated with systemic lupus erythematosus: clinical and laboratory profiles and comparison with primary Sjogren's syndrome. *Arthritis Rheum* 2004;50(3):882–891.
917. Palcoux JB, Janin-Mercier A, Campagne D, et al. Sjogren syndrome and lupus erythematosus nephritis. *Arch Dis Child* 1984;59(2):175–177.
918. Koike K, Utsunomiya Y, Ito Y, et al. A case of glomerulopathy showing podocytic infolding in association with Sjogren's syndrome and primary biliary cirrhosis. *Clin Exp Nephrol* 2008;12(6):489–493.
919. Kim CS, Choi YD, Choi JS, et al. EBV-positive diffuse large B-cell lymphoma in a patient with primary Sjogren's syndrome and membranous glomerulonephritis. *BMC Nephrol* 2012;13:149.
920. Ooms V, Decupere M, Lerut E, et al. Secondary renal amyloidosis due to long-standing tubulointerstitial nephritis in a patient with Sjögren syndrome. *Am J Kidney Dis* 2005;46(5):e75–e80.
921. el-Mallakh RS, Bryan RK, Masi AT, et al. Long-term low-dose glucocorticoid therapy associated with remission of overt renal tubular acidosis in Sjogren's syndrome. *Am J Med* 1985;79(4):509–514.
922. Rosenberg AM, Dyck RF, George DH. Intravenous pulse methylprednisolone for the treatment of a child with Sjogren's nephropathy. *J Rheumatol* 1990;17(3):391–394.
923. Saeki Y, Ohshima S, Ishida T, et al. Remission of the renal involvement in a patient with primary Sjogren's syndrome (SS) after pulse high-dose corticosteroid infusion therapy. *Clin Rheumatol* 2001;20(3):225–228.
924. Adu D, Berisa F, Howie AJ, et al. Glomerulonephritis in rheumatoid arthritis. *Br J Rheumatol* 1993;32(11):1008–1011.
925. Boers M, Croonen AM, Dijkmans BA, et al. Renal findings in rheumatoid arthritis: Clinical aspects of 132 necropsies. *Ann Rheum Dis* 1987;46(9):658–663.
926. Helin HJ, Korpela MM, Mustonen JT, et al. Renal biopsy findings and clinicopathologic correlations in rheumatoid arthritis. *Arthritis Rheum* 1995;38(2):242–247.
927. Thomas E, Symmons DP, Brewster DH, et al. National study of cause-specific mortality in rheumatoid arthritis, juvenile chronic arthritis, and other rheumatic conditions: a 20 year followup study. *J Rheumatol* 2003;30(5):958–965.
928. Sihvonen S, Korpela M, Mustonen J, et al. Renal disease as a predictor of increased mortality among patients with rheumatoid arthritis. *Nephron Clin Pract* 2004;96(4):c107–c114.
929. Klareskog L, Catrina AI, Paget S. Rheumatoid arthritis. *Lancet* 2009;373(9664):659–672.
930. Snir O, Widhe M, Hermansson M, et al. Antibodies to several citrullinated antigens are enriched in the joints of rheumatoid arthritis patients. *Arthritis Rheum* 2010;62(1):44–52.
931. Whiting PF, Smidt N, Sterne JA, et al. Systematic review: accuracy of anti-citrullinated Peptide antibodies for diagnosing rheumatoid arthritis. *Ann Intern Med.* 2010;152(7):456–464; W155—W166.
932. Winchester R, Dwyer E. MHC and autoimmune diseases: Susceptibility to rheumatoid arthritis associated with a hydrophobic strip of alpha helix encoded by several MHC alleles. *Immunol Ser* 1991;55:203–219.

933. van der Helm-van Mil AH, Huizinga TW. Advances in the genetics of rheumatoid arthritis point to subclassification into distinct disease subsets. *Arthritis Res Ther* 2008;10(2):205.
934. Palmer DG. The anatomy of the rheumatoid lesion. *Br Med Bull* 1995;51(2):286–295.
935. Zvaifler NJ, Boyle D, Firestein GS. Early synovitis—synoviocytes and mononuclear cells. *Semin Arthritis Rheum* 1994;23(6 Suppl 2):11–16.
936. Postigo AA, Garcia-Vicuna R, Laffon A, et al. The role of adhesion molecules in the pathogenesis of rheumatoid arthritis. *Autoimmunity* 1993;16(1):69–76.
937. Cutolo M, Sulli A, Barone A, et al. Macrophages, synovial tissue and rheumatoid arthritis. *Clin Exp Rheumatol* 1993;11(3):331–339.
938. Aletaha D, Neogi T, Silman AJ, et al. 2010 rheumatoid arthritis classification criteria: An American College of Rheumatology/European League Against Rheumatism collaborative initiative. *Ann Rheum Dis* 2010;69(9):1580–1588.
939. Sakellariou G, Scire CA, Zambon A, et al. Performance of the 2010 classification criteria for rheumatoid arthritis: a systematic literature review and a meta-analysis. *PLoS One* 2013;8(2):e56528.
940. Radner H, Neogi T, Smolen JS, et al. Performance of the 2010 ACR/EULAR classification criteria for rheumatoid arthritis: a systematic literature review. *Ann Rheum Dis* 2014;73:114–123.
941. Myllykangas-Luosujarvi R, Aho K, Kautiainen H, et al. Amyloidosis in a nationwide series of 1666 subjects with rheumatoid arthritis who died during 1989 in Finland. *Rheumatology (Oxford)* 1999;38(6):499–503.
942. Saag KG, Teng GG, Patkar NM, et al. American College of Rheumatology 2008 recommendations for the use of nonbiologic and biologic disease-modifying antirheumatic drugs in rheumatoid arthritis. *Arthritis Rheum* 2008;59(6):762–784.
943. Gertz MA, Kyle RA. Secondary systemic amyloidosis: response and survival in 64 patients. *Medicine (Baltimore)* 1991;70(4):246–256.
944. Lachmann HJ, Goodman HJ, Gilbertson JA, et al. Natural history and outcome in systemic AA amyloidosis. *N Engl J Med* 2007;356(23):2361–2371.
945. Bunker D, Gorevic P. AA amyloidosis: mount Sinai experience, 1997–2012. *Mt Sinai J Med* 2012;79(6):749–756.
946. Boers M. Renal disorders in rheumatoid arthritis. *Semin Arthritis Rheum* 1990;20(1):57–68.
947. Brun C, Olsen TS, Raaschou F, et al. Renal biopsy in rheumatoid arthritis. *Nephron* 1965;2(2):65–81.
948. Korpela M, Mustonen J, Helin H, et al. Immunological comparison of patients with rheumatoid arthritis with and without nephropathy. *Ann Rheum Dis* 1990;49(4):214–218.
949. Pasternack A, Wegelius O, Makisara P. Renal biopsy in rheumatoid arthritis. *Acta Med Scand* 1967;182(5):591–595.
950. Sellars L, Siamopoulos K, Wilkinson R, et al. Renal biopsy appearances in rheumatoid disease. *Clin Nephrol* 1983;20(3):114–120.
951. Gomez-Casanovas E, Sanmarti R, Sole M, et al. The clinical significance of amyloid fat deposits in rheumatoid arthritis: a systematic long-term followup study using abdominal fat aspiration. *Arthritis Rheum* 2001;44(1):66–72.
952. Wakhlu A, Krisnani N, Hissaria P, et al. Prevalence of secondary amyloidosis in Asian North Indian patients with rheumatoid arthritis. *J Rheumatol* 2003;30(5):948–951.
953. Okuda Y, Takasugi K, Oyama T, et al. [Amyloidosis in rheumatoid arthritis—clinical study of 124 histologically proven cases]. *Ryumachi* 1994;34(6):939–946.
954. Couverchel L, Maugars Y, Prost A. Outcomes of thirty-four rheumatoid arthritis patients with renal amyloidosis, including twelve given alkylating agents. *Rev Rhum Engl Ed* 1995;62(2):79–85.
955. Laiho K, Tiitinen S, Kaarela K, et al. Secondary amyloidosis has decreased in patients with inflammatory joint disease in Finland. *Clin Rheumatol* 1999;18(2):122–123.
956. Immonen K, Finne P, Gronhagen-Riska C, et al. A marked decline in the incidence of renal replacement therapy for amyloidosis associated with inflammatory rheumatic diseases - data from nationwide registries in Finland. *Amyloid* 2011;18(1):25–28.
957. Nakano M, Ueno M, Nishi S, et al. Analysis of renal pathology and drug history in 158 Japanese patients with rheumatoid arthritis. *Clin Nephrol* 1998;50(3):154–160.
958. Hiki Y, Horii A, Kokubo T, et al. A case of rheumatoid arthritis with renal tubular amyloidosis. *Nephron* 1994;68(3):394–395.
959. Kuroda T, Tanabe N, Kobayashi D, et al. Significant association between renal function and area of amyloid deposition in kidney biopsy specimens in reactive amyloidosis associated with rheumatoid arthritis. *Rheumatol Int* 2012;32(10):3155–3162.
960. Moroni G, Banfi G, Maccario M, et al. Extracapillary glomerulonephritis and renal amyloidosis. *Am J Kidney Dis* 1996;28(5):695–699.
961. Kaplan B, Vidal R, Kumar A, et al. Immunochemical microanalysis of amyloid proteins in fine-needle aspirates of abdominal fat. *Am J Clin Pathol* 1999;112(3):403–407.
962. Hazenberg BP, Bijzet J, Limburg PC, et al. Diagnostic performance of amyloid A protein quantification in fat tissue of patients with clinical AA amyloidosis. *Amyloid* 2007;14(2):133–140.
963. Sethi S, Vrana JA, Theis JD, et al. Laser microdissection and mass spectrometry-based proteomics aids the diagnosis and typing of renal amyloidosis. *Kidney Int* 2012;82(2):226–234.
964. Hawkins PN, Lavender JP, Pepys MB. Evaluation of systemic amyloidosis by scintigraphy with 123I-labeled serum amyloid P component. *N Engl J Med* 1990;323(8):508–513.
965. Hezemans RL, Krediet RT, Arisz L. Dialysis treatment in patients with rheumatoid arthritis. *Neth J Med* 1995;47(1):6–11.
966. Kofman T, Grimbert P, Canoui-Poitrine F, et al. Renal transplantation in patients with AA amyloidosis nephropathy: results from a French multicenter study. *Am J Transplant* 2011;11(11):2423–2431.
967. Chevrel G, Jenvrin C, McGregor B, et al. Renal type AA amyloidosis associated with rheumatoid arthritis: a cohort study showing improved survival on treatment with pulse cyclophosphamide. *Rheumatology (Oxford)* 2001;40(7):821–825.
968. Maezawa A, Hiromura K, Mitsuhashi H, et al. Combined treatment with cyclophosphamide and prednisolone can induce remission of nephrotic syndrome in a patient with renal amyloidosis, associated with rheumatoid arthritis. *Clin Nephrol* 1994;42(1):30–32.
969. Komatsuda A, Morita K, Ohtani H, et al. Remission of the nephrotic syndrome in a patient with renal amyloidosis due to rheumatoid arthritis treated with prednisolone and methotrexate. *Am J Kidney Dis* 1998;32(5):E7.
970. Nakamura T, Higashi S, Tomoda K, et al. Effectiveness of etanercept vs cyclophosphamide as treatment for patients with amyloid A amyloidosis secondary to rheumatoid arthritis. *Rheumatology (Oxford)* 2012;51(11):2064–2069.
971. Nakamura T, Higashi S, Tomoda K, et al. Etanercept can induce resolution of renal deterioration in patients with amyloid A amyloidosis secondary to rheumatoid arthritis. *Clin Rheumatol* 2010;29(12):1395–1401.
972. Keersmaekers T, Claes K, Kuypers DR, et al. Long-term efficacy of infliximab treatment for AA-amyloidosis secondary to chronic inflammatory arthritis. *Ann Rheum Dis* 2009;68(5):759–761.
973. Nishida S, Hagihara K, Shima Y, et al. Rapid improvement of AA amyloidosis with humanised anti-interleukin 6 receptor antibody treatment. *Ann Rheum Dis* 2009;68(7):1235–1236.
974. Manenti L, Tansinda P, Vaglio A. Eprodisate in AA amyloidosis. *N Engl J Med* 2007;357(11):1153; author reply -4.
975. Akhund L, Quinet RJ, Ishaq S. Celecoxib-related renal papillary necrosis. *Arch Intern Med* 2003;163(1):114–115.
976. Radford MG Jr, Holley KE, Grande JP, et al. Reversible membranous nephropathy associated with the use of nonsteroidal anti-inflammatory drugs. *JAMA* 1996;276(6):466–469.
977. Markowitz GS, Falkowitz DC, Isom R, et al. Membranous glomerulopathy and acute interstitial nephritis following treatment with celecoxib. *Clin Nephrol* 2003;59(2):137–142.
978. Cohen DJ, Appel GB. Cyclosporine: nephrotoxic effects and guidelines for safe use in patients with rheumatoid arthritis. *Semin Arthritis Rheum* 1992;21(6 Suppl 3):43–48.
979. Ludwin D, Alexopoulou I. Cyclosporin A nephropathy in patients with rheumatoid arthritis. *Br J Rheumatol* 1993;32(Suppl 1):60–64.
980. Eriksson LO, Sturfelt G, Thysell H, et al. Effects of sulindac and naproxen on prostaglandin excretion in patients with impaired renal function and rheumatoid arthritis. *Am J Med* 1990;89(3):313–321.
981. International Kidney Biopsy Registry of Cyclosporin (Scandimmun) in Autoimmune Diseases: renal morphology after cyclosporin A therapy in rheumatoid arthritis. *Br J Rheumatol* 1993;32(Suppl 1):65.

982. Ludwin D, Alexopoulou I, Esdaile JM, et al. Renal biopsy specimens from patients with rheumatoid arthritis and apparently normal renal function after therapy with cyclosporine. Canadian Multicentre Rheumatology Group. *Am J Kidney Dis* 1994;23(2):260–265.
983. Rodriguez F, Krayenbuhl JC, Harrison WB, et al. Renal biopsy findings and followup of renal function in rheumatoid arthritis patients treated with cyclosporin A. An update from the International Kidney Biopsy Registry. *Arthritis Rheum* 1996;39(9):1491–1498.
984. Empire Rheumatism Council Research Subcommittee. Gold therapy in rheumatoid arthritis: report of multicentre controlled trial. *Ann Rheum Dis* 1960;19:95.
985. Empire Rheumatism Council Research Subcommittee. Gold therapy in rheumatoid arthritis: report of multicentre controlled trial. *Ann Rheum Dis* 1961;20:315.
986. Katz WA, Blodgett RC Jr, Pietrusko RG. Proteinuria in gold-treated rheumatoid arthritis. *Ann Intern Med* 1984;101(2):176–179.
987. Luukkainen R. Chrysotherapy in rheumatoid arthritis with particular emphasis on the effect of chrysotherapy on radiographical changes and on the optimal time of initiation of therapy. *Scand J Rheumatol Suppl* 1980;34:1–56.
988. Silverberg DS, Kidd EG, Shnitka TK, et al. Gold nephropathy. A clinical and pathologic study. *Arthritis Rheum* 1970;13(6):812–825.
989. Lockie LM, Smith DM. Forty-seven years experience with gold therapy in 1,019 rheumatoid arthritis patients. *Semin Arthritis Rheum* 1985;14(4):238–246.
990. Hall CL, Fothergill NJ, Blackwell MM, et al. The natural course of gold nephropathy: long term study of 21 patients. *Br Med J (Clin Res Ed)* 1987;295(6601):745–748.
991. Hall CL. Gold nephropathy. *Nephron* 1988;50(4):265–272.
992. Hall CL. The natural course of gold and penicillamine nephropathy: a longterm study of 54 patients. *Adv Exp Med Biol* 1989;252:247–256.
993. Davies DJ, Dowling J, Xipell JM. Gold nephropathy. *Pathology* 1977;9(4):281–288.
994. Skrifvars BV, Tornroth TS, Tallqvist GN. Gold-induced immune complex nephritis in seronegative rheumatoid arthritis. *Ann Rheum Dis* 1977;36(6):549–556.
995. Tonroth T, Skrifvars B. Gold nephropathy prototype of membranous glomerulonephritis. *Am J Pathol* 1974;75(3):573–590.
996. Katz A, Little AH. Gold nephropathy. An immunopathologic study. *Arch Pathol* 1973;96(2):133–136.
997. Lee JC, Dushkin M, Eyring EJ, et al. Renal lesions associated with gold therapy; light and electron microscopic studies. *Arthritis Rheum* 1965;47:1–13.
998. Vaamonde CA, Hunt FR. The nephrotic syndrome as a complication of gold therapy. *Arthritis Rheum* 1970;13(6):826–834.
999. Viol GW, Minielly JA, Bistricki T. Gold nephropathy: tissue analysis by X-ray fluorescent spectroscopy. *Arch Pathol Lab Med* 1977;101(12):635–640.
1000. Watanabe I, Whittier FC Jr, Moore J, et al. Gold nephropathy. Ultrastructural, fluorescence, and microanalytic studies of two patients. *Arch Pathol Lab Med* 1976;100(12):632–635.
1001. Hall CL, Jawad S, Harrison PR, et al. Natural course of penicillamine nephropathy: a long term study of 33 patients. *Br Med J (Clin Res Ed)* 1988;296(6629):1083–1086.
1002. Day AT, Golding JR, Lee PN, et al. Penicillamine in rheumatoid disease: a long-term study. *Br Med J* 1974;1(900):180–183.
1003. Golding JR, Day AT, Tomlinson MR, et al. Rheumatoid arthritis treated with small doses of penicillamine. *Proc R Soc Med* 1977;70 (Suppl 3):131–135.
1004. Bacon PA, Tribe CR, Mackenzie JC, et al. Letter: D-penicillamine-induced nephropathy. *Lancet* 1975;2(7924):75.
1005. Hill HF. Treatment of rheumatoid arthritis with penicillamine. *Semin Arthritis Rheum* 1977;6(4):361–388.
1006. Kirby JD, Dieppe PA, Huskisson EC, et al. D-penicillamine and immune complex deposition. *Ann Rheum Dis* 1979;38(4):344–346.
1007. Almirall J, Alcorta I, Botey A, et al. Penicillamine-induced rapidly progressive glomerulonephritis in a patient with rheumatoid arthritis. *Am J Nephrol* 1993;13(4):286–288.
1008. Banfi G, Imbasciati E, Guerra L, et al. Extracapillary glomerulonephritis with necrotizing vasculitis in D-penicillamine-treated rheumatoid arthritis. *Nephron* 1983;33(1):56–60.
1009. Devogelaer JP, Pirson Y, Vandenbroucke JM, et al., Nagant de Deuxchaisnes C. D-penicillamine induced crescentic glomerulonephritis: report and review of the literature. *J Rheumatol* 1987;14(5):1036–1041.
1010. Gavaghan TE, McNaught PJ, Ralston M, et al. Penicillamine-induced "Goodpasture's syndrome": successful treatment of a fulminant case. *Aust N Z J Med* 1981;11(3):261–265.
1011. Gibson T, Burry HC, Ogg C. Letter: goodpasture syndrome and D-penicillamine. *Ann Intern Med* 1976;84(1):100.w
1012. Macarron P, Garcia Diaz JE, Azofra JA, et al. D-penicillamine therapy associated with rapidly progressive glomerulonephritis. *Nephrol Dial Transplant* 1992;7(2):161–164.
1013. Sadjadi SA, Seelig MS, Berger AR, et al. Rapidly progressive glomerulonephritis in a patient with rheumatoid arthritis during treatment with high-dosage D-penicillamine. *Am J Nephrol* 1985;5(3):212–216.
1014. Swainson CP, Thomson D, Short AI, et al. Plasma exchange in the successful treatment of drug-induced renal disease. *Nephron* 1982;30(3):244–249.
1015. Mathieson PW, Peat DS, Short A, et al. Coexistent membranous nephropathy and ANCA-positive crescentic glomerulonephritis in association with penicillamine. *Nephrol Dial Transplant* 1996;11(5):863–866.
1016. Nanke Y, Akama H, Terai C, et al. Rapidly progressive glomerulonephritis with D-penicillamine. *Am J Med Sci* 2000;320(6):398–402.
1017. Nagahama K, Matsushita H, Hara M, et al. Bucillamine induces membranous glomerulonephritis. *Am J Kidney Dis* 2002;39(4):706–712.
1018. Obayashi M, Uzu T, Harada T, et al. Clinical course of bucillamine-induced nephropathy in patients with rheumatoid arthritis. *Clin Exp Nephrol* 2003;7(4):275–278.
1019. Isozaki T, Kimura M, Ikegaya N, et al. Bucillamine (a new therapeutic agent for rheumatoid arthritis) induced nephrotic syndrome: a report of two cases and review of the literature. *Clin Investig* 1992;70(11):1036–1042.
1020. Yoshida A, Morozumi K, Suganuma T, et al. Clinicopathological findings of bucillamine-induced nephrotic syndrome in patients with rheumatoid arthritis. *Am J Nephrol* 1991;11(4):284–288.
1021. Emery P, Panayi GS, Huston G, et al. D-Penicillamine induced toxicity in rheumatoid arthritis: the role of sulphoxidation status and HLA-DR3. *J Rheumatol* 1984;11(5):626–632.
1022. Panayi GS, Wooley P, Batchelor JR. Genetic basis of rheumatoid disease: HLA antigens, disease manifestations, and toxic reactions to drugs. *Br Med J* 1978;2(6148):1326–1328.
1023. Speerstra F, Reekers P, van de Putte LB, et al. HLA-DR antigens and proteinuria induced by aurothioglucose and D-penicillamine in patients with rheumatoid arthritis. *J Rheumatol* 1983;10(6):948–953.
1024. Stein HB, Schroeder ML, Dillon AM. Penicillamine-induced proteinuria: risk factors. *Semin Arthritis Rheum* 1986;15(4):282–287.
1025. Wooley PH, Griffin J, Panayi GS, et al. HLA-DR antigens and toxic reaction to sodium aurothiomalate and D-penicillamine in patients with rheumatoid arthritis. *N Engl J Med* 1980;303(6):300–302.
1026. Ainsworth SK, Swain RP, Watabe N, et al. Gold nephropathy. Ultrastructural, fluorescent, and energy-dispersive x-ray microanalysis study. *Arch Pathol Lab Med* 1981;105(7):373–378.
1027. Ueda S, Wakashin M, Wakashin Y, et al. Experimental gold nephropathy in guinea pigs: detection of autoantibodies to renal tubular antigens. *Kidney Int* 1986;29(2):539–548.
1028. Nagi AH, Alexander F, Barabas AZ. Gold nephropathy in rats—light and electron microscopic studies. *Exp Mol Pathol* 1971;15(3):354–362.
1029. Doulton TW, Tucker B, Reardon J, et al. Antineutrophil cytoplasmic antibody-associated necrotizing crescentic glomerulonephritis in a patient receiving treatment with etanercept for severe rheumatoid arthritis. *Clin Nephrol* 2004;62(3):234–238.
1030. Mor A, Bingham C III, Barisoni L, et al. Proliferative lupus nephritis and leukocytoclastic vasculitis during treatment with etanercept. *J Rheumatol* 2005;32(4):740–743.
1031. Reitblat T, Reitblat O. Appearance of ANCA—associated vasculitis under Tumor necrosis factor-alpha inhibitors treatment. *Am J Case Rep* 2013;14:80–82.
1032. Ashok D, Dubey S, Tomlinson I. C-ANCA positive systemic vasculitis in a patient with rheumatoid arthritis treated with infliximab. *Clin Rheumatol* 2008;27(2):261–264.
1033. Hirohama D, Hoshino J, Hasegawa E, et al. Development of myeloperoxidase-antineutrophil cytoplasmic antibody-associated renal

vasculitis in a patient receiving treatment with anti-tumor necrosis factor-alpha. *Mod Rheumatol* 2010;20(6):602–605.
1034. Kaneko K, Nanki T, Hosoya T, et al. Etanercept-induced necrotizing crescentic glomerulonephritis in two patients with rheumatoid arthritis. *Mod Rheumatol* 2010;20(6):632–636.
1035. Simms R, Kipgen D, Dahill S, et al. ANCA-associated renal vasculitis following anti-tumor necrosis factor alpha therapy. *Am J Kidney Dis* 2008;51(3):e11–e14.
1036. Fournier A, Nony A, Rifard K. [Antineutrophil cytoplasmic antibody associated vasculitis in a patient treated with adalimumab for a rheumatoid arthritis]. *Nephrol Ther* 2009;5(7):652–657.
1037. Nishimura K, Saegusa J, Kawano S, et al. Tumor necrosis factor-alpha inhibitor-induced antiglomerular basement membrane antibody disease in a patient with rheumatoid arthritis. *J Rheumatol* 2012;39(9):1904–1905.
1038. Saint Marcoux B, De Bandt M. Vasculitides induced by TNFalpha antagonists: A study in 39 patients in France. *Joint Bone Spine* 2006;73(6): 710–713.
1039. Sokumbi O, Wetter DA, Makol A, et al. Vasculitis associated with tumor necrosis factor-alpha inhibitors. *Mayo Clin Proc* 2012;87(8):739–745.
1040. Asahina A, Ohshima N, Nakayama H, et al. Henoch-Schonlein purpura in a patient with rheumatoid arthritis receiving etanercept. *Eur J Dermatol* 2010;20(4):521–522.
1041. Duffy TN, Genta M, Moll S, et al. Henoch Schonlein purpura following etanercept treatment of rheumatoid arthritis. *Clin Exp Rheumatol* 2006;24(2 Suppl 41):S106.
1042. den Broeder AA, Assmann KJ, van Riel PL, et al. Nephrotic syndrome as a complication of anti-TNFalpha in a patient with rheumatoid arthritis. *Neth J Med* 2003;61(4):137–141.
1043. Matsuo Y, Mizoguchi F, Kohsaka H, et al. Tocilizumab-induced immune complex glomerulonephritis in a patient with rheumatoid arthritis. *Rheumatology (Oxford)* 2013;52(7):1341–1343.
1044. Sumida K, Ubara Y, Suwabe T, et al. Complete remission of myeloperoxidase-anti-neutrophil cytoplasmic antibody-associated crescentic glomerulonephritis complicated with rheumatoid arthritis using a humanized anti-interleukin 6 receptor antibody. *Rheumatology (Oxford)* 2011;50(10):1928–1930.
1045. Iijima T, Suwabe T, Sumida K, et al. Tocilizumab improves systemic rheumatoid vasculitis with necrotizing crescentic glomerulonephritis. *Mod Rheumatol* 2014, Feb 18. [Epub ahead of print].
1046. Michel M, Henri P, Vincent FB, et al. Mesangial immunoglobulin (Ig) A glomerulonephritis in a patient with rheumatoid arthritis treated with abatacept. *Joint Bone Spine* 2013;80:660–663.
1047. Iglesias J, D'Agati VD, Levine JS. Acute glomerulonephritis occurring during immunoadsorption with staphylococcal protein A column (Prosorba). *Nephrol Dial Transplant* 2004;19(12):3155–3159.
1048. Helin H, Mustonen J, Pasternack A, et al. IgM-associated glomerulonephritis. *Nephron* 1982;31(1):11–16.
1049. Helin H, Korpela M, Mustonen J, et al. Mild mesangial glomerulopathy—a frequent finding in rheumatoid arthritis patients with hematuria or proteinuria. *Nephron* 1986;42(3):224–230.
1050. Korpela M, Mustonen J, Pasternack A, et al. Mesangial glomerulopathy in rheumatoid arthritis patients. Clinical follow-up and relation to antirheumatic therapy. *Nephron* 1991;59(1):46–50.
1051. Korpela M, Mustonen J, Heikkinen A, et al. Isolated microscopic hematuria in patients with rheumatoid arthritis compared with age and sex matched controls. A population based study. *J Rheumatol* 1995;22(3):427–431.
1052. Zarza LP, Sanchez EN, Acin PA, et al. Membranous glomerulonephritis in rheumatoid arthritis unrelated to gold, D-penicillamine or other connective tissue disease. *Clin Rheumatol* 1996;15(4):385–388.
1053. Yun YS, Song HC, Lee K, et al. Fibrillary glomerulonephritis in rheumatoid arthritis. *Nephrology (Carlton)* 2010;15(2):266–267.
1054. Hordon LD, Sellars L, Morley AR, et al. Haematuria in rheumatoid arthritis: An association with mesangial glomerulonephritis. *Ann Rheum Dis* 1984;43(3):440–443.
1055. Myllymaki J, Saha H, Mustonen J, et al. IgM nephropathy: clinical picture and long-term prognosis. *Am J Kidney Dis* 2003;41(2):343–350.
1056. Pollet S, Depner T, Moore P, et al. Mesangial glomerulopathy and IgM rheumatoid factor in rheumatoid arthritis. *Nephron* 1989;51(1):107–111.
1057. Honkanen E, Tornroth T, Pettersson E, et al. Membranous glomerulonephritis in rheumatoid arthritis not related to gold or D-penicillamine therapy: a report of four cases and review of the literature. *Clin Nephrol* 1987;27(2):87–93.
1058. Breedveld FC, Valentijn RM, Westedt ML, et al. Rapidly progressive glomerulonephritis with glomerular crescent formation in rheumatoid arthritis. *Clin Rheumatol* 1985;4(3):353–359.
1059. Kuznetsky KA, Schwartz MM, Lohmann LA, et al. Necrotizing glomerulonephritis in rheumatoid arthritis. *Clin Nephrol* 1986;26(5):257–264.
1060. Ludmerer KM, Kissane JM. Abdominal pain, purpura, and death in an elderly woman. *Am J Med* 1985;78(5):839–849.
1061. Mustila A, Korpela M, Mustonen J, et al. Perinuclear antineutrophil cytoplasmic antibody in rheumatoid arthritis: a marker of severe disease with associated nephropathy. *Arthritis Rheum* 1997;40(4):710–717.
1062. Naschitz JE, Yeshurun D, Scharf Y, et al. Recurrent massive alveolar hemorrhage, crescentic glomerulonephritis, and necrotizing vasculitis in a patient with rheumatoid arthritis. *Arch Intern Med* 1989;149(2):406–408.
1063. Qarni MU, Kohan DE. Pauci-immune necrotizing glomerulonephritis complicating rheumatoid arthritis. *Clin Nephrol* 2000;54(1):54–58.
1064. Tebib JG, Trolliet P, Bouvier M, et al. Necrotizing glomerulonephritis as a complication of rheumatoid arthritis vasculitis. *Br J Rheumatol* 1993;32(8):765–766.
1065. Yorioka N, Taniguchi Y, Amimoto D, et al. Chronic rheumatoid arthritis complicated by myeloperoxidase antineutrophil cytoplasmic antibody-associated nephritis. *Am J Nephrol* 1999;19(4):527–529.
1066. Messiaen T, M'Bappe P, Boffa JJ, et al. MPO-ANCA necrotizing glomerulonephritis related to rheumatoid arthritis. *Am J Kidney Dis* 1998;32(5):E6.
1067. Harper L, Cockwell P, Howie AJ, et al. Focal segmental necrotizing glomerulonephritis in rheumatoid arthritis. *QJM* 1997;90(2):125–132.
1068. Belot A, Bader-Meunier B, Niaudet P, et al. ANCA-associated glomerulonephritis in systemic-onset juvenile idiopathic arthritis. *Am J Kidney Dis* 2012;59(3):439–443.
1069. Moreland L, DiBartolomeo A, Brick J. Rheumatoid vasculitis with intrarenal aneurysm formation. *J Rheumatol* 1988;15(5):845–849.
1070. Murakami H, Ura N, Nagao K, et al. Rheumatoid arthritis associated with renal amyloidosis and crescentic glomerulonephritis. *Intern Med* 1998;37(1):94–97.
1071. Kratochvil D, Amann K, Bruck H, et al. Membranoproliferative glomerulonephritis complicating Waldenström's macroglobulinemia. *BMC Nephrol* 2012;13:172.
1072. Nomura M, Okada J, Tateno S, et al. Renal thrombotic microangiopathy in a patient with rheumatoid arthritis and antiphospholipid syndrome: successful treatment with cyclophosphamide pulse therapy and anticoagulant. *Intern Med* 1994;33(8):484–487.
1073. Kim YG, Kim HW, Cho YM, et al. The difference between lupus nephritis class IV-G and IV-S in Koreans: focus on the response to cyclophosphamide induction treatment. *Rheumatology (Oxford)* 2008;47(3):311–314.
1074. Daleboudt GM, Bajema IM, Goemaere NN, et al. The clinical relevance of a repeat biopsy in lupus nephritis flares. *Nephrol Dial Transplant* 2009;24(12):3712–3717.
1075. Wang GB, Xu ZJ, Liu HF, et al. Changes in pathological pattern and treatment regimens based on repeat renal biopsy in lupus nephritis. *Chin Med J (Engl)* 2012;125(16):2890–2894.
1076. Graham RR, Hom G, Ortmann W, et al. Review of recent genome-wide association scans in lupus. *J Intern Med* 2009;265(6):680–688.
1077. Han S, Guthridge JM, Harley IT, et al. Osteopontin and systemic lupus erythematosus association: a probable gene-gender interaction. *PLoS One* 2008;3(3):e0001757.
1078. Lee YH, Woo JH, Choi SJ, et al. Association of programmed cell death 1 polymorphisms and systemic lupus erythematosus: a meta-analysis. *Lupus* 2009;18(1):9–15.
1079. Kyogoku C, Dijstelbloem HM, Tsuchiya N, et al. Fcgamma receptor gene polymorphisms in Japanese patients with systemic lupus erythematosus: contribution of FCGR2B to genetic susceptibility. *Arthritis Rheum* 2002;46(5):1242–1254.
1080. Wu J, Edberg JC, Redecha PB, et al. A novel polymorphism of FcgammaRIIIa (CD16) alters receptor function and predisposes to autoimmune disease. *J Clin Invest* 1997;100(5):1059–1070.
1081. Bodano A, Gonzalez A, Ferreiros-Vidal I, et al. Association of a non-synonymous single-nucleotide polymorphism of DNASEI with SLE susceptibility. *Rheumatology (Oxford)* 2006;45(7):819–823.
1082. Hartung K, Baur MP, Coldewey R, et al. Major histocompatibility complex haplotypes and complement C4 alleles in systemic lupus erythematosus. Results of a multicenter study. *J Clin Invest* 1992;90(4):1346–1351.

CHAPTER 15

J. Charles Jennette • Volker Nickeleit

# Anti–Glomerular Basement Membrane Glomerulonephritis and Goodpasture Syndrome



## BACKGROUND INFORMATION

### Anti-GBM Disease

In 1919, Ernest Goodpasture (1) reported an 18-year-old male patient who developed hemoptysis and acute renal failure after a flu-like illness. The patient died and at autopsy was found to have massive pulmonary hemorrhage and glomerulonephritis with "fibrinous exudates in the Bowman space" and necrotizing vasculitis in the spleen and gut. Four decades later, Stanton and Tange (2) coined the eponym Goodpasture syndrome for the association of pulmonary hemorrhage with glomerulonephritis. This eponym was initially used to indicate any form of glomerulonephritis combined with pulmonary hemorrhage. The use of the term *Goodpasture syndrome* now should be confined to those examples of pulmonary-renal syndrome that are caused by anti–glomerular basement membrane (anti-GBM) antibodies (3). Glomerulonephritis that is caused by anti-GBM occurs not only as a component of Goodpasture syndrome but also in the absence of pulmonary disease. In this setting, it should not be diagnosed as Goodpasture syndrome but simply as anti-GBM glomerulonephritis. A few patients have anti-GBM–mediated pulmonary hemorrhage without glomerulonephritis (4). Anti-GBM disease is the generic term for any clinical expression of disease caused by anti-GBM antibodies and includes Goodpasture syndrome, isolated anti-GBM glomerulonephritis, and isolated anti-GBM pulmonary hemorrhage (5). Grouping these clinically distinct syndromes in a unifying disease category is justified based on the finding that patients with these varied expressions of disease have autoantibodies with specificity for the identical portion of the α3 chain of type IV collagen in vascular basement membranes (6).

The term Goodpasture syndrome is used in this chapter only for anti-GBM antibody–mediated pulmonary-renal syndrome. However, the patient whom Goodpasture described in his publication probably did not have anti-GBM antibody–mediated pulmonary-renal syndrome. He more likely had pulmonary-renal syndrome caused by antineutrophil cytoplasmic antibodies (ANCA) because the pulmonary alveolar capillaries had more intense neutrophilic infiltration than is usually seen with anti-GBM disease, and, even more compelling, there was a necrotizing small-vessel vasculitis in the spleen and gut (7).

Anti-GBM antibodies were first detected by immunofluorescence microscopy using antibodies to immunoglobulins that revealed linear localization of IgG along GBMs in patients with pulmonary hemorrhage and crescentic glomerulonephritis (CGN) (Fig. 15.1). Scheer and Grossman, in 1964 (8), reported the linear immunostaining for γ globulin along the GBMs of two additional patients with Goodpasture syndrome. The next year, Sturgill and Westervelt (9) reported linear staining of pulmonary as well as glomerular capillary basement membranes for γ globulin in a patient with Goodpasture syndrome. This was followed by the landmark article by Lerner et al. (10) that established anti-GBM antibodies as a cause for pulmonary-renal syndrome as well as for CGN in the absence of pulmonary hemorrhage. In 1971, Martinez and Kohler (3) proposed that the term Goodpasture syndrome should be used only for patients with anti-GBM antibody–mediated pulmonary-renal vasculitic syndrome. At that time, it was clear that other pathogenic mechanisms could cause concurrent glomerulonephritis and pulmonary

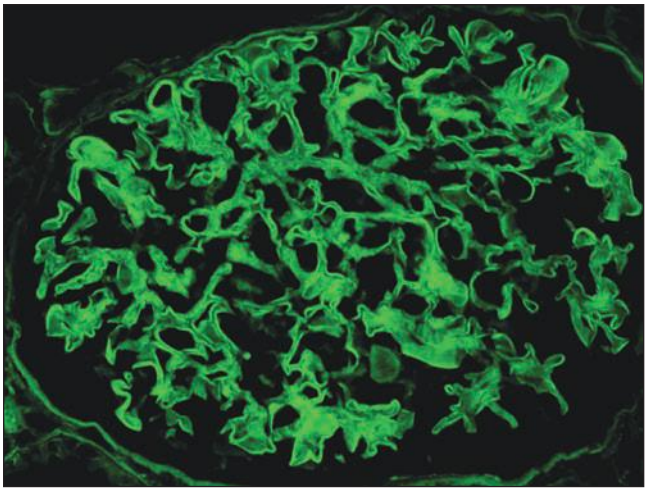


**FIGURE 15.1** Global linear staining for IgG indicative of diffuse binding of anti-GBM antibodies to type 4 collagen in glomerular basement membranes. (FITC anti-IgG.)

hemorrhage. Unless Goodpasture syndrome is restricted to one pathogenic category, the term does not specify a clinically and pathogenetically distinct entity.

Once immunofluorescence microscopy became a routine method for examining renal biopsy specimens, glomerulonephritis with intense linear staining of capillary walls that was indicative of anti-GBM disease was noted to be associated with glomerular crescent formation and thus CGN. CGN is not a specific disease but rather is a morphologic manifestation of severe glomerular injury that can be caused by many different etiologic and pathogenic mechanisms, one of which is anti-GBM disease. The definition of a glomerular crescent that is endorsed by the World Health Organization (WHO) is two or more layers of cells that are partially or completely filling the Bowman space (Fig. 15.2). The term *crescentic glomerulonephritis* often is used generically for glomerulonephritis with any degree of crescent formation; however, it is advisable to use this term on the diagnostic line only when 50% or more of glomeruli have crescents (11). Lesser degrees of crescent formation should be stated in the diagnosis as the percentage of glomeruli involved. By this approach, a specimen with anti-GBM glomerulonephritis that has crescents in 70% of glomeruli would be diagnosed as crescentic anti-GBM glomerulonephritis, whereas a specimen with anti-GBM glomerulonephritis that has crescents in 15% of glomeruli would be diagnosed as anti-GBM glomerulonephritis with 15% crescents.

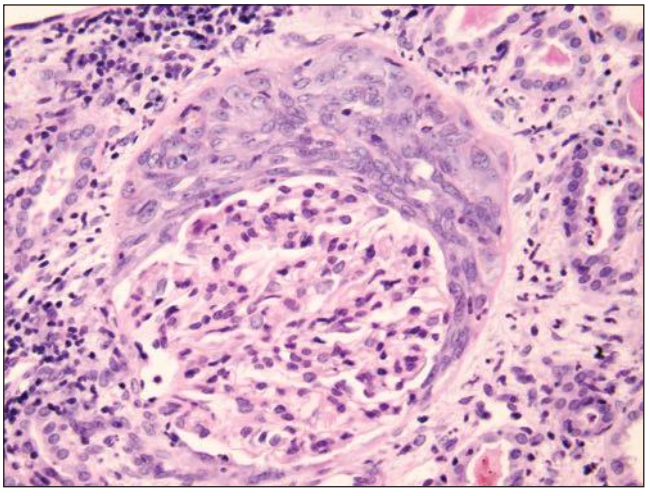


**FIGURE 15.2 Anti-GBM glomerulonephritis with a large cellular crescent forming a cap over the glomerular tuft.** Note also the edema and inflammation in the periglomerular interstitium. (H&E.)

## Crescentic Glomerulonephritis

Glomerular crescents have been described in the medical literature since the 19th century. For example, Langhans (12) and Purdy (13) provided excellent illustrations of glomerular crescents in publications that appeared in 1879 and 1886, respectively. By the turn of the century, a number of investigators, including Volhard and Fahr (14), had recognized that glomerular crescents often correlated with poor outcome. This association was confirmed by many investigators, and the close relationship between rapid clinical progression of glomerulonephritis and the pathologic finding of glomerular crescents became well established. The term "rapidly progressive glomerulonephritis" has become the standard designation for clinical manifestations that suggest severe glomerulonephritis. Most patients with this clinical syndrome have severe glomerulonephritis with crescent formation, but some have other diseases that can mimic CGN, such as thrombotic microangiopathy. A conclusive diagnosis of CGN can be made only by evaluation of a renal biopsy specimen.

With the advent of immunofluorescence microscopy and electron microscopy, the heterogeneity of the glomerular diseases that cause crescent formation was recognized (15,16). This allowed Steen Olsen (15) to conclude that CGN "might constitute a common final and fatal pathway of several etiologically and pathogenetically different glomerular diseases." Thus, the light microscopic recognition of CGN in a renal biopsy specimen is only the beginning of an adequate pathologic analysis. Further elucidation of pathogenic mechanisms that are causing the glomerulonephritis is equally or more important in predicting the prognosis and selecting the appropriate treatment strategy for the patient. A pathologic diagnosis of CGN is incomplete unless the disease is categorized further, which usually requires immunofluorescence microscopy (or immunohistochemistry) and electron microscopy. Accurate and prompt categorization of CGN is critical for optimal patient outcome, because one of the major positive prognostic factors in the most aggressive forms of CGN, including anti-GBM glomerulonephritis, is rapid institution of immunosuppressive treatment (17).

CGN is categorized by immunohistology into anti-GBM CGN with linear GBM staining for immunoglobulin, immune complex CGN with granular staining of glomeruli for immunoglobulin or complement, or crescentic glomerulonephritis with little or no glomerular staining for immunoglobulin or complement (i.e., pauci-immune CGN) (Fig. 15.3) (18,19). These three immunopathologic categories are sometimes designated types I, II, and III, respectively; however, this terminology is not widely used and thus may not be understood when used in a diagnosis. Anti-GBM disease is a special form

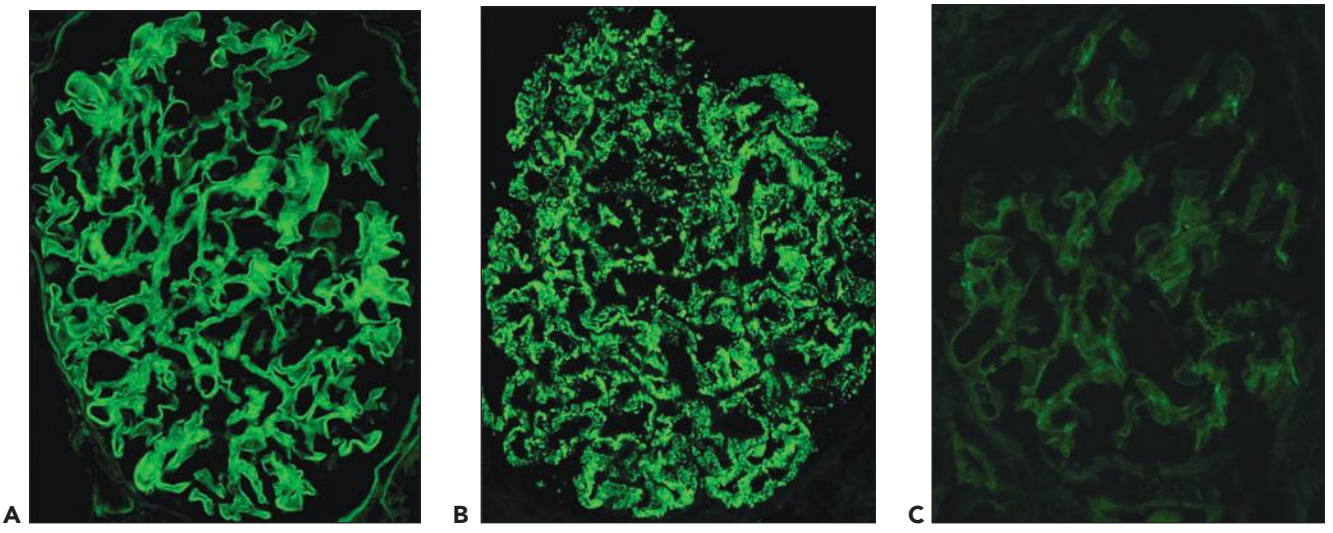



**FIGURE 15.3 Immunofluorescence microscopy patterns of glomerular staining for IgG that are indicative of anti-GBM (A), immune complex (B), and pauci-immune (C) glomerulonephritis.** Note the linear staining of anti-GBM disease compared with the granular staining of immune complex disease and the scanty background staining of pauci-immune disease. (FITC anti-IgG.)

of immune complex disease caused by in situ binding of anti-GBM antibodies to GBM antigens, but it is traditionally separated from other forms of immune complex glomerulonephritis when categorizing CGN. Throughout this chapter, the term *immune complex glomerulonephritis* is used for glomerulonephritis caused by immune complexes other than anti-GBM immune complexes.

Crescents also occur in categories of glomerular injury that are not included in the three major categories of anti-GBM, immune complex, or ANCA disease. For example, C3 glomerulopathy (including dense deposit disease and C3 glomerulonephritis), which is caused by dysregulated alternative pathway complement activation rather than immune complexes, may have crescent formation (20). Rarely, crescents occur with thrombotic microangiopathy and diabetic glomerulosclerosis apparently caused by capillary injury that results directly from these diseases. However, whenever crescents are observed in the context of a glomerular disease that rarely has crescents, the possibility of a concurrent disease (e.g., ANCA disease) that may be causing the crescents should be considered.

Table 15.1 lists the frequency of the categories of CGN among patients whose renal biopsies were evaluated in the University of North Carolina Nephropathology Laboratory (18). Anti-GBM glomerulonephritis is uncommon at any age, accounting for about 15% of CGN. Pauci-immune CGN is the most common category of glomerulonephritis in patients who have more than 50% of glomeruli involved by crescents. Approximately 90% of patients with pauci-immune CGN have circulating antineutrophil cytoplasmic autoantibodies (ANCA) (21). Pauci-immune CGN (i.e., ANCA-CGN) is by far the most common category of CGN in older patients (18). In contrast, immune complex glomerulonephritis with crescents is more common in younger patients, which is not surprising considering that many of the most common immune complex glomerulonephritides occur most frequently in children or young adults, such as IgA nephropathy, IgA vasculitis (Henoch-Schönlein purpura), lupus nephritis, poststreptococcal glomerulonephritis, and membranoproliferative glomerulonephritis. For example, in an analysis in London of 30 children with CGN, Jardim et al. (22) identified IgA vasculitis (Henoch-Schönlein purpura) glomerulonephritis in 9, membranoproliferative glomerulonephritis in 7, poststreptococcal glomerulonephritis in 2, and lupus glomerulonephritis in 1. The remaining patients had microscopic polyangiitis (MPA) in four, granulomatosis with polyangiitis (GPA) (Wegener granulomatosis) in one, anti-GBM disease in two, and idiopathic (pauci-immune) CGN in four.

Although reports from North America and Europe are generally in agreement with the data in Table 15.1 (23–25), the relative frequency of different types of CGN may vary substantially in different geographic locations. For example, a study of 172 patients with CGN from China noted 8.7% anti-GBM CGN, 22.7% pauci-immune CGN, and 68.6% immune complex CGN (half of which were lupus nephritis) (26). Another study of a cohort of 106 patients with CGN from China, although more recent and from a more industrialized region, observed 16% anti-GBM disease, 40.6% immune complex disease, and 43.4% pauci-immune disease (27). An analysis of 28 patients with CGN from the Republic of Macedonia identified 22 cases of poststreptococcal glomerulonephritis, 1 case of poststaphylococcal glomerulonephritis, 1 case of anti-GBM glomerulonephritis, and 4 cases of ANCA-positive pauci-immune CGN (28). This higher proportion of immune complex CGN from some regions may be caused by a higher frequency of nephritogenic infections in this region.

Even in the same country, demographic differences in cohorts affect the frequency of different causes for CGN. For example, in a study from India of all patients (predominantly adults) with ≥50% crescents, 72% (33/46) had pauci-immune CGN (29), whereas another study from India of only children (≤18 years old) with ≥50% crescents found that 86% (19/22) had immune complex glomerulonephritis (30).

In general, anti-GBM glomerulonephritis and ANCA glomerulonephritis have a higher frequency and severity of

**TABLE 15.1 Frequency of different types of Crescentic Glomerulonephritis[a] in renal biopsy specimens evaluated by the University of North Carolina Nephropathology Laboratory**

| Age (years) | *n* | Anti-GBM crescentic glomerulonephritis (%) | Pauci-immune crescentic glomerulonephritis (%) | Immune complex crescentic glomerulonephritis (%) | Other crescentic glomerulonephritis (%) |
|---|---|---|---|---|---|
| All | 632 | 15 | 60 | 24 | 1 |
| 1–20 | 73 | 12 | 42 | 45 | 0 |
| 21–60 | 303 | 15 | 48 | 35 | 3 |
| 61–100 | 256 | 15 | 79 | 6 | 0 |

[a]Anti-GBM crescentic glomerulonephritis was defined as glomerulonephritis with ≥50% crescents and ≥2+ linear GBM staining for IgG by direct immunofluorescence microscopy. Pauci-immune crescentic glomerulonephritis was defined as glomerulonephritis with ≥50% crescents and ≤2+ staining of glomeruli for any immunoglobulin. Immune complex crescentic glomerulonephritis was defined as glomerulonephritis with ≥50% crescents and ≥2+ nonlinear GBM staining for any immunoglobulin by direct immunofluorescence microscopy. Crescentic membranoproliferative and postinfectious glomerulonephritis, which typically have substantial staining for C3 but little or no staining for immunoglobulin, also were included in the immune complex category. The other category includes all other glomerular diseases, such as thrombotic microangiopathy, diabetic glomerulosclerosis, monoclonal immunoglobulin deposition disease, and so on.

Modified from Jennette JC. Rapidly progressive crescentic glomerulonephritis. *Kidney Int* 2003;63:1164.

crescent formation than immune complex glomerulonephritis (Table 15.2 and Fig.15.4) (18,19). This finding suggests that the pathogenic mechanisms that underlie anti-GBM and ANCA glomerulonephritis are more destructive than the mechanisms that underlie immune complex glomerulonephritis. However, some categories of immune complex glomerulonephritis tend to have a greater frequency of crescent formation than do others. In general, the extent of glomerular subendothelial, as opposed to subepithelial or mesangial, immune complex localization correlates with severe inflammation and crescent formation among patients with immune complex glomerulonephritis. This suggests that the proximity of subendothelial immune complexes to the inflammatory mediator systems in the circulation is more effective at causing the glomerular injury that results in crescent formation than are immune complexes in the mesangium or subepithelial zones of glomeruli. This could in part explain the severity of anti-GBM glomerulonephritis because the autoantibodies form immune complexes in situ with the GBM antigens at the subendothelial interface between the GBM and the plasma.

This chapter reviews the clinical and pathologic features of anti-GBM disease, including Goodpasture syndrome and anti-GBM glomerulonephritis in the absence of pulmonary disease. In the context of this discussion of an archetype of CGN, the pathogenesis of crescent formation that is initiated by many aggressive forms of glomerulonephritis in addition to anti-GBM disease is summarized. The specific pathogenic events that are unique to anti-GBM disease are reviewed.

**TABLE 15.2 Frequency of glomerular crescents, necrosis, and endocapillary hypercellularity in different types of glomerular disease evaluated by the University of North Carolina Nephropathology Laboratory**

| Type of glomerular disease | % With any crescents | % With >50% crescents | Average % glomerular crescents[a] | Glomerular necrosis (0–4+) | Glomerular hypercellularity (0–4+) |
|---|---|---|---|---|---|
| Anti-GBM glomerulonephritis | 97.1 | 84.8 | 77 | 1.7+ | 0.8+ |
| ANCA glomerulonephritis | 89.5 | 50.3 | 49 | 1.2+ | 0.8+ |
| Lupus glomerulonephritis (III and IV) | 56.5 | 12.9 | 31 | 1.7+ | 2.2+ |
| Henoch-Schönlein purpura glomerulonephritis | 61.3 | 9.7 | 27 | 0.4+ | 1.5+ |
| IgA nephropathy | 32.5 | 4.0 | 21 | 0.1+ | 1.4+ |
| Postinfectious glomerulonephritis | 33.3 | 3.3 | 19 | 0.3+ | 2.7+ |
| Type I membranoproliferative glomerulonephritis | 23.8 | 4.6 | 25 | 0.2+ | 2.8+ |
| DDD/C3 glomerulopathy | 43.8 | 18.8 | 48 | 0.2+ | 1.8+ |
| Fibrillary glomerulonephritis | 22.8 | 5.0 | 26 | 0+ | 0.6+ |
| Monoclonal immunoglobulin deposition disease | 5.6 | 0 | 13 | 0+ | 0.3+ |
| Thrombotic microangiopathy | 5.6 | 0.9 | 26 | 0.4+ | 0.3+ |
| Diabetic glomerulosclerosis | 3.2 | 0.3 | 20 | 0+ | 0.3+ |
| Nonlupus membranous glomerulopathy | 3.2 | 0.1 | 15 | 0+ | 0.1+ |

[a]ANCA glomerulonephritis was defined as glomerulonephritis with ≤2+ staining of glomeruli for any immunoglobulin in a patient who is positive for MPO-ANCA or PR3-ANCA by ELISA.

Modified from Jennette JC. Rapidly progressive crescentic glomerulonephritis. *Kidney Int* 2003;63:1164.

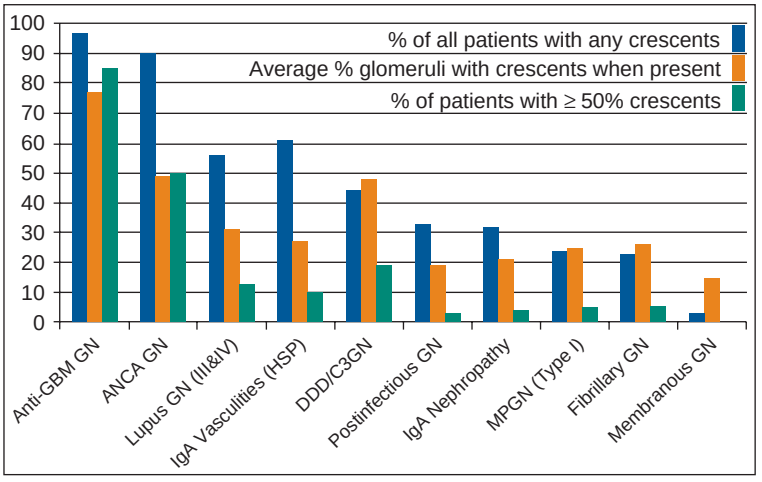



**FIGURE 15.4 Frequency of glomerular crescents in different types of glomerular disease evaluated in the University of North Carolina Nephropathology Laboratory.** For each category of disease, the data are expressed as (i) the % of patients who had any crescents in biopsy specimens, (ii) the average % of crescents in specimens that had crescent formation, and (iii) the % of patients who had specimens with ≥50% crescents. (Data derived from Jennette JC. Rapidly progressive crescentic glomerulonephritis. *Kidney Int* 2003;63:1164.)

## CLINICAL PRESENTATION

Anti-GBM disease is rare with an incidence of approximately 1/1,000,000 (compared to greater than 1/100,000 for ANCA disease), an equal male-to-female ratio, and a broad bimodal age of onset with one peak in older children and young adults and another in older adults (31–38). Anti-GBM GN is rare in children but does occur (39). Glomerulonephritis is the most common initial clinical manifestation of anti-GBM disease. Anti-GBM glomerulonephritis usually manifests with rapidly progressive glomerulonephritis, and renal biopsy reveals more than 50% glomerular crescents in most patients. For example, a cohort of 28 patients with anti-GBM but no ANCA (see Table 15.2) had more than 80% of patients with crescents in 50% or more of glomeruli in the initial renal biopsy (18). Multiple other studies confirm that more than 80% of patients with anti-GBM disease have more than 50% of glomeruli with crescents in the initial renal biopsy (34–38). The data in Tables 15.2 and 15.3 demonstrate that anti-GBM glomerulonephritis is pathologically and clinically the most severe form of glomerulonephritis (18). Hematuria is virtually always present. Proteinuria is common. Although most patients with anti-GBM disease present with severe renal disease, severe pulmonary disease, or both, patients will rarely present with hematuria and proteinuria in the absence of renal insufficiency or pulmonary hemorrhage (40).

The frequency of pulmonary hemorrhage among patients with anti-GBM disease varies among cohorts, but most series show a frequency of 50% ± 15% (33–38). For example, in a cohort of 71 patients from the United Kingdom evaluated by Savage et al. (33), 35% had Goodpasture syndrome at presentation, although approximately 50% eventually had some pulmonary hemorrhage. In a cohort of 53 North American patients with native kidney anti-GBM disease reported by Wilson and Dixon (31), 60% had Goodpasture syndrome. In a series of 40 patients from Ireland, 62% had pulmonary involvement (34). In a large cohort from China, 45% (80/176) of patients with anti-GBM disease had hemoptysis (37). Pulmonary involvement typically causes hemoptysis, dyspnea, rales, and rhonchi. Most patients with extensive pulmonary hemorrhage have pallor because of anemia. Many patients have a history of a recent flu-like illness or other infection before the onset of anti-GBM disease (8,31,37,41), and a minority of patients have a history of hydrocarbon exposure although this frequency is higher than in the general population (42–44). Patients have a higher frequency of cigarette smoking than does the general population. For example, in a study of 23 anti-GBM disease patients from New Zealand, 50% were regular

**TABLE 15.3 Features at the time of presentation of different types of Crescentic Glomerulonephritis[a] Evaluated by the University of North Carolina Nephropathology Laboratory**

| | Mean age | Male:female | Creatinine | Proteinuria (g/24 h) |
|---|---|---|---|---|
| Anti-GBM crescentic glomerulonephritis | 52 ± 21 (14–84) | 1:1.0<br>45:47 | 9.7 ± 7.2 (0.8–50) | 1.67 ± 3.35 (0.20–16.20) |
| Pauci-immune crescentic glomerulonephritis | 56 ± 20 (2–92) | 1:0.9<br>202:177 | 6.5 ± 4.0 (0.8–22.1) | 1.94 ± 2.95 (0.11–18.00) |
| Immune complex crescentic glomerulonephritis | 33 ± 17 (4–77) | 1:1.6<br>61:95 | 4.9 ± 3.8 (0.8–21.7) | 4.39 ± 4.77 (0.30–22.00) |

[a]Anti-GBM crescentic glomerulonephritis was defined as glomerulonephritis with ≥50% crescents and ≥2+ linear GBM staining for IgG by direct immunofluorescence microscopy. Pauci-immune crescentic glomerulonephritis was defined as glomerulonephritis with ≥50% crescents and ≤2+ nonlinear staining of glomeruli for any immunoglobulin. Immune complex crescentic glomerulonephritis was defined as glomerulonephritis with ≥50% crescents and ≥2+ nonlinear GBM staining for any immunoglobulin by direct immunofluorescence microscopy (plus membranoproliferative and postinfectious glomerulonephritis).

Jennette JC. Rapidly progressive crescentic glomerulonephritis. *Kidney Int* 2003;63:1164.

smokers compared to 17% of the general population, and only 14% had never smoked compared to 56% of the general population ($P < 0.001$) (38). Savage et al. (33) observed that there is a tendency for young men with anti-GBM disease to present with Goodpasture syndrome (i.e., combined pulmonary hemorrhage and nephritis) and for older women to present with glomerulonephritis alone. We have observed a similar pattern in the patients at the University of North Carolina. Overall, however, the male-to-female ratio is equal (see Table 15.3). Anti-GBM glomerulonephritis is rare in African Americans.

Approximately one quarter to one third of anti-GBM patients have ANCA, usually MPO-ANCA (Table 15.4) (18,37,45–49). Most patients with concurrent anti-GBM and ANCA have renal limited disease or microscopic polyangiitis (MPA); however, some have granulomatosis with polyangiitis (GPA) (Wegener's) (50) or eosinophilic granulomatosis with polyangiitis (EGPA) (Churg-Strauss) (51). In a study of anti-GBM patients from China, patients who were 65 years or older at the time of diagnosis were more often ANCA positive (46.0% vs. 14.6%; $P < 0.001$) and had less hemoptysis (26.0% vs. 46.2%; $P = 0.01$) (52).

With current antirejection regimens, anti-GBM disease only very rarely recurs in transplants (53). Alport posttransplantation anti-GBM glomerulonephritis, which is mediated by alloantibodies against the GBM antigens that are lacking in Alport patients, occurs after kidney transplantation in a minority of patients with Alport syndrome (54,55). The clinical presentation is the same as new onset of disease, and patients have positive serology for anti-GBM antibodies, although the epitope specificity of the anti-GBM alloantibodies in Alport patients is different from that of the autoantibodies in recurrent disease (55).

## PATHOLOGIC FINDINGS

### Gross Pathology

As with all severe forms of glomerulonephritis, during the acute phase, the kidneys are of normal size or slightly enlarged and have tiny red dots scattered over the outer and cut cortical surfaces, referred to as a flea-bitten appearance (Fig. 15.5). Most of these red dots correspond to blood within tubular lumens or within the Bowman capsule. However, in severe necrotizing disease, a few spots are caused by rupture of the Bowman capsule with hemorrhage into the adjacent interstitium. Advanced chronic disease is characterized by progressive shrinkage and scarring of the cortical parenchyma.

The lungs from patients with pulmonary involvement have focal to diffuse dark red consolidation caused by intra-alveolar hemorrhage. Chronic pulmonary injury has a paler consolidation caused by fibrosis and may be rust colored from the accumulation of hemosiderin. Discrete nodule formation or cavitation should raise the possibility of concurrent GPA (Wegener's).

### Light Microscopy

#### Glomeruli

Glomerular fibrinoid necrosis and crescent formation are the histologic hallmarks of acute anti-GBM glomerulonephritis (18,19,32,36,56). Even though anti-GBM antibodies are bound diffusely to intact GBMs, glomerular tuft necrosis more often is segmental rather than global (Figs. 15.6 and 15.7). Foci of glomerular fibrinoid necrosis are characterized by replacement of the normal architecture by ragged, deeply acidophilic fibrinoid material. Special stains that demarcate GBM (e.g., periodic acid–Schiff [PAS], Jones silver methenamine) are helpful for identifying glomerular necrosis by demonstrating areas of GBM rupture and also are helpful for identifying crescent formation by demarcating where the glomerular tuft ends and the crescent begins (Figs. 15.6 to 15.9). Masson trichrome stains are useful for differentiating between areas of fibrinoid necrosis, which are red (fuchsinophilic), and areas of sclerosis, which are blue or green depending on the counterstain used (Fig. 15.10). Masson trichrome staining can be useful in identifying small segmental foci of necrosis that might be overlooked on hematoxylin and eosin (H&E)–stained sections (Figs. 15.10 and 15.11). Some biopsy specimens from patients with anti-GBM antibody–mediated pulmonary disease, especially from patients with little or no renal dysfunction, have normal light microscopic histologic patterns when immunofluorescence microscopy documents extensive diffuse global staining for anti-GBM antibodies (4). Fuchsinophilic fibrin occurs not only in foci of fibrinoid necrosis but also in glomerular capillary thrombi and in the interstices between the cells of crescents (Fig. 15.12).

**TABLE 15.4 Frequency of serum ANCA, anti-GBM, and anti-GBM plus ANCA in crescentic glomerulonephritis with linear GBM IGG (anti-GBM glomerulonephritis) versus a paucity of immunoglobulin (pauci-immune glomerulonephritis) evaluated by the University of North Carolina Nephropathology Laboratory**

| | Anti-GBM–positive, ANCA-negative | Anti-GBM–positive, ANCA-positive | ANCA-positive, anti-GBM–negative | ANCA-negative, anti-GBM–negative |
|---|---|---|---|---|
| Linear IgG (anti-GBM) crescentic glomerulonephritis | 60.9% | 26.1% (26.1% MPO-ANCA, 0.0% PR3-ANCA) | 0% | 13.0% |
| Pauci-immune crescentic glomerulonephritis | 1.5% | 6.8% (4.5% MPO-ANCA, 2.3% PR3-ANCA) | 77.7% (52.2% MPO-ANCA, 20.5% PR3-ANCA) | 18.9% |

Data in all columns were derived from patients with ELISA serology results for MPO-ANCA, PR3-ANCA, and anti-GBM. Pauci-immune crescentic glomerulonephritis ($n = 132$) was defined as glomerulonephritis with ≥50% crescents and ≤2+ nonlinear staining of glomeruli for any immunoglobulin. Linear IgG crescentic glomerulonephritis ($n = 46$) was defined as glomerulonephritis with ≥50% crescents and ≥2+ linear GBM staining for IgG by direct immunofluorescence microscopy.

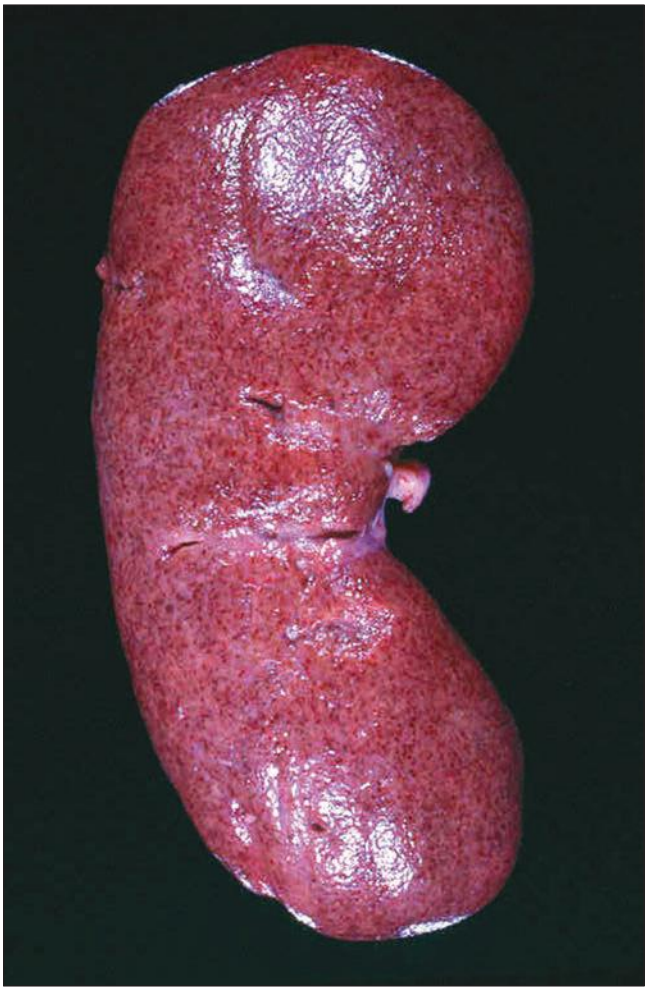


**FIGURE 15.5 Kidney from a patient with Goodpasture syndrome who died of massive pulmonary hemorrhage and was found to have crescentic anti-GBM glomerulonephritis.** Notice the tiny dark dots on the surface that correspond mainly to blood in tubular lumens and in the Bowman spaces. A few of the dots correspond to periglomerular hemorrhage at sites of severe disruption of the Bowman capsules.

Even in glomeruli with crescents, the histologic changes in capillary tufts vary from little or no change (see Fig. 15.2) to extensive necrotizing destruction of the tuft (see Fig. 15.8). Foci of glomerular fibrinoid necrosis may contain scattered karyorrhectic debris (see Fig. 15.7). Glomerular neutrophilic infiltration is variable. Extensive neutrophilic infiltration is uncommon and is usually seen with severely necrotizing disease (see Fig. 15.9). Unlike many forms of immune complex CGN, the intact capillary walls in anti-GBM glomerulonephritis often have normal thickness (see Figs. 15.2, 15.10, and 15.11) because there is no bulky accumulation of immune complex deposits. Thus, the best-preserved glomerular tufts help predict the immunopathologic category because immune complex CGN typically has marked hypercellularity and thickening of capillary walls in the intact tufts, whereas anti-GBM or ANCA-CGN typically has capillary walls of normal thickness and minimal hypercellularity in the most intact segments and glomeruli.

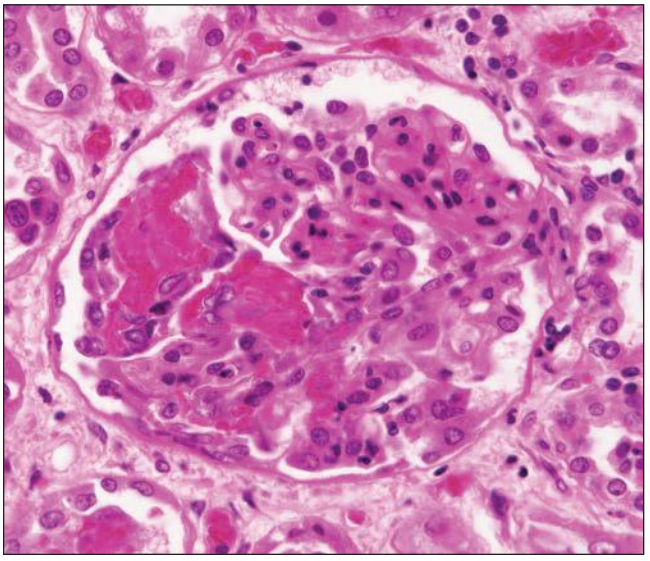


**FIGURE 15.6 Anti-GBM glomerulonephritis with segmental fibrinoid necrosis manifesting as irregular, deeply acidophilic material replacing the normal architecture of about a third of the glomerular tuft.** There are reactive changes in the adjacent podocytes but no well-defined crescent formation. There is slight periglomerular interstitial edema. (H&E)

More than 90% of patients with anti-GBM disease who undergo renal biopsy are found to have some degree of crescent formation (see Table 15.2) (18). When there are crescents, on average, about 80% of glomeruli have crescents; however, this ranges from less than 5% to 100%. The percentage

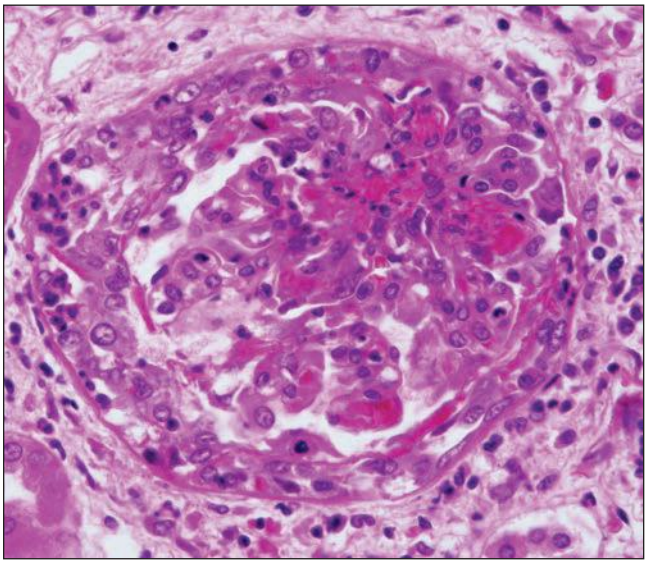


**FIGURE 15.7 Anti-GBM glomerulonephritis with segmental fibrinoid necrosis and a circumferential cellular crescent.** There are dark fragments of karyorrhectic debris in the area of necrosis. The crescent appears to have an admixture of epithelial cells and leukocytes, including some neutrophils. There is a greater degree of periglomerular interstitial edema and inflammation than in Figure 15.5. (H&E)

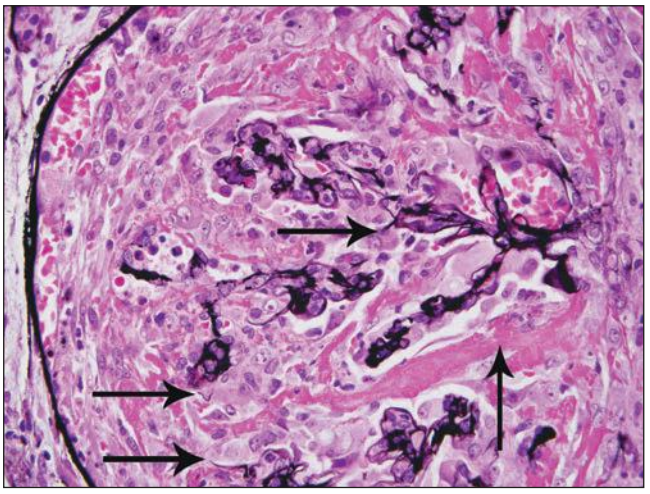


**FIGURE 15.8 Jones silver methenamine stain with an H&E counterstain of a glomerulus from a patient with anti-GBM glomerulonephritis showing a large cellular crescent and extensive glomerular necrosis with disruption of glomerular basement membranes.** The *horizontal arrows* point to fragments of GBM with "ends" that indicate GBM rupture. The *vertical arrow* points to fibrinoid material in the glomerular tuft that corresponds to an area of fibrinoid necrosis. Tinctorially identical fibrinoid material (fibrin) also is admixed with some of the crescent cells.

crescents correlate with serum creatinine level at the time of biopsy (36). Biopsy specimens without crescents typically have focal segmental fibrinoid necrosis that is relatively mild (see Fig. 15.11). Crescents may be segmental (see Fig. 15.9) or large and circumferential (see Fig. 15.12). Breaks in the

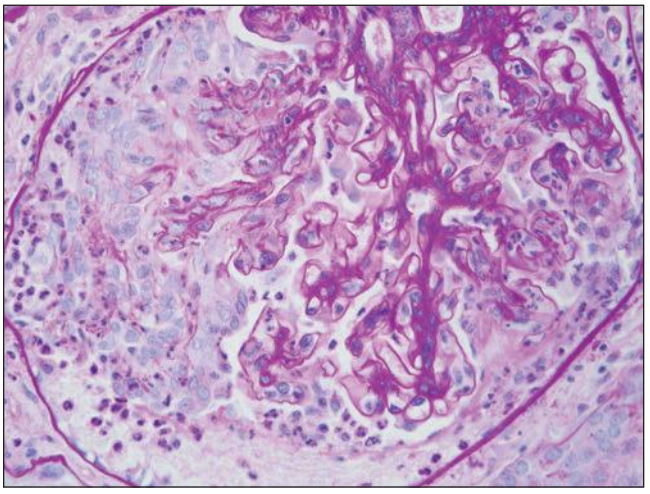


**FIGURE 15.9 Periodic acid–Schiff (PAS)–stained glomerulus from a patient with anti-GBM glomerulonephritis showing a large cellular crescent that contains an unusually prominent component of neutrophils.** The PAS stain allows the identification of segments that have extensive dissolution of glomerular basement membranes. Note also the prominence of neutrophils within some capillaries, especially in segments with extensive necrosis.

Bowman capsule may accompany crescent formation and can be identified best with special stains that highlight the basement membrane of the Bowman capsule, such as Jones silver methenamine or PAS (Fig. 15.13). The proliferative changes that form crescents occasionally extend into the origin of the proximal tubule (Fig. 15.14). The appearance of crescents in anti-GBM glomerulonephritis ranges from relatively orderly layers of cells with features consistent with epithelial cells (see Figs. 15.7, 15.8, and 15.12) to very disorderly crescents containing mainly cells with features consistent with macrophages or other leukocytes, occasionally including multinucleated giant cells (57) (Fig. 15.15).

Magil and Wadsworth (58) observed that macrophages are more common in the crescents of anti-GBM glomerulonephritis than in the crescents of immune complex glomerulonephritis. Disorderly crescents with numerous macrophages occur in glomeruli with disruption of the Bowman capsule (59) and often are accompanied by and continuous with periglomerular inflammation that may have a granulomatous appearance. Both anti-GBM glomerulonephritis and ANCA glomerulonephritis often have extensive fibrinoid necrosis, focal destruction of the Bowman capsule, disordered crescents, and occasional periglomerular giant cells (58,60). In contrast, immune complex CGN typically has little or no fibrinoid necrosis, more orderly crescents, and intact Bowman capsules. Thus, the identification of severe necrotizing glomerulonephritis with disorderly crescents and periglomerular inflammation favors a diagnosis of anti-GBM glomerulonephritis, ANCA glomerulonephritis, or concurrent anti-GBM and ANCA disease rather than immune complex glomerulonephritis.

Periglomerular interstitial inflammation may accompany severe glomerular injury, especially when there is disruption of the Bowman capsule (see Fig. 15.15). This infiltrate often contains numerous monocytes and macrophages, with variable numbers of admixed neutrophils and lymphocytes. In a few specimens, the periglomerular inflammation includes multinucleated giant cells (59–61). Periglomerular granulomatous inflammation occurs in anti-GBM glomerulonephritis with concurrent ANCA and also in anti-GBM glomerulonephritis without ANCA. In a study by Rutgers et al. (45), periglomerular granulomas were identified in 11% of patients with MPO-ANCA, 40% of patients with concurrent MPO-ANCA and anti-GBM, and in 0% of 13 patients with anti-GBM but no ANCA. However, other investigators have observed periglomerular granulomas in anti-GBM glomerulonephritis alone (36,57). In a study of 80 patients with anti-GBM glomerulonephritis, 13% had periglomerular granulomatous inflammation (36). We also have observed this phenomenon in patients with anti-GBM but no ANCA. Interstitial eosinophilic infiltrates also have been reported with anti-GBM glomerulonephritis, but this is rare and could be secondary to concurrent hypersensitivity tubulointerstitial nephritis (57).

Histologically, anti-GBM glomerulonephritis with concurrent ANCA is of intermediate severity between anti-GBM glomerulonephritis without ANCA, which is more severe, and ANCA glomerulonephritis without anti-GBM, which is less severe. Table 15.5 shows that in our cohort, anti-GBM glomerulonephritis with concurrent ANCA has on average 67% of glomeruli with crescents compared with 84% in anti-GBM glomerulonephritis without ANCA and less than 50% in ANCA glomerulonephritis without anti-GBM. Rutgers et al.

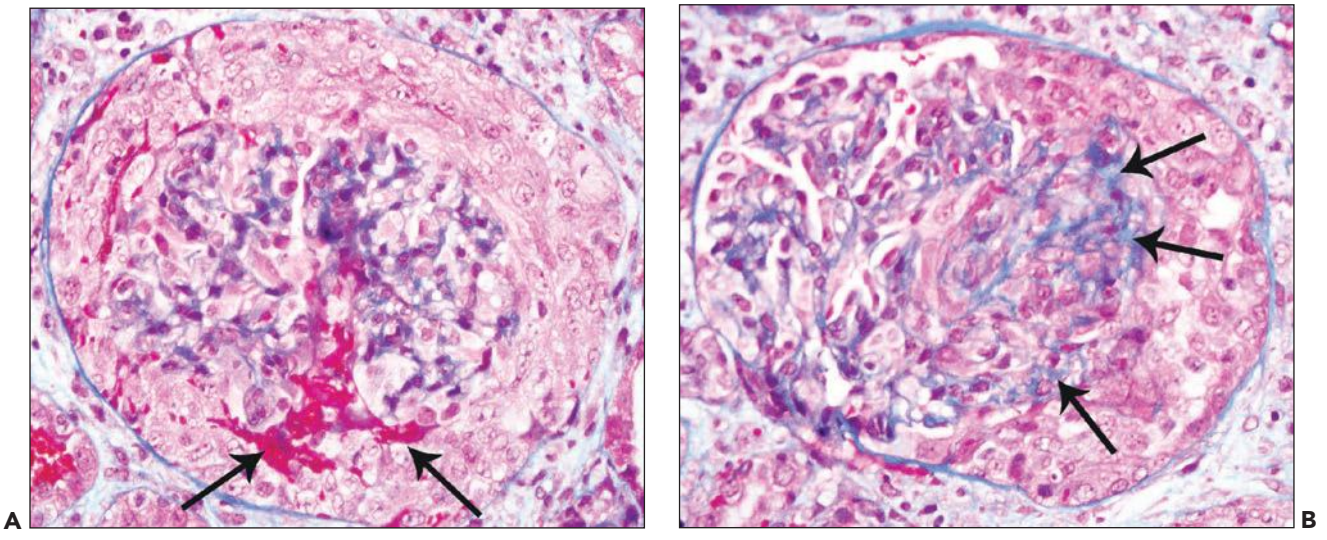



**FIGURE 15.10 Masson trichrome staining of two different glomeruli from the same patient with anti-GBM disease.** One glomerulus **(A)** has segmental fibrinoid necrosis with an adjacent cellular crescent. The necrosis is marked by *dark red* (fuchsinophilic) staining. The other glomerulus **(B)** has segmental sclerosis with adjacent epithelial proliferation and an adhesion to the Bowman capsule. The collagenous matrix of the sclerosis stains *blue*.

(45) reported similar results with anti-GBM glomerulonephritis with concurrent ANCA having on average 58% of glomeruli with crescents compared with 72% in anti-GBM glomerulonephritis without ANCA and less than 45% in ANCA glomerulonephritis without anti-GBM.

The acute necrotizing glomerular lesions evolve into sclerotic lesions, ranging from segmental sclerosis with adhesions to the Bowman capsule to global sclerosis, depending on the severity of the antecedent acute injury. Sclerotic lesions may be present along with necrotizing lesions in the same specimen (see Fig. 15.10); however, the degree of activity versus chronicity often is relatively synchronous. This differs from ANCA glomerulonephritis, which more often has an admixture of active necrotizing lesions and sclerotic lesions. Acute cellular crescents (see Figs. 15.2, 15.7, and 15.12) evolve into fibrocellular (see Fig. 15.10B) and fibrous crescents (Fig. 15.16) as the cellular components are progressively replaced by collagen. The initial cause of injury may be difficult to discern with advanced global sclerosis. Special stains that highlight residual basement membranes are helpful because they can demarcate disruption of GBMs or the Bowman capsule (see Fig. 15.16), which

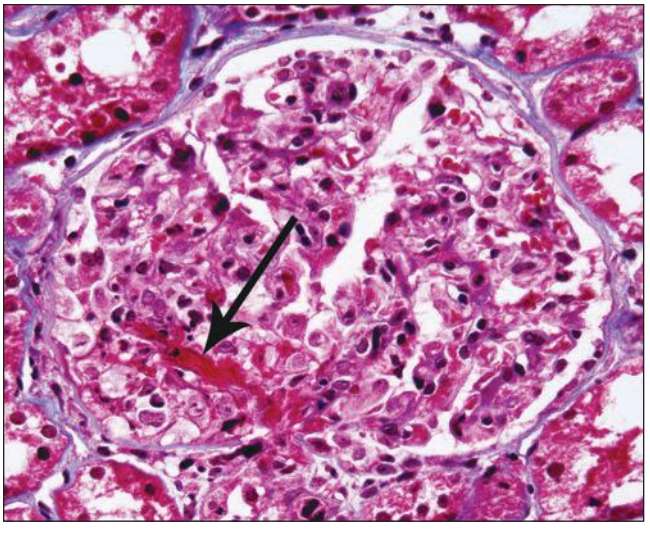


**FIGURE 15.11** Masson trichrome stain demonstrating a very small focus of fuchsinophilic fibrinoid necrosis (*arrow*) in a glomerulus from a patient with Goodpasture syndrome who had a renal biopsy that demonstrated intense diffuse global linear GBM staining for IgG by immunofluorescence microscopy but had only slight segmental fibrinoid necrosis in 25% of glomeruli and no crescents.

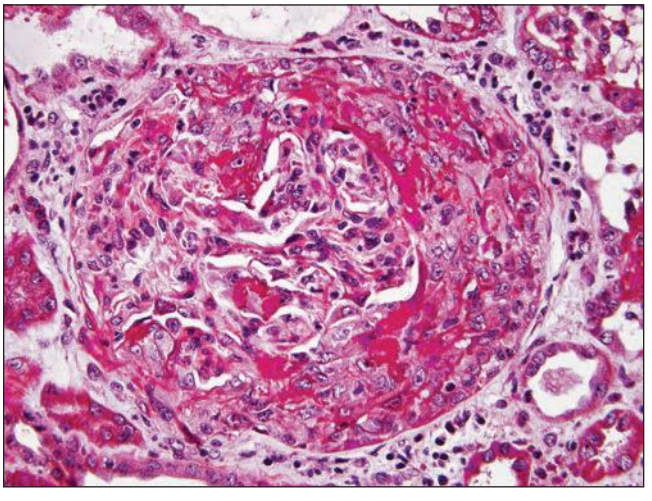


**FIGURE 15.12** Masson trichrome–stained glomerulus from a patient with anti-GBM disease showing a large circumferential cellular crescent with strands of fuchsinophilic fibrin interspersed with the cells of the crescent. Note also the periglomerular interstitial edema and inflammation.

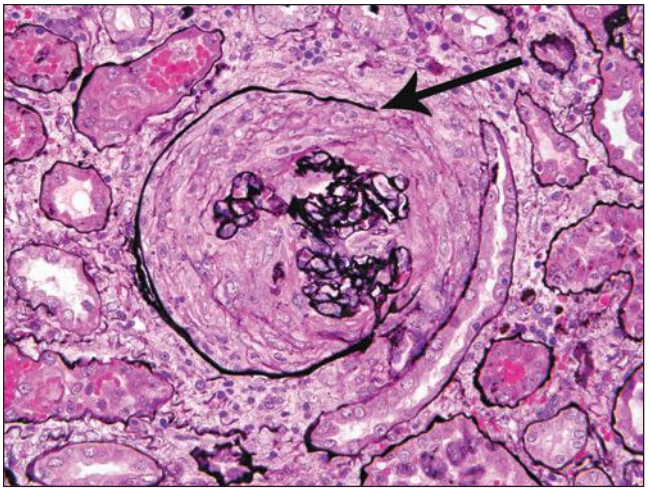


**FIGURE 15.13** Jones methenamine silver–stained glomerulus that shows the demarcation between the glomerular tuft basement membranes, which are stained black, and the large cellular crescent in a patient with anti-GBM glomerulonephritis. Note also the focal disruption of the Bowman capsule (*arrow*).

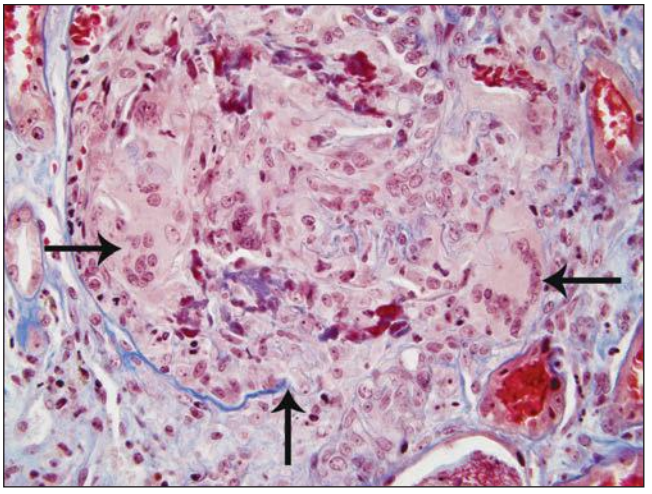


**FIGURE 15.15 Masson trichrome–stained glomerulus from a patient with anti-GBM disease showing a very disordered cellular crescent with multinucleated giant cells (*horizontal arrows*).** The giant cell on the left is in a zone of periglomerular inflammation that is continuous with the crescent through a large gap in the Bowman capsule that begins at the point of the *vertical arrow*. This patient had intense linear GBM staining for IgG by immunofluorescence microscopy and definitive serologic results for anti-GBM antibodies; however, multiple serologic tests for ANCA before and after renal biopsy all were negative.

supports the conclusion that the glomerular scarring has been caused by necrotizing inflammation rather than less destructive processes, such as ischemia, which has more wrinkling and collapse of GBMs rather than segmental fragmentation of GBMs.

## Tubules

In mild or early disease, tubules typically are normal or have focal acute injury as indicated by epithelial flattening (simplification) and interstitial edema. With more severe acute disease, there is more pronounced acute tubular injury with associated interstitial influx of leukocytes. Epithelial flattening is caused primarily by sloughing of apical cytoplasm. Flattening caused by atrophy is usually accompanied by interstitial fibrosis. The acute tubular epithelial flattening and necrosis may closely resemble ischemic acute renal failure ("acute tubular necrosis") and may be caused, at least in part, by impaired perfusion in peritubular capillaries as a consequence of severe obstruction to blood flow through glomeruli. Focal acute tubulitis may occur, but this is usually mild. Breaks in tubular basement membranes and tubulitis may develop, especially in patients who have linear staining of glomerular and tubular basement membranes by immunofluorescence microscopy (62). Epithelial cells at the origins of proximal tubules occasionally proliferate in concert with other epithelial cells in the crescents (62) (see Fig. 15.14). As acute anti-GBM glomerulonephritis evolves to chronic disease, tubules in areas of severe injury undergo atrophy and some disappear (Figs. 15.17 and 15.18).

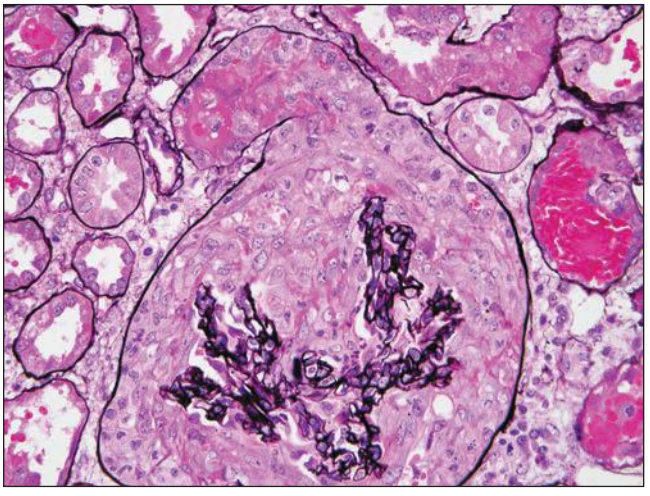


**FIGURE 15.14** Jones methenamine silver–stained glomerulus that shows the proliferation of epithelial cells extending into the proximal tubule in a patient with anti-GBM glomerulonephritis.

## Interstitium

Various degrees of interstitial inflammation and subsequent fibrosis occur and usually parallel the severity, activity, and chronicity of the glomerular disease. Andres et al. (62) reported that patients who have anti-GBM antibodies that bind to glomerular and tubular basement membranes have more severe tubulointerstitial inflammation than those who have antibodies that bind only to glomeruli.

With acute disease, interstitial edema is more pronounced (Figs. 15.19 and 15.20), whereas with chronic disease, interstitial fibrosis predominates (see Figs. 15.17 and 15.18). Masson trichrome staining is helpful in distinguishing interstitial edema from interstitial fibrosis because the latter has more collagen, which causes more blue (or green) staining.

Interstitial infiltrates are composed of neutrophils, eosinophils, lymphocytes, monocytes, and macrophages (36,62,63). Lymphocytes are most numerous and are predominantly CD4 lymphocytes (63). There are occasional interstitial

**TABLE 15.5 Comparison of clinical and pathologic features of glomerulonephritis in patients with different ANCA and anti-GBM (AGBM) serology findings evaluated by the University of North Carolina Nephropathology Laboratory**

| | Anti-GBM–positive, ANCA-negative | MPO-ANCA–positive, anti-GBM–negative | PR3-ANCA–positive, anti-GBM–negative | Anti-GBM–positive, ANCA-positive |
|---|---|---|---|---|
| | *n* = 28 | *n* = 102 | *n* = 52 | *n* = 25 |
| Age | 41 ± 21 | 62 ± 15 | 54 ± 18 | 68 ± 13 |
| Creatinine | 10.0 ± 9.1 | 6.9 ± 7.7 | 5.6 ± 4.6 | 9.6 ± 5.3 |
| Anti-GBM titer | 579.7 | <20 | <20 | 350.5 |
| ANCA titer | <20 | 86.0 ± 22 | 92.9 ± 39 | 72.3 ± 25 |
| More than 50% crescents | 93% | 44% | 38% | 62% |
| Mean % crescents[a] | 84 ± 21 | 48 ± 29 | 46 ± 30 | 67 ± 32 |
| Glomerular necrosis[b] | 2.1+ | 1.2+ | 1.8+ | 2.1+ |
| Glomerular sclerosis[b] | 1.1+ | 1.7+ | 1.3+ | 1.2+ |

[a]Mean percentage of crescents when crescents are present.
[b]Mean of scores of 0 to 4+, with 0 being none and 4+ being severe.

multinucleated giant cells (62). Accentuated periglomerular inflammation, which occasionally has a granulomatous appearance, occurs around glomeruli with extensive disruption of the Bowman capsule (see Fig. 15.15). This may have a starburst appearance because of the epithelioid macrophages palisading around the inflamed glomerulus. As noted earlier, periglomerular granulomatous inflammation with multinucleated giant cells may occur (45,57,61). Interstitial fibrosis begins while the glomerulonephritis is still active and becomes pronounced in those kidneys that sustain severe injury (see Fig. 15.17).

## Blood Vessels

Glomerular fibrinoid necrosis and inflammation occasionally extend into the most proximal part of contiguous hilar arterioles. Otherwise, acute inflammation of renal vessels, other than glomerular capillaries, is not typical for anti-GBM glomerulonephritis, unless the patient has concurrent ANCA (47). Patients with anti-GBM and ANCA can manifest the pathologic features of ANCA disease, including necrotizing small-vessel vasculitis in the kidney (Figs. 15.21 and 15.22). Any of the expressions of ANCA small-vessel vasculitis can occur in the kidneys of patients with concurrent anti-GBM and ANCA disease, including necrotizing arteriolitis, necrotizing arteritis, and leukocytoclastic medullary angiitis. The presence of necrotizing small-vessel vasculitis affecting vessels other than glomerular or alveolar capillaries in a patient with anti-GBM antibodies should raise the possibility of concurrent ANCA disease (Fig. 15.21).

## Lungs

Alveolar hemorrhage is the dominant histologic feature of acute lung disease (41,64) (Fig. 15.23). Some investigators have not been impressed by inflammatory changes in alveolar septa (41), but others have found neutrophilic infiltration and leukocytoclasia in the septa (64). In our experience, alveolar septal neutrophilic infiltration and leukocytoclasia are much less conspicuous in anti-GBM disease than in ANCA pulmonary disease. Thus, if neutrophils are conspicuous in a lung specimen from a patient with anti-GBM pulmonary disease, ANCA testing should be performed. The number of hemosiderin-laden macrophages in the areas of hemorrhage is an index of chronicity and/or recurrence.

Additional patterns of pulmonary injury include alveolar septal edema and hyaline membrane formation. An uncommon finding is a general pattern of diffuse alveolar damage with hyaline membrane formation with only focal areas of alveolar hemorrhage (64). The later phases of anti-GBM lung injury are characterized by progressive loss of intra-alveolar blood and a reciprocal increase in hemosiderin-laden macrophages. Intra-alveolar and septal fibrosis may develop but usually are relatively mild.

## Immunofluorescence Microscopy

The characteristic immunohistologic finding in anti-GBM glomerulonephritis is linear staining of GBMs for IgG (Figs. 15.1, 15.24, and 15.25), usually accompanied by much lesser amounts of granular to discontinuous linear staining for C3

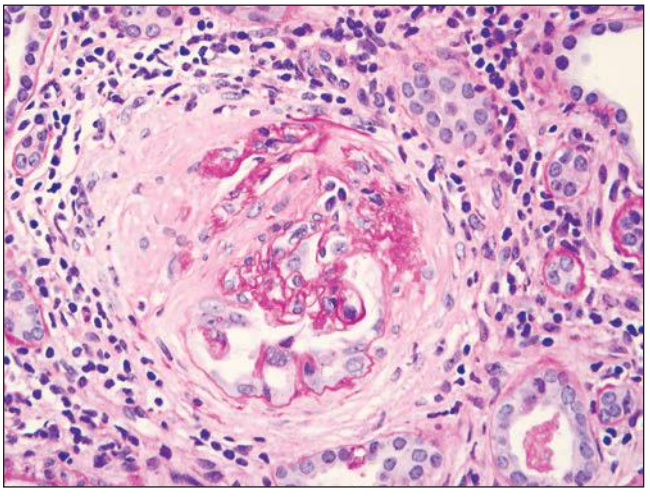


**FIGURE 15.16 Chronic anti-GBM glomerulonephritis with extensive sclerosis of the glomerular tuft and an adjacent fibrotic crescent.** The Bowman capsule has been almost totally destroyed. There is marked periglomerular interstitial fibrosis and chronic inflammation. This destructive, asymmetric pattern of sclerosis is not consistent with ischemic sclerosis. (PAS.)

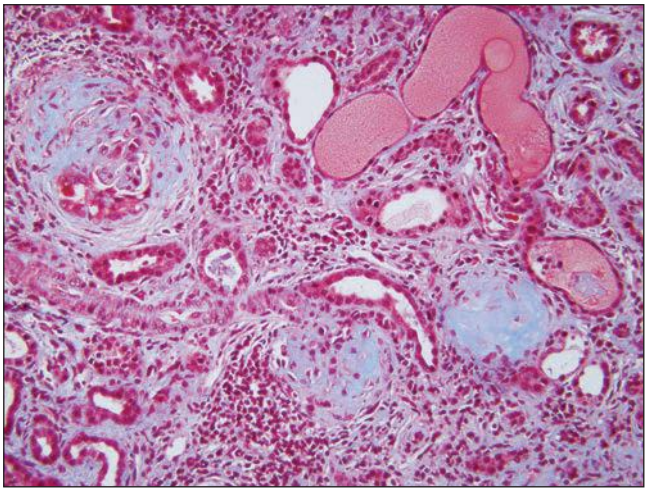


**FIGURE 15.17 Chronic anti-GBM glomerulonephritis with global sclerosis of three glomeruli, marked interstitial fibrosis and tubular atrophy, and focally variable interstitial infiltration by chronic inflammatory cells.** In some areas, there has been total disappearance (dropout) of tubules. (Masson trichrome stain.)

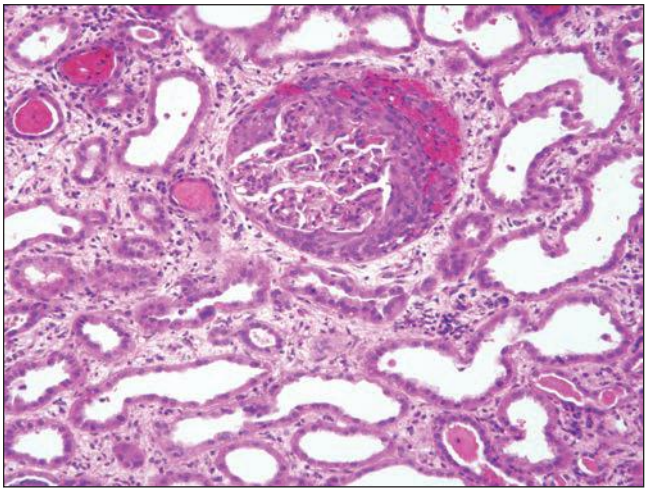


**FIGURE 15.19 Acute anti-GBM CGN with widespread tubular epithelial flattening (simplification), diffuse interstitial edema, mild interstitial infiltration of mononuclear leukocytes, and red blood cell casts in a few tubular lumens.** Note the hemorrhage that is admixed with the cells of the crescent. (H&E.)

(Fig. 15.26) (3,30–33,36,56,65,66) (Table 15.6). Biopsy specimens from patients with anti-GBM antibody–mediated pulmonary disease that have no clinical evidence for renal disease nevertheless have diffuse global linear staining for IgG along GBMs but no lesions by light microscopy (4). In most instances, however, the linear staining is accompanied by extensive necrotizing glomerulonephritis that causes varying degrees of disruption of GBMs. Occasional specimens have such severe destruction of GBMs that a misdiagnosis of pauci-immune CGN may be made because no glomerular staining is observed (see Fig. 15.25). Intact GBMs must be identified by carefully examining the background fluorescence before concluding that there is no glomerular staining. The mesangial matrix does not contain the α3 chain of type IV collagen, which is the target antigen of anti-GBM, and thus does not stain. Distal tubular basement membranes, which contain type IV collagen with α3 chains, may have linear staining as well (62,67), which may induce tubulointerstitial injury.

Table 15.4 shows the serologic results in 46 patients with CGN who had 2+ or greater linear staining of GBMs by

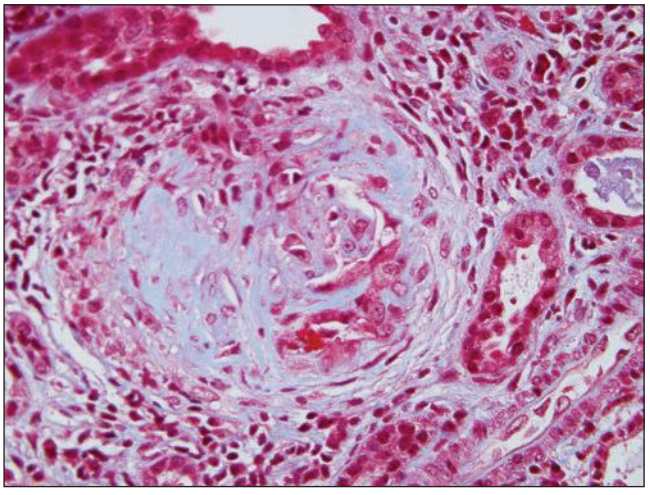


**FIGURE 15.18 Higher magnification of the same specimen as in Figure 15.16 showing slight residual cellularity in a mostly fibrotic crescent and a few residual glomerular cells in the adjacent segment of the tuft.** In the segment with residual cellularity, there is a small focus of red (fuchsinophilic) material representing slight residual fibrinoid necrosis. (Masson trichrome stain.)

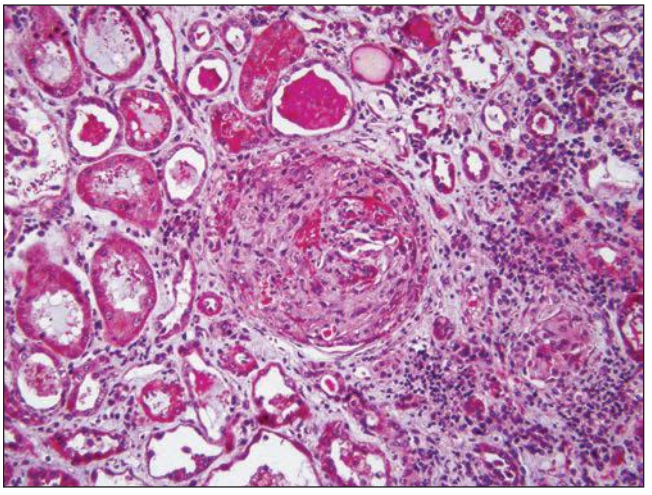


**FIGURE 15.20 Masson trichrome stain of subacute anti-GBM CGN with focal tubular epithelial flattening (simplification), focal tubular atrophy, diffuse interstitial edema, and marked focal interstitial infiltration of mononuclear leukocytes.** The lack of substantial blue staining in the interstitium indicates that the expansion is caused by edema rather than fibrosis. Contrast this with the bluer interstitium in Figure 15.16.

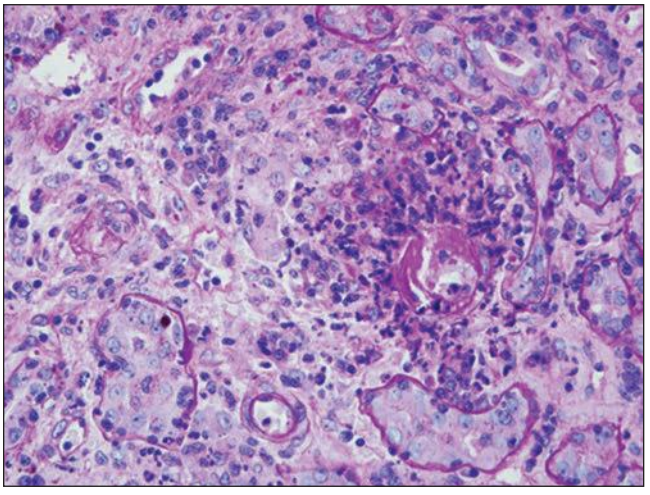


**FIGURE 15.21 Necrotizing vasculitis affecting a small interlobular artery in a patient with anti-GBM glomerulonephritis and ANCA.** Note the PAS-positive fibrinoid material in the vessel wall and the perivascular leukocyte infiltration with leukocytoclasia.

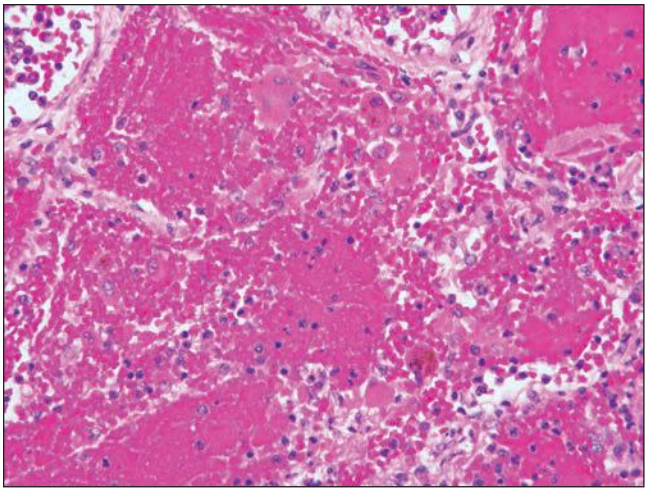


**FIGURE 15.23** Massive pulmonary intra-alveolar hemorrhage with a few hemosiderin-laden macrophages and scattered neutrophils in a patient with Goodpasture syndrome. (H&E.)

immunofluorescence microscopy. Enzyme-linked immunosorbent assay (ELISA) testing for anti-GBM was positive in 87% but negative in 13%. This is similar to another North American cohort of 32 patients with typical immunofluorescence microscopy and light microscopy features of anti-GBM disease who were 84% positive and 16% negative for anti-GBM antibodies by standard ELISA (36). However, when multiple highly sensitive assays are used, approximately 95% of patients with typical pathologic and immunofluorescence microscopy features of anti-GBM disease have positive serology for α3 chains of type IV collagen in at least one assay system although approximately 10% are negative in at least one assay (68). The serologically negative patients probably have CGN that is mediated by anti-GBM antibodies that are not detected in the standard ELISA, possibly because they had specificity for cryptic α3 chain determinants that were not displayed in the substrate (69) or had specificities for other GBM constituents, such as entactin (25).

Linear staining for IgM and IgA occurs, but is less common and rarely as intense as the staining for IgG (see Table 15.6) (31,33,36,66). This staining usually is less than 1+ intensity on a scale of 0 to 4+. However, rare patients with anti-GBM disease have linear glomerular staining only for IgA and have circulating IgA anti-GBM antibodies in the absence of IgG anti-GBM antibodies in the serum or deposited in glomeruli (36,70–74). In a series of 80 specimens, linear IgA-dominant staining was observed in one specimen (36). Borza et al. (72) reported an interesting patient with monoclonal IgA-kappa

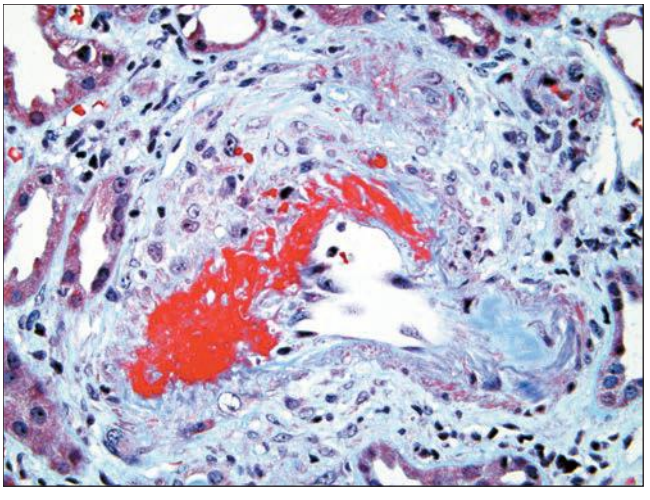


**FIGURE 15.22 Necrotizing vasculitis affecting an arcuate artery in a patient with anti-GBM glomerulonephritis and ANCA.** Note the fuchsinophilic (*red*) zone of fibrinoid necrosis. (Masson trichrome stain.)

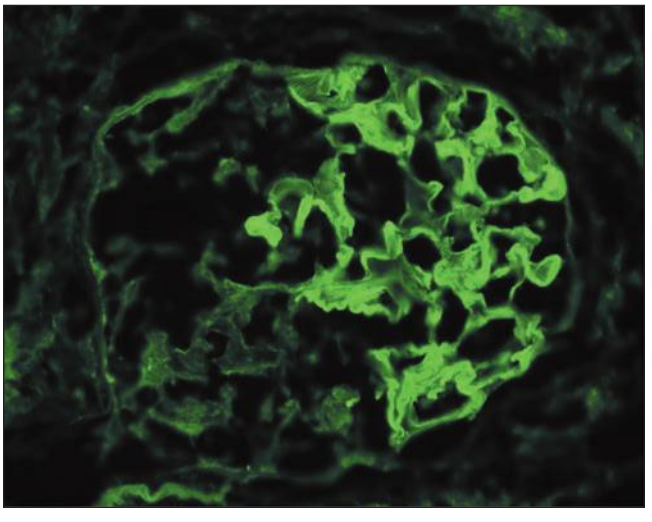


**FIGURE 15.24 Linear immunofluorescence for IgG along the glomerular capillary walls in a patient with anti-GBM glomerulonephritis.** The tuft is displaced to one side of the Bowman space by a crescent that does not stain for IgG. (FITC anti-IgG.)

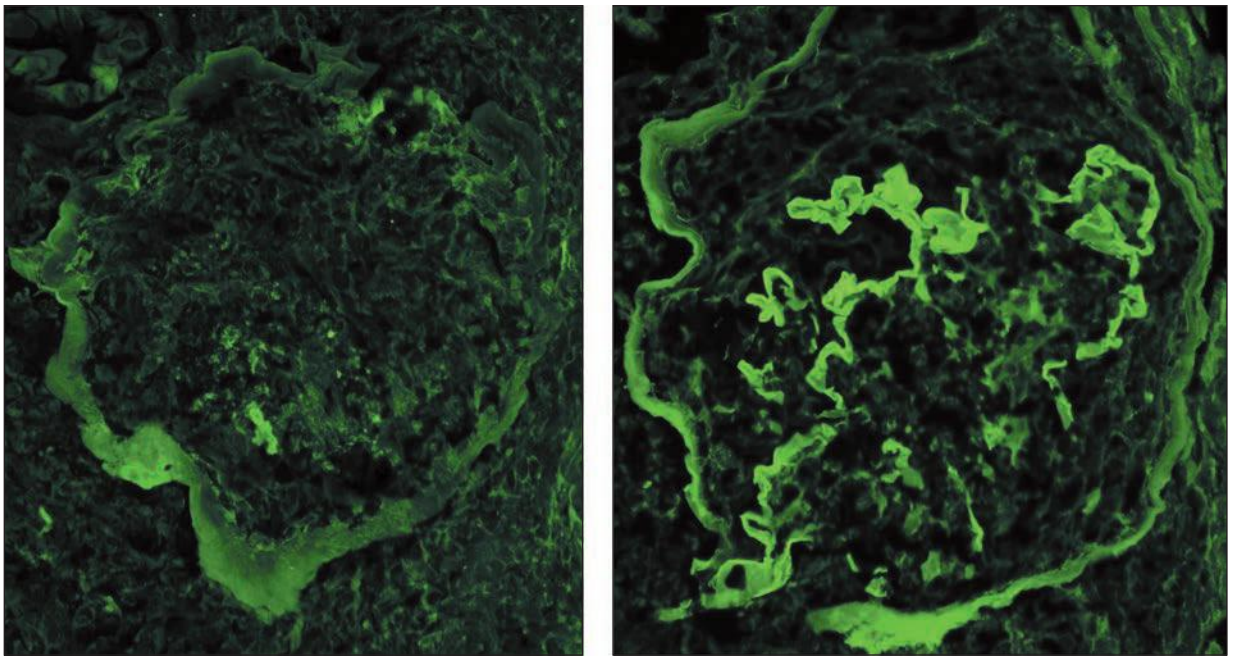



**FIGURE 15.25** These two levels of section of a glomerulus from a patient with anti-GBM glomerulonephritis show that at one level of section **(A)**, only a minute fragment of GBM is staining and could be missed, resulting in a misdiagnosis of pauci-immune glomerulonephritis, whereas at another level of section **(B)**, more fragments of GBM with linear staining for IgG are observed. (FITC anti-IgG.)

anti-GBM antibodies that were specific for the α1/α2 chains of type IV collagen. This patient not only developed end-stage renal disease caused by anti-GBM disease but also developed recurrent crescentic glomerulonephritis and pulmonary hemorrhage after renal transplantation.

The predominant IgG subclass in glomeruli in anti-GBM glomerulonephritis is IgG3 (66). In a study of 46 patients, linear GBM deposits were positive for IgG3 in 100%, IgG2 in 48%, IgG1 in 20%, and IgG4 in 15% (66). IgG3 also had the greatest average intensity. The subclass distribution of circulating anti-GBM antibodies is less restricted (66).

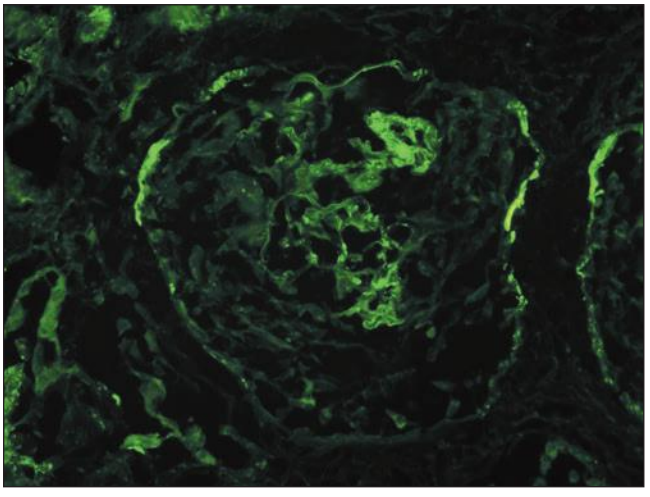


**FIGURE 15.26 Segmental low-intensity granular staining for C3 in the same specimen with anti-GBM glomerulonephritis illustrated in Figure 15.24.** The tuft is displaced to the center of the Bowman space by a large circumferential crescent that does not stain for C3. (FITC anti-C3.)

Although discontinuous linear or granular staining for C3 is observed in most specimens (see Fig. 15.26), glomerular staining for C3 is negative for some specimens. Wilson and Dixon (31) observed no glomerular staining for C3 in 4 of 22 specimens. We observed C3 staining in 96% of specimens with anti-GBM glomerulonephritis; however, the intensity of staining usually is much less that for IgG (see Table 15.6), and the distribution may be segmental rather than global.

Foci of glomerular necrosis and cellular crescents have irregular staining for fibrin (Figs. 15.27 and 15.28). These foci correspond to the fuchsinophilic (red) material that is highlighted by Masson trichrome staining (see Figs. 15.10A and 15.12). As crescents become fibrotic, they progressively lose staining for fibrin.

Linear staining of distal tubular basement membranes for IgG occurs in some patients with anti-GBM disease (Fig. 15.27). Tubular basement membrane staining typically is restricted to distal tubules that express the α3 chain of type IV collagen. For example, Lehman et al. (67) observed this in 79% of 47 patients, Andres et al. (62) in 50% of 18 patients, and Qu et al. (66) in 67% of 46 patients.

Rare specimens will have anti-GBM disease combined with another disease that has an additional distinctive pattern of glomerular immunofluorescence staining. The most common concurrent process, other than ANCA disease, is membranous glomerulonephritis (75–80). Membranous glomerulonephritis can be confirmed by electron microscopic demonstration of numerous subepithelial electron-dense deposits (Fig. 15.28A). With concurrent anti-GBM and membranous

**TABLE 15.6 Immunofluorescence microscopy findings in 58 patients with anti-GBM glomerulonephritis evaluated in the University of North Carolina Nephropathology Laboratory**

| | IgG | IgA | IgM | Kappa | Lambda | C3 | C1q |
|---|---|---|---|---|---|---|---|
| Percentage of specimens positive | 100 | 39 | 55 | 100 | 100 | 96 | 10 |
| Mean intensity when positive | 3.4+ | 0.9+ | 0.8+ | 2.9+ | 2.5+ | 1.6+ | 0.7+ |

glomerulonephritis, careful examination at high magnification reveals inner linear and outer granular staining (Fig. 15.28B). Both diseases may be present on the initial biopsy, or an initial process may show only one disease, usually anti-GBM disease, and a later biopsy shows concurrent disease.

Linear staining of glomerular and tubular basement membranes for IgG and other plasma proteins, which may be very intense, is a frequent feature of diabetic glomerulosclerosis and is not indicative of anti-GBM antibodies. Nondiagnostic, low-level, linear IgG staining of GBMs is observed in older patients, especially those with arteriosclerosis, and it is accentuated in autopsy material. Fivush et al. (81) also observed linear GBM staining for IgA in patients who had no evidence for anti-GBM disease.

Direct immunofluorescence microscopy of lung tissue from patients with anti-GBM disease reveals linear to discontinuous linear staining of alveolar capillary basement membranes, predominantly for IgG (Fig. 15.29A), with less frequent, more granular, and lower-intensity staining for C3 (32,82). Alveolar spaces often contain masses of fibrin that stain intensely with antifibrin antibodies (Fig. 15.29B). Interpretation of lung immunofluorescence microscopy results is more difficult than for renal immunofluorescence because the pattern of IgG staining is more irregular and is scattered diffusely through the tissue, rather than showing the well-defined localization to glomerular and tubular basement membranes that is observed in the kidney. Lung tissue also has more autofluorescence and more nonspecific background staining caused by tissue and plasma immunoglobulin than kidney tissue.

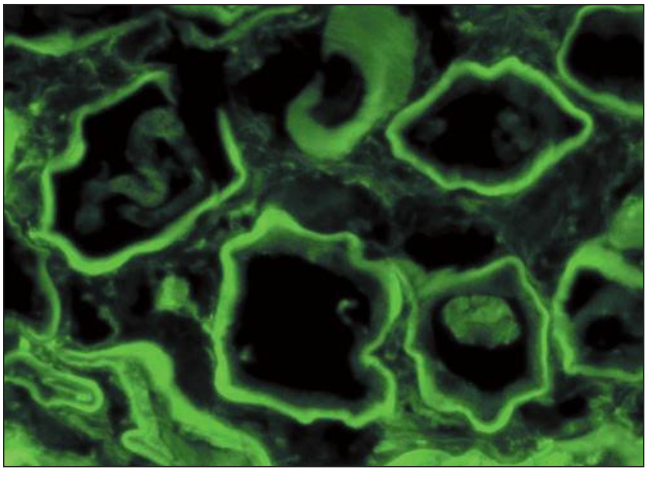


**FIGURE 15.27** Linear immunofluorescence for IgG along distal tubular basement membranes in a patient with anti-GBM glomerulonephritis.

## Electron Microscopy

The characteristic ultrastructural features of anti-GBM glomerulonephritis are segmental necrosis and crescent formation in the acute phase and sclerosis in the chronic phase (32,36,56,65). The ultrastructural appearance of anti-GBM CGN is indistinguishable from that of pauci-immune CGN, but it differs from immune complex glomerulonephritis by the absence of any discernible glomerular immune complex–type electron-dense deposits, except in specimens with concurrent anti-GBM and immune complex disease.

During the acute phase, there often is focally and segmentally variable endothelial swelling, mild lucent expansion of the subendothelial zone (lamina rara interna) (Fig. 15.30), rupture of GBMs (Figs. 15.31 and 15.32) and the Bowman capsule, focal effacement of epithelial foot processes, and accumulation of epithelial cells and macrophages in the Bowman space. Neutrophils may be present, especially in and adjacent to sites of necrosis and at sites of capillary endothelial denudation. Any profile of GBM or Bowman capsule basement membrane that comes to an end (see Figs. 15.31 and 15.32) is indicative of GBM rupture because the normal GBM is endless. The GBM extends uninterrupted around all the capillary loops and paramesangial regions, is continuous with the Bowman capsule at the hilum, and eventually transitions into the basement membrane of the proximal tubule. The breaks in GBMs that are observed by transmission electron microscopy can be identified rather dramatically by scanning electron microscopy of isolated glomeruli that have had the cells removed by detergents (83). Occasionally, neutrophils are identified in capillaries, especially at sites where there is disruption of GBMs (see Fig. 15.32).

Electron-dense fibrin tactoids are found within capillary lumens at sites of thrombosis, in zones of fibrinoid necrosis, and between crescent cells in the Bowman space (Fig. 15.33). The fibrin tactoids are very electron dense, irregular, curvilinear masses of polymerized fibrin that may have periodicity of approximately 10 nm. These accumulations of fibrin tactoids correspond to fuchsinophilic (red) deposits seen in Masson trichrome stains (see Fig. 15.12) and areas of positive staining for fibrin by immunofluorescence microscopy (Figs. 15.34 and 15.35).

The presence of immune complex–type electron-dense deposits in a patient with anti-GBM glomerulonephritis indicates a concurrent immune complex glomerulonephritis. The form of immune complex glomerulonephritis that most often accompanies anti-GBM glomerulonephritis is membranous glomerulonephritis with numerous subepithelial dense deposits (75–80) (see Fig. 15.28). In patients who have membranous glomerulonephritis before the onset of anti-GBM disease, the pathogenic link may be release of immunogenic GBM antigens

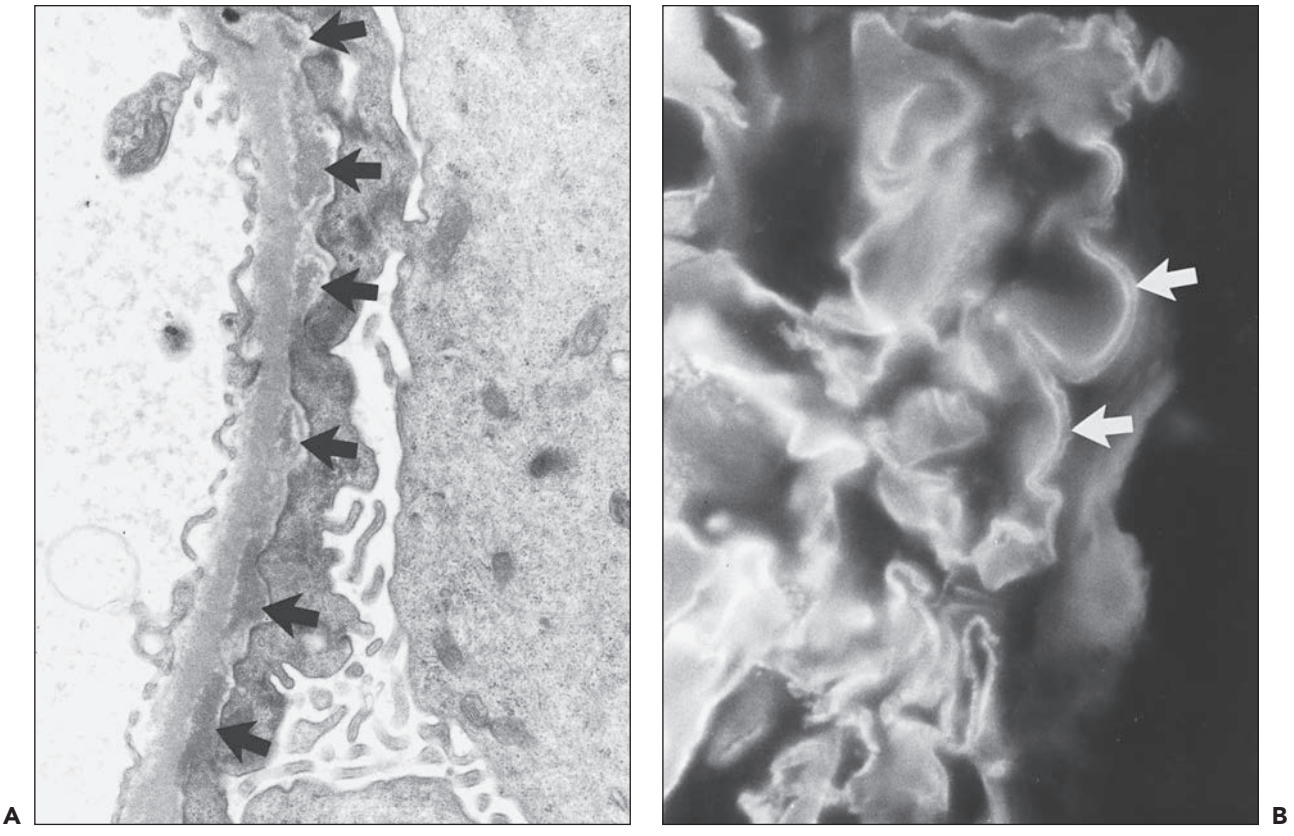



**FIGURE 15.28** **A:** Subepithelial electron-dense deposits (*arrows*) in a patient with concurrent membranous glomerulonephritis and anti–glomerular basement membrane glomerulonephritis (×9000). **B:** Double-contour capillary wall staining in the same patient. The inner linear staining is caused by anti–glomerular basement membrane antibodies bound to the glomerular basement membrane, and the outer finely granular staining is caused by the subepithelial deposits (*arrows*). (×1200.) (From Jennette JC, Lamanna RW, Burnette JP, et al. Concurrent anti–glomerular basement membrane antibody and immune complex mediated glomerulonephritis. *Am J Clin Pathol* 1982;78:381.)

by membranous glomerulonephritis that induce an anti-GBM immune response. Care should be taken not to misinterpret electron-dense fibrin tactoids at sites of necrosis or electron-dense insudative lesions at sites of sclerosis as immune complex–type electron-dense deposits. If dense material at sites of severe injury suggests immune complex deposition, try to confirm this in the most structurally intact glomerular segments.

Unbanded collagenous matrix material progressively replaces foci of glomerular necrosis and the cellular elements of crescents as the glomerulonephritis evolves into a chronic phase. In more intact segments, GBMs become thickened and wrinkled and the mesangial matrix expands.

## ETIOLOGY AND PATHOGENESIS

The pathogenesis of anti-GBM glomerulonephritis begins with the induction of the anti-GBM autoimmune response followed by the mediation of vascular inflammation. Although the initiation of the inflammatory process is different for anti-GBM disease, immune complex disease, and ANCA disease, the pathogenic pathways eventually converge to a substantial degree with a final common pathway of injury (Fig. 15.36) that results in acute glomerular inflammation and crescent formation. The following review will first consider the development of CGN in general and then the events that are specific to anti-GBM glomerulonephritis. The pathogenesis of ANCA disease will be reviewed in detail in Chapter 16.

### Mediation of Crescentic Glomerulonephritis

There has been controversy about the origin of the cells that constitute crescents. Some investigators have contended that the major cell type is epithelial, and others have argued that the major cell type is derived from monocytes. Part of the problem derives from the fact that the composition of crescents varies over time in a given disease (e.g., macrophages initially followed by epithelial cells and fibroblasts) and varies among pathogenetically different human diseases and experimental disease models. For example, crescents in immune complex–mediated glomerulonephritis are composed predominantly of epithelial cells, whereas crescents in severe necrotizing anti-GBM glomerulonephritis and ANCA glomerulonephritis, which have extensive disruption of the Bowman capsule, have a high content of macrophages (60,84).

Because of the histologic appearance of the cells within crescents, they were initially considered to be composed of epithelial cells (12–14). This conclusion is supported by electron microscopic evidence, which has demonstrated distinctive

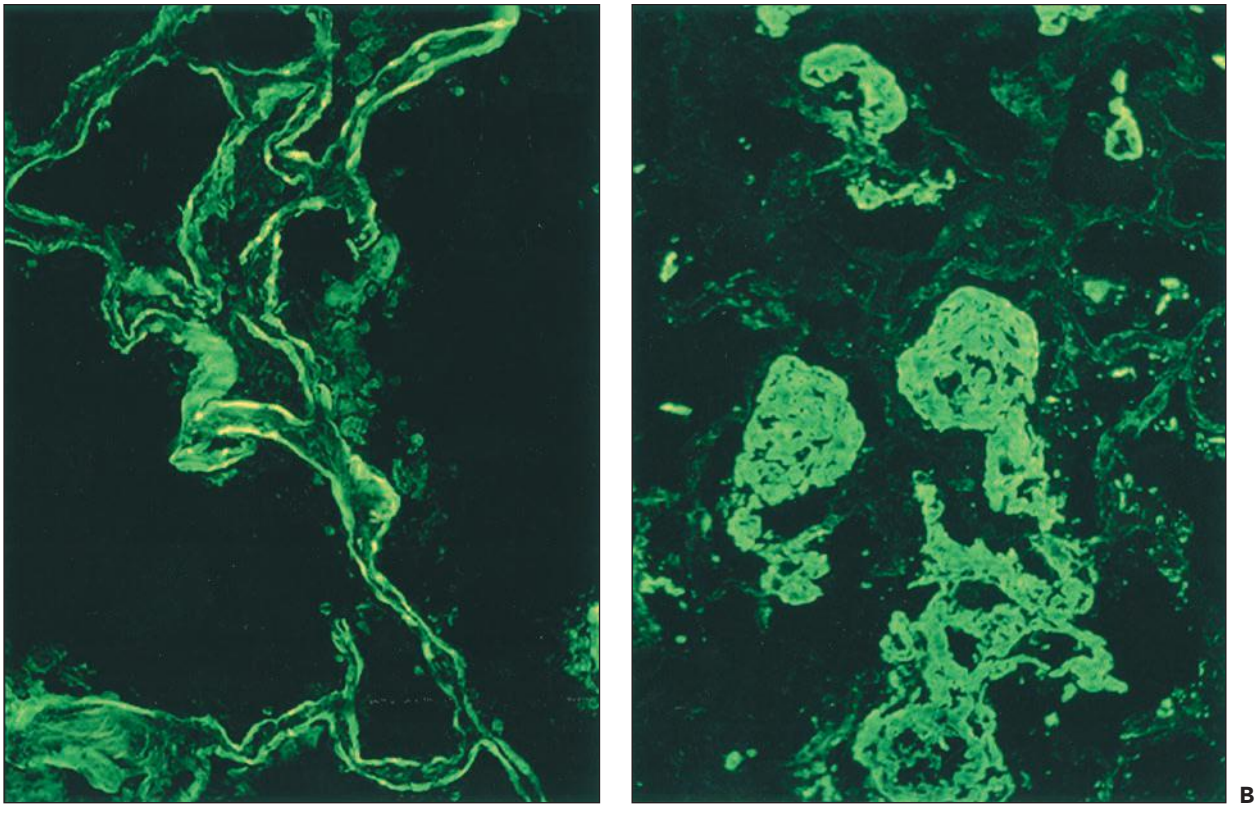



**FIGURE 15.29** Linear immunofluorescence for IgG along pulmonary alveolar capillary basement membranes in a patient with Goodpasture syndrome **(A)** and irregular intra-alveolar staining for fibrin in the lung of the same patient **(B)**.

ultrastructural epithelial features such as cell junctions in cells within crescents and the absence of macrophage features such as distinctive lysosomes (58,85–87). Electron microscopy revealed an association between crescent formation and breaks in GBMs and documented the presence of fibrin within crescents. Fibrin within crescents also has been observed by light microscopy and immunofluorescence microscopy (88).

Immunohistologic studies of human renal biopsy specimens have confirmed the epithelial phenotype of most cells in some but not all mature cellular crescents (60,84,89–92), although even when epithelial cells predominate, at least a few cells are macrophages and other leukocytes (60,84,89,90,93). Even when monocytes and macrophages are not the major cell type comprising mature crescents, they probably are important, if not necessary, for mediating crescent formation. In general, macrophages have accounted for a higher proportion of crescent cells in experimental animal models of CGN than in human CGN, particularly in experimental models of disease induced by anti-GBM antibodies. Crescents in patients with anti-GBM glomerulonephritis and ANCA glomerulonephritis, especially when there is extensive disruption of the Bowman capsule, have a higher proportion of macrophages than crescents in patients with immune complex glomerulonephritis, which usually has little or no disruption of the Bowman capsule (60,84). For example, in an immunohistologic study of human CGN by Boucher et al. (84), 55% to 95% of crescent cells were epithelial cells when the Bowman capsule was intact. When the Bowman capsule was ruptured, epithelial cells were outnumbered by mononuclear leukocytes, especially macrophages. Glomeruli in patients with CGN who have extensive disruption of the Bowman capsule also are more likely to have a periglomerular granulomatous reaction (59,94).

One of the earliest detailed hypotheses about the pathogenesis of crescent formation was formulated by Arnold Rich in the 1950s (95). He proposed that crescents resulted from the proliferation of glomerular capsular epithelial cells into clotted blood within the Bowman space. Fibrin often can be demonstrated between the cells of crescents by immunofluorescence microscopy (see Fig. 15.35). Prevention of crescent formation in experimental models of CGN by anticoagulation and defibrinogenation using pit viper venom, streptokinase, or tissue plasminogen activator has supported the importance of fibrin in the morphogenesis of crescents (96–99). Within the Bowman capsule, fibrin probably acts as a scaffolding and stimulus for the proliferation of epithelial cells, as it does at sites of wound healing. The absence of fibrin in the Bowman space may prevent crescent formation because of the absence of supporting and mitogenic effects. Prevention of crescent formation, however, does not reduce inflammatory injury to glomerular capillaries, because crescent formation is a manifestation rather than a cause of glomerular inflammation.

The ubiquitous presence of fibrin in the Bowman space in CGN indicates that there has been a breach of capillary integrity that has allowed plasma proteins, including coagulation proteins, to enter the Bowman space. As a result of destructive

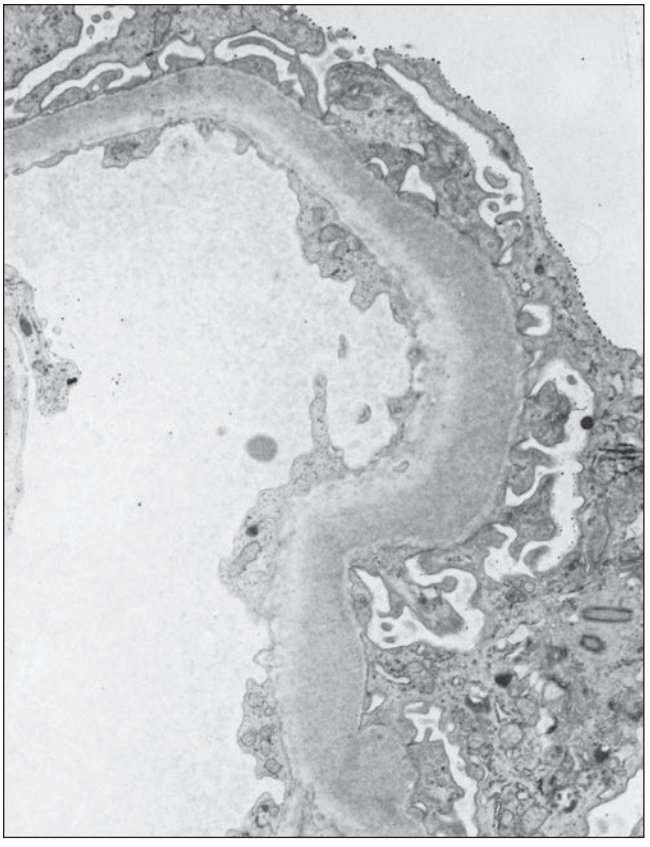


**FIGURE 15.30 Electron micrograph of tissue from a patient with Goodpasture syndrome.** No electron-dense immune deposits are seen, but there is an electron-lucent zone on the endothelial side of the glomerular basement membrane. (×10,000.) (Courtesy of Dr. T. Antonovych, Armed Forces Institute of Pathology, Washington, DC.)

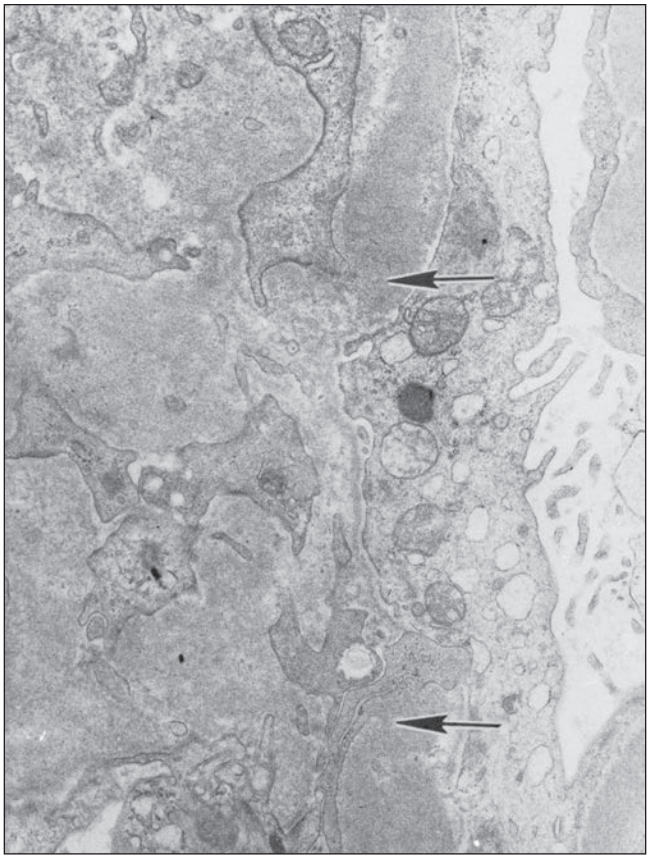


**FIGURE 15.31 Glomerular basement membrane from a patient with Goodpasture syndrome showing a break in the continuity between the two *arrows*.** (×10,000.) (Courtesy of Dr. T. Antonovych, Armed Forces Institute of Pathology, Washington, DC.)

inflammation, the coagulation proteins contact procoagulant, or thrombogenic, surfaces and molecules, such as those generated by tissue necrosis (e.g., collagen fragments) and activated cells (e.g., epithelial cells, monocytes, macrophages) including tissue factor (100–104). Epithelial cells and macrophages release procoagulant tissue factors (102–104). Once fibrin polymerizes within the Bowman space, it is not removed as efficiently by fibrinolytic mechanisms as is fibrin in vascular thrombi, and it persists and participates in crescent formation (104,105).

The presence of fibrin in the Bowman space is an effector of crescent formation and a marker that other molecular and cellular inflammatory mediator systems have been activated within the Bowman space. These activated molecular and cellular mediator systems generate factors that stimulate epithelial proliferation, such as thrombin generated by coagulation (106) and growth factor cytokines released by monocytes and platelets (107). Cytokines, such as epidermal growth factor, interleukin-1, and interleukin-2, probably play a role in orchestrating crescent formation and evolution (107–111). The epithelial cells within crescents form a relatively cohesive mass by interactions between up-regulated surface membrane adhesion molecules, such as integrins and immunoglobulin superfamily adhesion molecules (112,113).

Transmission (see Figs. 15.31 and 15.32) and scanning (83) electron microscopy (see Fig. 15.31) have demonstrated that CGN has holes in the GBMs. These holes probably are the doorways for molecular and cellular inflammatory mediators to enter the Bowman space, resulting in stimulation of epithelial proliferation. In severe necrotizing glomerulonephritis, which occurs most often with anti-GBM glomerulonephritis and ANCA glomerulonephritis, gaps in the Bowman capsule can allow leukocytes, especially macrophages, to enter the Bowman space from the interstitium. Perforation of the Bowman capsule may be caused by leukocytes within the Bowman space and by periglomerular inflammatory cells (114).

Activated neutrophils and monocytes are the likely culprits for causing the perforations through GBMs and the Bowman capsule that are associated with crescent formation. Proinflammatory and injurious factors generated by activated neutrophils and monocytes include granule enzymes (e.g., elastase, collagenase, proteinase 3, cathepsins, myeloperoxidase), oxygen metabolites (e.g., superoxide, hydroxyl radical, hydrogen peroxide, hypochlorous acid), lipid metabolites (e.g., eicosanoids, platelet-activating factor), and cytokines (e.g., interleukin-1, interleukin-8, tumor necrosis factor, transforming growth factor–β) (115,116). Proteinases such as elastase, cathepsin G, PR3, and collagenase can digest basement

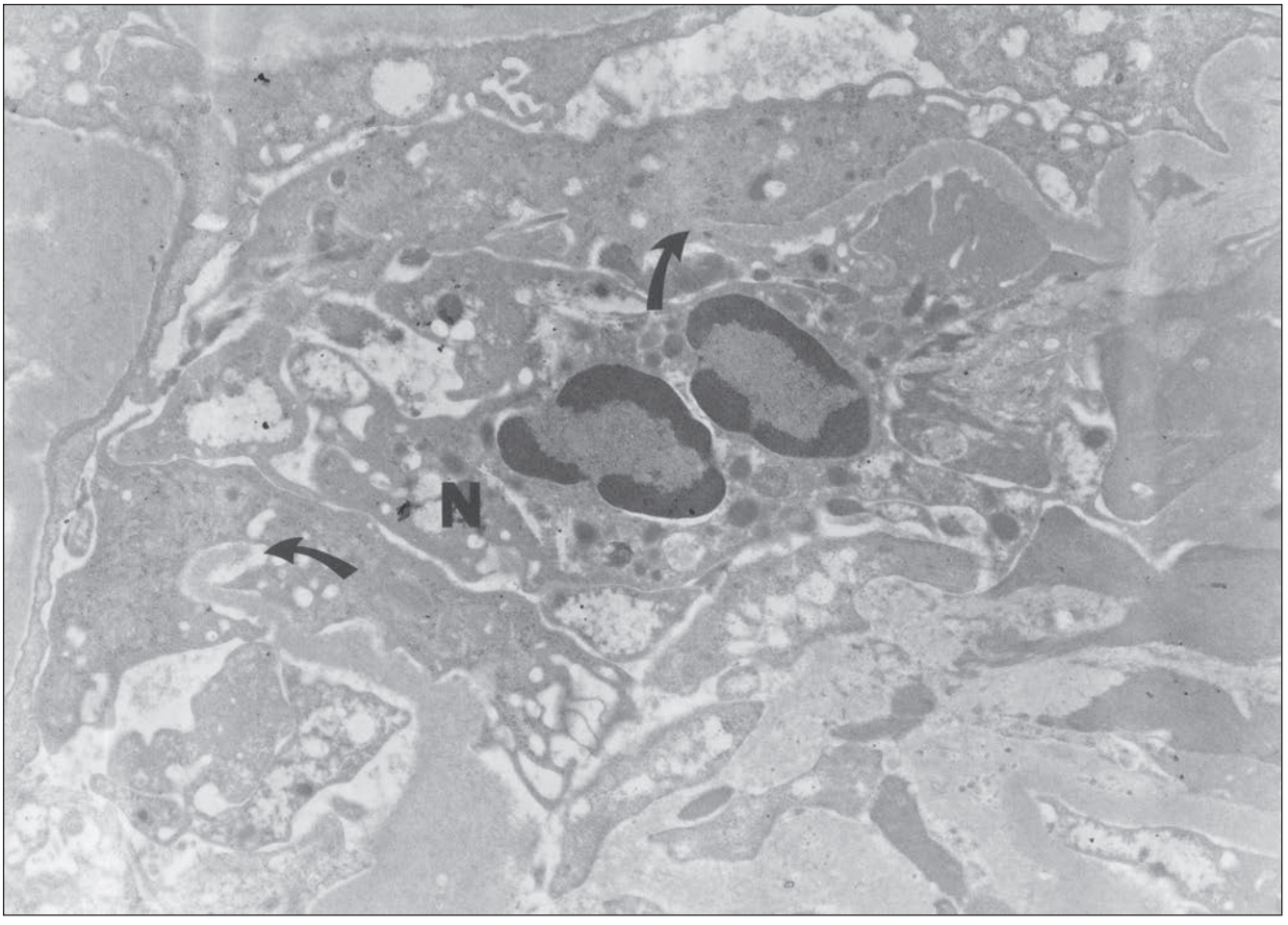



**FIGURE 15.32 Electron micrograph of a glomerulus from a patient with anti-GBM glomerulonephritis demonstrating a break in the glomerular basement membrane (between curved *arrows*).** A neutrophil (N) with two nuclear lobes is in the gap between the GBM ends. (×6000.)

membranes in glomerular capillaries and the Bowman capsule. This is facilitated by inactivation of antiproteinases by oxygen metabolites.

Over time, cellular crescents resolve through apoptosis or evolve into fibrocellular and fibrotic (see Figs. 15.16 and 15.17) crescents (87,107,112,117,118). This process involves collagen synthesis by epithelial cells (119) and, in some patients, collagen synthesis by fibroblasts that infiltrate from the periglomerular interstitium through gaps in the Bowman capsule (120,121). In CGN, fibronectin, initially derived from the plasma but later from epithelial cells, appears to be an important fibroblast chemotactic factor (117), and transforming growth factor–β enhances collagen production and fibrosis (107,112,122,123). Type IV collagen, laminin, fibronectin, and thrombospondin are matrix materials in crescents that are produced by epithelial cells (124,125), and type I and type III collagen are matrix materials produced by fibroblasts from the interstitium (120,121). Transforming growth factor–β enhances fibrosis and inhibits epithelial proliferation, promoting transformation of epithelial crescents to fibrous crescents (107). As the fibrosis develops, the epithelial cells are removed by apoptosis (118). Macrophages play a major role in orchestrating the progression from acute inflammatory to chronic sclerosing glomerular lesions (126).

For neutrophils and monocytes to attack and perforate glomerular capillary walls during the initiation phase of CGN, the stereotypic sequence of events that mediates all forms of acute microvascular inflammation must be initiated. This involves leukocyte adhesion to glomerular capillary followed by activation with release of injurious products that disrupt the capillary walls (116). Many different pathogenic mechanisms share this final common pathway of microvascular injury. The three major factors that initiate this final common pathway of injury in patients with CGN are anti-GBM antibodies, immune complexes, and ANCA.

## Etiology and Pathogenesis of Anti-GBM Disease

In 1967, Lerner et al. (10) reported that the serum of kidneys of patients with Goodpasture syndrome and of some patients with CGN without pulmonary hemorrhage contained antibodies that reacted with the GBM of humans and animals. They also demonstrated that these anti-GBM antibodies caused glomerulonephritis when injected into monkeys (10).

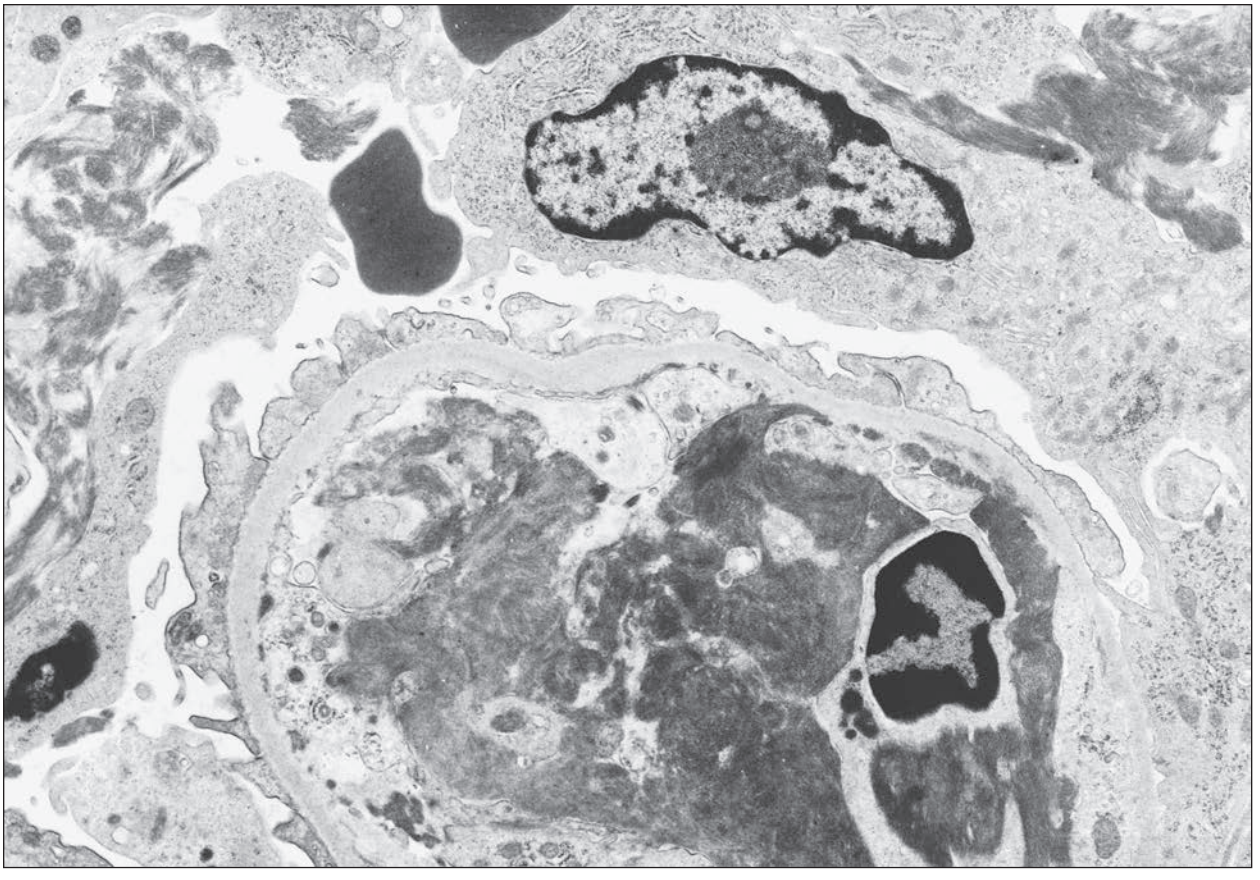


**FIGURE 15.33 Capillary lumen containing electron-dense fibrin in a patient with anti–glomerular basement membrane crescentic glomerulonephritis.** There are also accumulations of fibrin in the Bowman space between the epithelial cells of a crescent. (×5000.)

Even before the pathogenic potential of anti-GBM antibodies was recognized in humans, it had been documented in experimental animals (124–128). The pathologic and experimental data support a pathogenic mechanism that entails binding of anti-GBM antibodies to the NC1 domain of type IV collagen in GBMs, activation of complement, recruitment of neutrophils and monocytes, destruction of glomerular capillary walls, and initiation of the final common pathway of crescent formation that was reviewed earlier.

The pathogenicity of anti-GBM antibodies is illustrated in experimental models and in patients with Alport hereditary nephritis who develop anti-GBM glomerulonephritis in transplanted kidneys (129–132). The allografts express an antigen that is not expressed in the defective GBMs of Alport patients (54,55). These patients have a mutation in the genes for α3, α4, or α5 chain of type IV collagen. No matter which chain is defective, the whole type IV collagen molecule cannot be assembled, and thus the anti-GBM epitope on the α3 chain is not displayed to the immune system (54,55). The transplant recipient develops antibodies to this "foreign" GBM antigen (alloantigen); the antibodies bind to the GBMs of the renal allograft and induce glomerulonephritis. This is compelling evidence for the pathogenicity of an anti-GBM immune response. However, only a few hereditary nephritis patients who receive renal transplants develop anti-GBM disease in the allograft, which suggests that a genetic susceptibility must exist to allow the development of the anti-GBM immune response. The anti-GBM antibodies that develop in transplanted patients with hereditary nephritis are accessible on the surface of type IV collagen, whereas those in patients with spontaneous anti-GBM disease are cryptic epitopes that are inaccessible to binding to anti-GBM antibodies (55). This suggests that some synergistic pathogenic process must alter the GBM to allow the development of anti-GBM disease.

The search for the target antigen of the anti-GBM antibodies was progressively narrowed to the α3 chain in the C-terminal noncollagenous globular domain of type IV collagen (α3NCI) (54,133–137) and to a lesser degree, on the α5NC1 (55). In patients with anti-GBM disease, approximately 1% of total IgG is anti-NC1 antibodies, and 90% of these antibodies are specific for α3NC1 chains, although 80% of patients have low levels of antibodies to other α chains (136). The target antigen of anti-GBM is in the kidney, in the lung, and in several other tissues, such as the choroid plexus and eye (138).

Collagen type IV has a lattice-like structure composed of triple helices of α3, α4, and α5 collagen chains, terminating in short globular noncollagenous domains (NC1 and NC2). The αNCI epitopes are sequestered within the cross-linked structure of the collagen and are not available to initiate an immune response or interact with anti-GBM autoantibodies and thus are cryptic epitopes. A pathogenic event, such as disruption of a

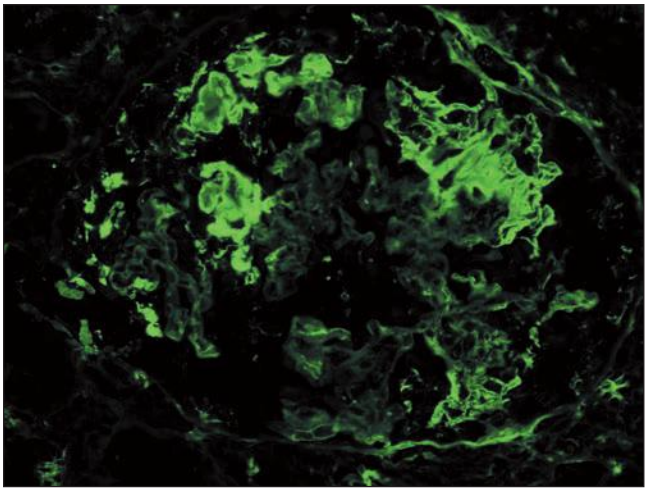


**FIGURE 15.34** Segmental irregular immunofluorescence staining for fibrin in a patient with anti-GBM glomerulonephritis corresponding to foci of segmental necrosis and early crescent formation. (FITC antifibrin.)

molecular bond, is required to produce a modified conformation (conformer) that incites the autoimmune response and allows binding of the autoantibodies and initiation of pathogenic events. Anti-GBM disease thus is a "conformeropathy" (55).

Although autoantibodies against conformational collagen IV epitopes clearly are very important in pathogenesis, patients with anti-GBM disease also appear to have antibodies directed against linear type IV collagen epitopes (139).

The pathogenic importance of an alteration in the quaternary conformation of type IV collagen is consistent with the observation that certain environmental factors are associated with risk for anti-GBM disease. A role for hydrocarbons in the induction of anti-GBM disease is suggested by patients in whom an intense exposure to hydrocarbons, such as glue or solvent sniffing, is accompanied by the development of anti-GBM disease (42–44). This finding raises the possibility that the solvents expose or alter type IV collagen in GBMs resulting in the exposure of cryptic epitopes, causing an immune response to the previously sequestered antigens and accessibility to binding by pathogenic autoantibodies (54,55). The antigen specificity is identical in patients with Goodpasture syndrome compared with those with anti-GBM glomerulonephritis without associated pulmonary hemorrhage or pulmonary hemorrhage without glomerulonephritis (6). Factors other than the antigen specificity of the autoantibodies therefore must be responsible for these different phenotypes of anti-GBM disease. Hydrocarbon exposure and cigarette smoking may influence the development of pulmonary involvement (38,43,140,141). In an analysis of 51 patients with anti-GBM disease by Donaghy and Rees (141), all 37 patients who smoked cigarettes had pulmonary and renal disease, but only 2 of 10 nonsmokers had pulmonary involvement. This suggests that hydrocarbons or some other noxious component of the smoke predisposed the lung tissue to attack by the anti-GBM antibodies. Once again, a possible mechanism is damage to alveolar capillary basement membranes, for example, by toxic oxygen radicals, resulting in the exposure of pathogenic cryptic epitopes. Alternatively, injury to pneumocytes could result in production of altered basement membranes or greater access to basement membranes by autoantibodies in the plasma.

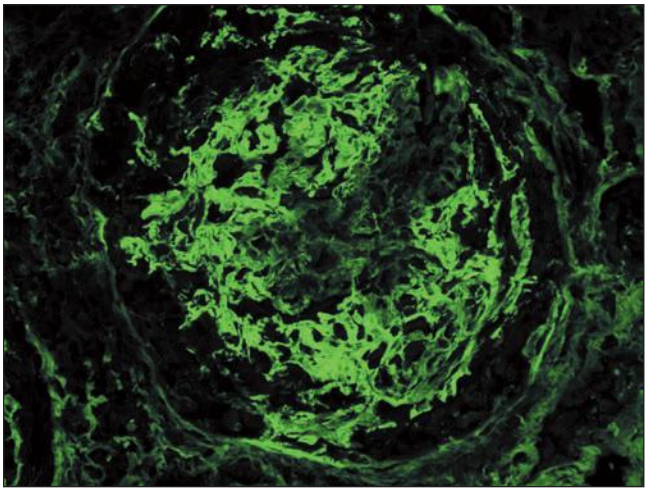


**FIGURE 15.35** Irregular immunofluorescence staining for fibrin in a large circumferential crescent in a patient with anti-GBM CGN. (FITC antifibrin.)

An association between extracorporeal shock wave lithotripsy and anti-GBM disease is rare but intriguing (142,143). Conceptually, shock waves could disrupt the GBM collagen to produce the pathogenic conformers.

Conceptually, anti-GBM antibodies must be present prior to the onset of anti-GBM disease. In fact, many healthy individuals appear to have low titers of natural anti-GBM autoantibodies that can only be detected by highly sensitive analytical methods (144). Another study demonstrated preexisting antibodies in patients who developed anti-GBM disease (145). Serum samples from the Department of Defense Serum Repository from 30 patients prior to the onset of anti-GBM disease and from 30 matched healthy controls were tested for anti-GBM antibodies. Low levels of anti-GBM antibodies were detected in at least one serum sample prior to disease onset in 70% of anti-GBM patients versus 17% of controls ($P < 0.001$). Only anti-GBM patients had detectable anti-GBM levels in multiple samples before diagnosis (50% vs. 0%, $P < 0.001$). The shift from nonpathogenic to pathogenic anti-GBM antibodies may result from a qualitative or quantitative shift from preexisting natural to pathogenic autoantibodies.

Genetic factors appear to play a role in susceptibility to anti-GBM disease. This is supported by the strong association between anti-GBM disease and DR and DQ human leukocyte antigens, including in the patients who develop anti-GBM disease following lithotripsy (146–148). This association suggests a role for antigen-specific T cells in either the immunogenesis or the pathogenesis of anti-GBM disease, or both. HLA-DRB1*1501 is strongly associated with anti-GBM disease (147,148). In Chinese patients, the combined presence of DRB1*1501 and absence of DPB1*0401 might have an even higher risk to anti-GBM disease than HLA-DRB1*1501 alone (148) suggesting a protective effect by DPB1*0401.

There is evidence that T lymphocytes and macrophages are involved in the induction and mediation of anti-GBM disease (149–152). Peripheral $CD4^+$ T cells in anti-GBM patients

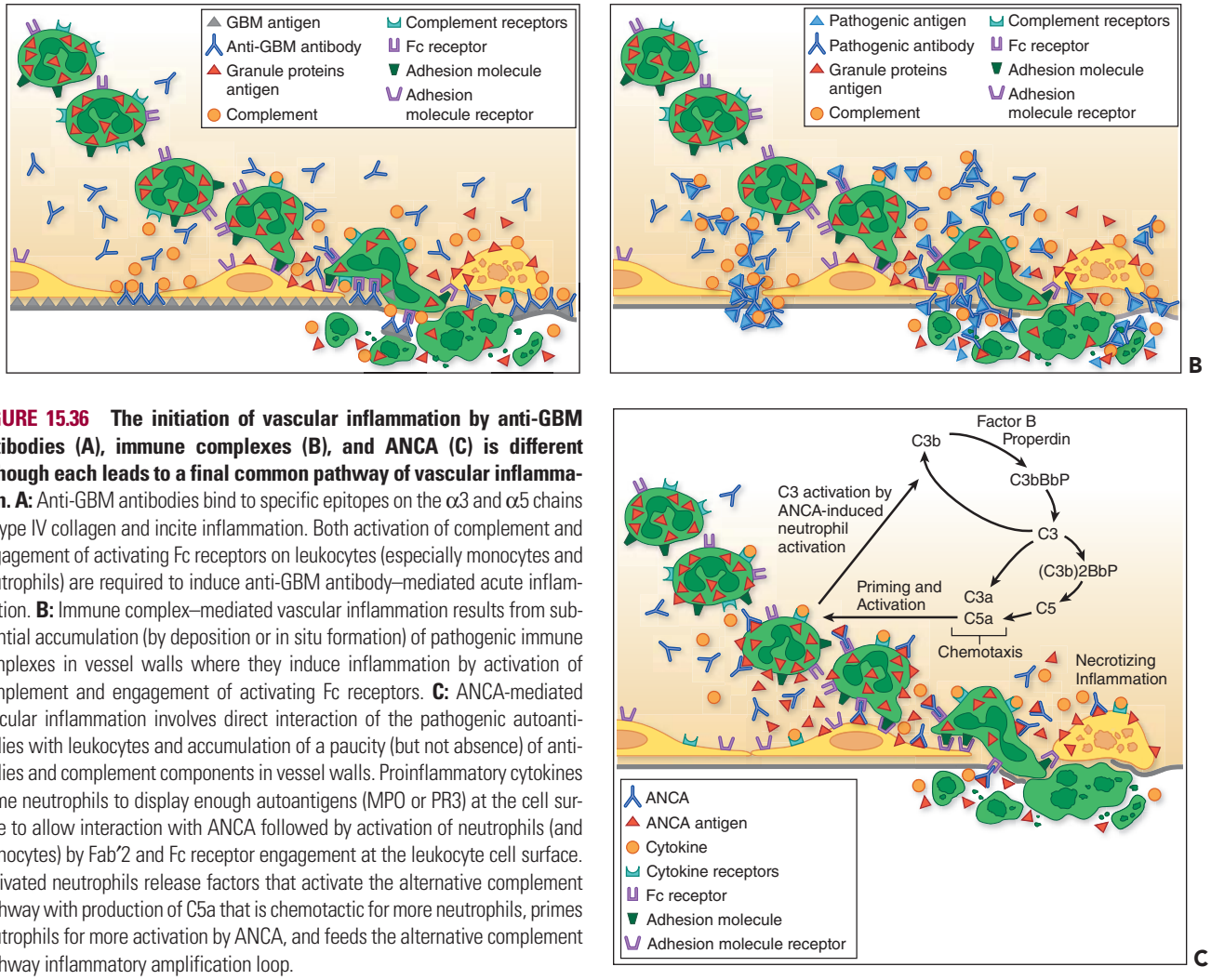



**FIGURE 15.36 The initiation of vascular inflammation by anti-GBM antibodies (A), immune complexes (B), and ANCA (C) is different although each leads to a final common pathway of vascular inflammation. A:** Anti-GBM antibodies bind to specific epitopes on the α3 and α5 chains of type IV collagen and incite inflammation. Both activation of complement and engagement of activating Fc receptors on leukocytes (especially monocytes and neutrophils) are required to induce anti-GBM antibody–mediated acute inflammation. **B:** Immune complex–mediated vascular inflammation results from substantial accumulation (by deposition or in situ formation) of pathogenic immune complexes in vessel walls where they induce inflammation by activation of complement and engagement of activating Fc receptors. **C:** ANCA-mediated vascular inflammation involves direct interaction of the pathogenic autoantibodies with leukocytes and accumulation of a paucity (but not absence) of antibodies and complement components in vessel walls. Proinflammatory cytokines prime neutrophils to display enough autoantigens (MPO or PR3) at the cell surface to allow interaction with ANCA followed by activation of neutrophils (and monocytes) by Fab′2 and Fc receptor engagement at the leukocyte cell surface. Activated neutrophils release factors that activate the alternative complement pathway with production of C5a that is chemotactic for more neutrophils, primes neutrophils for more activation by ANCA, and feeds the alternative complement pathway inflammatory amplification loop.

are responsive to the same autoantigen of α3NC1 that is the target for anti-GBM antibodies (151). Because the anti-GBM immune response is predominantly IgG, T-cell help must be involved for the shift to γ heavy-chain usage. T lymphocytes are found at sites of glomerulonephritis, but this could be owing to the recruitment of T cells with no specificity for GBM as part of the innate inflammatory response to injury. A more direct role is suggested by experimental models of anti-GBM disease in which T lymphocytes seem to be critically important for induction of injury (150–166).

Animal models have helped elucidate pathogenetic mechanisms involved in anti-GBM disease. Many species have been used, but mice and rats have been used most often (152–167). Nephrotoxic models are induced by intravenous injection of anti-GBM antibodies (nephrotoxic antibodies) from one species (e.g., rabbits or sheep) into another species (e.g., rats or mice) causing two phases of glomerular injury. The first phase (the heterologous phase) is transient and is the direct effect of the heterologous anti-GBM antibodies binding to the GBM. The second phase (the autologous phase) is the result of production of antibodies in the recipient animal against the foreign anti-GBM antibodies. These antibodies bind to the foreign anti-GBM antibodies that already are planted in the glomerular capillary walls. Thus, the secondary autologous phase is more like immune complex disease than human anti-GBM disease. Although this difference between human anti-GBM glomerulonephritis and glomerulonephritis induced by heterologous anti-GBM antibodies must be kept in mind, this model is a useful tool for investigating the role of various inflammatory mediators in the induction of glomerulonephritis.

Mice that are deficient in C3 and C4 are protected from the development of glomerulonephritis after injection of heterologous anti-GBM, suggesting an important role for both the classic and the alternative complement pathway activation in this model (161). In accord with this animal model observation, there is evidence for complement activation in patients with active anti-GBM disease (167). Although plasma C3 and C4 levels are normal, levels of C5a and SC5b-9 were increased in plasma in 15% and 30% of patients respectively, and they were increased in urine from almost all patients (100% and 92%). The levels of plasma SC5b-9 and urinary C5a were

positively correlated with the serum creatinine at presentation ($P$ = 0.01, $P$ = 0.02, respectively) and the percentage of glomerular crescents ($P$ = 0.005; $P$ = 0.005, respectively) (168).

Once IgG binds to antigen, the Fc region can engage Fc receptors on leukocytes, resulting in stimulation or inhibition of inflammatory events. The absence of the IgG Fc receptors that cause activation of inflammation when engaged (i.e., Fc gamma RIII) results in less severe anti-GBM antibody–mediated disease in mice, whereas the absence of IgG Fc receptors that cause inhibition of inflammation when engaged (i.e., Fc gamma RIIb) leads to more severe disease (162–164). This exacerbation is the result of the absence of FcgRIIb on myeloid cells and results in more glomerular neutrophils and crescents in nephrotoxic nephritis (164).

In some respects, the nephrotoxic model, especially in the autologous phase, is more analogous to immune complex disease than to anti-GBM disease. Alternative models of anti-GBM disease have been developed using direct immunization of mice and rats with GBM antigens. For example, Sado et al. (165,166) and Kohda et al. (167) have induced anti-GBM glomerulonephritis and pulmonary hemorrhage in rats immunized with bovine GBM. Anti-GBM glomerulonephritis could be induced by the passive transfer of anti-GBM antibodies from immunized to naïve rats, supporting the ability of antibody alone to cause CGN (166). Anti-GBM glomerulonephritis and pulmonary hemorrhage have been induced in rats by injection of monoclonal anti-GBM antibodies (167). These studies also demonstrated that the severity of renal and pulmonary disease was dependent on the titer and subclass of nephritogenic anti-GBM antibodies.

Kalluri et al. (155) and Hopfer et al. (154) induced anti-GBM glomerulonephritis by immunizing susceptible strains of mice with the noncollagenous domain of the α3 chain of type IV collagen. Lymphocytes or anti-GBM antibodies were harvested from mice that developed glomerulonephritis and were transferred into syngeneic recipient mice. Transfer of both antibodies and T cells resulted in glomerulonephritis; however, transfer of the anti-GBM antibodies alone failed to cause glomerulonephritis. These observations suggest that anti-GBM antibodies required assistance from T lymphocytes to induce disease (155). This may be related to the production by the activated T cells of cytokines that augment the inflammatory events (154).

Wu et al. (152,153) have reported even more compelling evidence for the involvement of T cells in the induction of anti-GBM glomerulonephritis in experimental animals. They immunized rats with a synthetic peptide for the noncollagenous domain (NC1) or the α3 chain of type IV collagen. They generated from these immunized rats a $CD4^+$ T-cell line with specificity for this antigen, which thus had anti-GBM specificity. They injected these anti-GBM–specific T cells into unimmunized rats and observed the development of glomerulonephritis with crescents but no immunoglobulin deposits (152). Thus, at least in this animal model, T cells with specificity for GBM antigens appear to be nephritogenic. Wu et al. (153) also were able to induce severe CGN by immunizing rats with a synthetic peptide that represented a single T-cell epitope.

Even though some effector T cells may be involved in mediating or facilitating anti-GBM injury, other regulatory T cells may be able to suppress the development of anti-GBM disease. Wolf et al. (157) have provided evidence for this in a mouse model of accelerated anti-GBM glomerulonephritis that was induced by injecting rabbit anti-GBM into mice that had been immunized against rabbit IgG. Mice that receive regulatory $CD4^+/CD25^+$ T cells during the immunization with rabbit IgG but before the injection of anti-GBM developed less severe disease than mice that received $CD4^+/CD25^-$ T cells. This observation suggests that regulatory T cells are involved in maintaining tolerance for GBM antigens and that a disturbance in regulatory T cells may be involved in the induction of the anti-GBM autoimmune response. The relevance of this experimental animal observation to human disease is supported by the observation of Salama et al. (156) that a population of $CD25^+$ T cells emerges in patients following acute anti-GBM disease and is able to diminish the responsiveness of GBM-specific effector T cells.

## DIFFERENTIAL DIAGNOSIS

The two major clinical presentations for anti-GBM disease are pulmonary-renal syndrome and rapidly progressive glomerulonephritis without pulmonary hemorrhage. Isolated pulmonary hemorrhage is a much less common presentation. The differential diagnosis for pulmonary-renal vasculitic syndrome is analogous to that for CGN and includes anti-GBM disease (Goodpasture syndrome), immune complex disease, and pauci-immune disease (169). Goodpasture syndrome accounts for only a minority of patients with a presentation of concurrent pulmonary hemorrhage and rapidly progressive glomerulonephritis. For example, in a study of 88 patients with pulmonary-renal vasculitic syndrome by Niles et al. (169), 55% had ANCA without anti-GBM antibodies, 7% had anti-GBM without ANCA, and 8% had ANCA and anti-GBM antibodies. The proportion of patients with anti-GBM antibodies who also have ANCA varies among series, but it is approximately one third (47–49). Immune complex–mediated pulmonary-renal syndrome is a very rare manifestation of a number of systemic immune complex diseases, including systemic lupus erythematosus, Henoch-Schönlein purpura, and cryoglobulinemia. Pathologic evaluation of renal biopsy specimens is usually much more definitive than lung biopsy in distinguishing among the various causes of pulmonary-renal syndrome.

Anti-GBM CGN must be distinguished from immune complex CGN and ANCA-CGN. Of course, immunohistology is a better means for resolving this differential diagnosis, but findings by light microscopy can indicate the most likely diagnosis. The light microscopic appearance of the glomerular tufts in immune complex CGN is dictated by the underlying type of immune complex glomerulonephritis. Histologic examination of relatively intact glomeruli or glomerular segments may suggest that the glomerulonephritis is immune complex mediated and suggest what type of immune complex glomerulonephritis is most likely. For example, IgA nephropathy and lupus nephritis usually have focal to diffuse glomerular hypercellularity with variable thickening of capillary walls; poststreptococcal glomerulonephritis has marked diffuse global endocapillary hypercellularity with conspicuous neutrophils;

membranoproliferative glomerulonephritis has lobular accentuation with thickening of capillary walls and marked mesangial expansion and subendothelial interposition; and membranous glomerulonephritis has thick capillary walls with little or no endocapillary hypercellularity. There is a greater degree of glomerular tuft necrosis in crescentic immune complex glomerulonephritis than in immune complex glomerulonephritis without crescents; however, immune complex CGN only very rarely has as much fibrinoid necrosis as is typically observed in active anti-GBM and ANCA glomerulonephritis. In other words, the glomerular hypercellularity and thickening of capillary walls that are characteristic of immune complex CGN are more pronounced than in anti-GBM CGN and ANCA-CGN, whereas glomerular fibrinoid necrosis is more pronounced in anti-GBM CGN and ANCA-CGN than in immune complex glomerulonephritis (see Table 15.2) (18).

The crescents of immune complex glomerulonephritis tend to be more compact and demarcated by an intact Bowman capsule, whereas those of anti-GBM and ANCA glomerulonephritis tend to be disorderly because of disruption of the Bowman capsule (59). The disruption of the Bowman capsule also results in more conspicuous periglomerular inflammation in anti-GBM and ANCA-CGN compared with immune complex CGN. The cellular crescents of immune complex CGN may evolve into fibrocellular crescents and fibrotic crescents.

Linear GBM staining for immunoglobulin also occurs in diabetic glomerulosclerosis and monoclonal immunoglobulin deposition disease; however, these diseases have diagnostic clinical manifestations and characteristic pathologic findings that readily distinguish them from anti-GBM disease. Pathologically, both have nodular sclerosing glomerular lesions rather than necrotizing and CGN. A rare differential consideration is a nodular sclerosing glomerular lesion with intense polyclonal GBM staining in patients who have no diabetes and lack circulating autoantibodies to type IV collagen α3NCI. These patients may have an autoantibody against other GBM constituents.

## CLINICAL COURSE, PROGNOSIS, THERAPY, AND CLINICOPATHOLOGIC CORRELATIONS

Anti-GBM glomerulonephritis, whether it is a component of Goodpasture syndrome or occurs alone, is almost always an aggressive disease that requires rapid institution of intense immunosuppressive therapy for optimum outcome (19,34,37,170–173). Treatment typically includes high-dose corticosteroids, cytotoxic drugs, and plasma exchange (apheresis). Unlike many autoimmune diseases, anti-GBM disease, with rare exceptions, has one major episode rather than a relapsing and remitting course. Anti-GBM antibodies become undetectable within months of institution of therapy and, even without therapy, disappear after several years (156).

In a study from China of 221 anti-GBM patients, the addition of plasmapheresis to treatment with corticosteroids and cyclophosphamide had an overall beneficial effect on both patient survival (HR for patient mortality, 0.31; $P = 0.001$) and renal survival (HR for renal failure, 0.60; $P = 0.032$), particularly patient survival for those with Goodpasture syndrome (HR for patient mortality, 0.29; $P = 0.004$) and renal survival for those with anti-GBM nephritis with initial serum creatinine over 6.8 mg/dL (HR for renal failure, 0.52; $P = 0.014$) (37).

The role of targeted B-cell therapy (e.g., anti-CD20 monoclonal antibody) in anti-GBM disease has not been well defined, but anecdotal reports suggest a possible role (174).

The severity of renal injury and the serum creatinine at the time treatment is begun are the best predictors of outcome (37,171,173). In a study of 71 patients with anti-GBM glomerulonephritis who were treated with prednisolone, cyclophosphamide, and plasma exchange (173), patients with initial creatinine level less than 5.7 mg/dL had 95% renal survival and 100% patient survival at 1 year. Patients with creatinine levels greater than 5.7 mg/dL and not requiring dialysis at the time of presentation had renal and patient survival of 82% and 83% at 1 year. Patients who required dialysis at presentation had renal and patient survival of 8% and 65% at 1 year.

Segelmark et al. (35) correlated clinical outcome with anti-GBM titer and affinity in 79 patients with anti-GBM disease. Better renal survival correlated with lower titers of anti-GBM antibodies and a lower proportion of antibodies with specificity for an immunodominant GBM epitope. In a subset of 57 patients with renal biopsy data available, serum creatinine was greater than 600 mol/L (6.8 mg/dL) at the time of biopsy in 100% of 25 patients who had more than 80% crescents compared with 41% of patients with less than 80% crescents.

Anti-GBM glomerulonephritis has a worse prognosis than immune complex glomerulonephritis or pauci-immune (ANCA) glomerulonephritis. Surprisingly, in one study, a patient with anti-GBM disease who has a positive ANCA has a better prognosis than an anti-GBM patient with a negative ANCA (47). This is consistent with the pathologic findings at the time of presentation as indicated in Table 15.5. Patients who were anti-GBM–positive and ANCA-negative had crescents in an average of 84% of glomeruli, whereas patients who were positive for both anti-GBM and ANCA had crescents in an average of 67% of glomeruli. However, this still was worse than patients who were ANCA positive but anti-GBM negative who had crescents in less than 50% of glomeruli. Rutgers et al. (45) reported similar results with an average of 72% of glomeruli with crescents in patients who had anti-GBM without ANCA, 58% in patients who had anti-GBM and ANCA, and 45% in patients who had ANCA without anti-GBM. The respective renal survival at 1 year in their three groups was 15%, 10%, and 65%. Thus, they did not observe the same intermediate survival for concurrent anti-GBM and ANCA disease that was reported by Bosch et al. (47) but rather observed that anti-GBM disease with ANCA had an outcome more like that of anti-GBM alone and significantly worse than ANCA disease alone (45).

Once the initial episode of acute injury enters remission, recurrence of active disease is uncommon but may occur even many years later (156,175–178). Anti-GBM glomerulonephritis can recur in renal allografts (179), but this is uncommon if transplantation is delayed until anti-GBM antibodies are undetectable in serum.

There is general agreement that renal outcome is best if treatment is started before patients are dialysis dependent and before the serum creatinine is 5 mg/dL or higher (34,171,173). Some studies have found a correlation between the degree of crescent formation and renal outcome (34,171,180), but others have not (181). In a study of 40 patients with anti-GBM disease by Daly et al. (34), the odds ratio for needing dialysis for a 10% increase in crescent formation was 1.8 ± 1.3

($P$ = 0.02), which further substantiates the critical importance of timely diagnosis and timely institution of appropriate immunosuppressive therapy. In a retrospective study of 29 patients with anti-GBM disease, Herody et al. (180) concluded that predictors of poor outcome included a high serum creatinine, oliguria, absence of normal glomeruli on renal biopsy, high percentage of circumferential cellular crescents, and high titer of circulating anti-GBM antibodies. Some histologically normal glomeruli were present in 100% of patients with a good renal outcome compared with only 10% of patients with a poor renal outcome. Circumferential cellular crescents affected a mean of 15% of glomeruli in patients with a good renal outcome compared with 81% of glomeruli in patients with a poor renal outcome.

## REFERENCES


1. Goodpasture WE. The significance of certain pulmonary lesions in relation to the etiology of influenza. *Am J Med Sci* 1919;158:863.
2. Stanton MC, Tange JD. Goodpasture's syndrome (pulmonary haemorrhage associated with glomerulonephritis). *Australas Ann Med* 1958; 7:132.
3. Martinez JS, Kohler PF. Variant "Goodpasture's syndrome"? The need for immunologic criteria in rapidly progressive glomerulonephritis and hemorrhagic pneumonitis. *Ann Intern Med* 1971;75:67.
4. Saraf P, Berger HW, Thung SN. Goodpasture's syndrome with no overt renal disease. *Mt Sinai J Med* 1978;45:451.
5. Jennette JC, Falk RJ, Bacon PA, et al. 2012 revised International Chapel Hill Consensus Conference Nomenclature of Vasculitides. *Arthritis Rheum* 2013;65:1.
6. Kalluri R, Wilson CB, Weber M, et al. Identification of the alpha 3 chain of type IV collagen as the common autoantigen in antibasement membrane disease and Goodpasture's syndrome. *J Am Soc Nephrol* 1995;6:1178.
7. Jones DA, Jennette JC, Falk RJ. Goodpasture's syndrome revisited: A new perspective on glomerulonephritis and alveolar hemorrhage. *N C Med J* 1990;51:411.
8. Scheer RL, Grossman MA, for the Armed Forces Institute of Pathology. Immune aspects of the glomerulonephritis associated with pulmonary hemorrhage. *Ann Intern Med* 1964;60:1009.
9. Sturgill BC, Westervelt FB. Immunofluorescence studies in a case of Goodpasture's syndrome. *JAMA* 1965;194:914.
10. Lerner RA, Glassock RJ, Dixon FJ. The role of anti-glomerular basement membrane antibody in the pathogenesis of human glomerulonephritis. *J Exp Med* 1967;126:989.
11. Churg J, Bernstein J, Glassock RJ. *Renal Disease: Classification and Atlas of Glomerular Diseases*, 2nd ed. New York: Igaku-Shoin, 1995.
12. Langhans T. Uber die veranderungen der glomeruli bei der nephritis nebst einigen bemerkungen uber die entstehung der fibrin cylinder. *Arch Pathol Physiol Klin Med* 1879;76:85.
13. Purdy CW. *Bright's Disease and Allied Affections of the Kidneys*. Philadelphia: Lea Brothers, 1886.
14. Volhard F, Fahr T. *Die brightsche Nierenkrankheit: Klink, Pathologic und Atlas*. Berlin: Springer, 1914.
15. Olsen S. Extracapillary glomerulonephritis: a semiquantitative light microscopial study of 59 patients. *Acta Pathol Microbiol Scand A* 1974; 82(Suppl 249):7.
16. Spargo BH, Ordonez NG, Ringus JC. The differential diagnosis of crescentic glomerulonephritis: the pathology of specific lesions with prognostic implications. *Hum Pathol* 1977;8:187.
17. Cassidy MJ, Gaskin G, Savill J, et al. Towards a more rapid diagnosis of rapidly progressive glomerulonephritis. *Br Med J* 1990;301:329.
18. Jennette JC. Rapidly progressive crescentic glomerulonephritis. *Kidney Int* 2003;63:1164.
19. Kambhan N. Crescentic glomerulonephritis: an update on pauci-immune and anti-GBM disease. *Adv Anat Pathol* 2012;19:111.
20. Nasr SH, Valeri AM, Appel GB, et al. Dense deposit disease: clinicopathologic study of 32 pediatric and adult patients. *Clin J Am Soc Nephrol* 2009;4:22.
21. Falk RJ, Nachman PH, Hogan SL, et al. ANCA glomerulonephritis and vasculitis: a Chapel Hill perspective. *Semin Nephrol* 2000;20:233.
22. Jardim HM, Leake J, Risdon RA, et al. Crescentic glomerulonephritis in children. *Pediatr Nephrol* 1992;6:231.
23. Andrassy K, Kuster S, Waldherr R, et al. Rapidly progressive glomerulonephritis: analysis of prevalence and clinical course. *Nephron* 1991;59:206.
24. Angangco R, Thiru S, Esnault VL, et al. Does truly "idiopathic" crescentic glomerulonephritis exist? *Nephrol Dial Transplant* 1994;9:630.
25. Saxena R, Bygren P, Rasmussen N, et al. Circulating autoantibodies in patients with extracapillary glomerulonephritis. *Nephrol Dial Transplant* 1991;6:389.
26. Tang Z, Wu Y, Wang Q, et al. Clinical spectrum of diffuse crescentic glomerulonephritis in Chinese patients. *Chin Med J (Engl)* 2003;116:1737.
27. Lin W, Chen M, Cui Z, et al. The immunopathological spectrum of crescentic glomerulonephritis: a survey of 106 patients in a single Chinese center. *Nephron Clin Pract* 2010;116:c65.
28. Grcevska L, Polenakovic M. Crescentic glomerulonephritis as renal cause of acute renal failure. *Ren Fail* 1995;17:595.
29. Gupta R, Singh L, Sharma A, et al. Crescentic glomerulonephritis: A clinical and histomorphological analysis of 46 cases. *Indian J Pathol Microbiol* 2011;54:497.
30. Dewan D, Gulati S, Sharma RK, et al. Clinical spectrum and outcome of crescentic glomerulonephritis in children in developing countries. *Pediatr Nephrol* 2008;23:389.
31. Wilson CB, Dixon FJ. Anti-glomerular basement membrane antibody-induced glomerulonephritis. *Kidney Int* 1973;3:74.
32. Teague CA, Doak PB, Simpson IJ, et al. Goodpasture's syndrome: an analysis of 29 cases. *Kidney Int* 1978;13:492.
33. Savage CO, Pusey CD, Bowman C, et al. Antiglomerular basement membrane antibody mediated disease in the British Isles 1980–4. *Br Med J (Clin Res Ed)* 1986;292:301.
34. Daly C, Conlon PJ, Medwar W, et al. Characteristics and outcome of anti-glomerular basement membrane disease: a single-center experience. *Ren Fail* 1996;18:105.
35. Segelmark M, Hellmark T, Wieslander J. The prognostic significance in Goodpasture's disease of specificity, titre and affinity of anti-glomerular-basement-membrane antibodies. *Nephron Clin Pract* 2003;94:c59.
36. Fischer EG, Lager DJ. Anti-glomerular basement membrane glomerulonephritis: a morphologic study of 80 cases. *Am J Clin Pathol* 2006;125:445.
37. Cui Z, Zhao J, Jia XY, et al. Anti-glomerular basement membrane disease: outcomes of different therapeutic regimens in a large single-center Chinese cohort study. *Medicine (Baltimore)* 2011;90:303.
38. Taylor DM, Yehia M, Simpson IJ, et al. Anti-glomerular basement membrane disease in Auckland. *Intern Med J* 2012;42:672.
39. Poddar B, Singhal S, Azim A, et al. Goodpasture's syndrome in children. *Saudi J Kidney Dis Transpl* 2010;21:935.
40. Knoll G, Rabin E, Burns BF. Antiglomerular basement membrane antibody-mediated nephritis with normal pulmonary and renal function: a case report and review of the literature. *Am J Nephrol* 1993;13:494.
41. Proskey AJ, Weatherbee L, Easterling RE, et al. Goodpasture's syndrome: a report of five cases and review of the literature. *Am J Med* 1970;48:162.
42. Bombassei GJ, Kaplan AA. The association between hydrocarbon exposure and anti-glomerular basement membrane antibody-mediated disease (Goodpasture's syndrome). *Am J Ind Med* 1992;21:141.
43. Bonzel KE, Muller-Wiefel DE, Ruder H, et al. Anti-glomerular basement membrane antibody-mediated glomerulonephritis due to glue sniffing. *Eur J Pediatr* 1987;146:296.
44. Kleinknecht D, Morel-Maroger L, Callard P. Antiglomerular basement membrane nephritis after solvent exposure. *Arch Intern Med* 1980;140:230.
45. Rutgers A, Slot M, van Paassen P, et al. Coexistence of anti-glomerular basement membrane antibodies and myelope-roxidase-ANCAs in crescentic glomerulonephritis. *Am J Kidney Dis* 2005;46:253.
46. Jayne DR, Marshall PD, Jones SJ, et al. Autoantibodies to GBM and neutrophil cytoplasm in rapidly progressive glomerulonephritis. *Kidney Int* 1990;37:965.
47. Bosch X, Mirapeix E, Font J, et al. Prognostic implication of anti-neutrophil cytoplasmic autoantibodies with myeloperoxidase specificity in anti-glomerular basement membrane disease. *Clin Nephrol* 1991;36:107.

48. Bosch X, Mirapeix E, Font J, et al. Anti-myeloperoxidase autoantibodies in patients with necrotizing glomerular and alveolar capillaritis. *Am J Kidney Dis* 1992;20:231.
49. Short AK, Esnault VL, Lockwood CM. Anti-neutrophil cytoplasm antibodies and anti-glomerular basement membrane antibodies: two coexisting distinct autoreactivities detectable in patients with rapidly progressive glomerulonephritis. *Am J Kidney Dis* 1995;26:439.
50. Mulpuru S, Touchie C, Karpinski J, et al. Coexistent Wegener's granulomatosis and Goodpasture's disease. *J Rheumatol* 2010;37:1786.
51. Gleeson CM, Levy JB, Cook HT, et al. An atypical cutaneous presentation of vasculitis with features of Churg-Strauss syndrome, associated with anti-neutrophil cytoplasmic antibodies and anti-glomerular basement membrane antibodies. *Clin Exp Dermatol* 2009;34:e577.
52. Cui Z, Zhao J, Jia XY, et al. Clinical features and outcomes of anti-glomerular basement membrane disease in older patients. *Am J Kidney Dis* 2011;57:575.
53. Khandelwal M, McCormick BB, Lajoie G, et al. Recurrence of anti-GBM disease 8 years after renal transplantation. *Nephrol Dial Transplant* 2004;19:491.
54. Borza DB, Neilson EG, Hudson BG. Pathogenesis of Goodpasture's syndrome: a molecular perspective. *Semin Nephrol* 2003;23:522.
55. Pedchenko V, Bondar O, Fogo AB, et al. Molecular architecture of the Goodpasture autoantigen in anti-GBM nephritis. *N Engl J Med* 2010;363:343–354.
56. McPhaul JJ Jr, Mullins JD. Glomerulonephritis mediated by antibody to glomerular basement membrane: immunological, clinical, and histopathological characteristics. *J Clin Invest* 1976;57:351.
57. Ohashi N, Sugiura T, Isozaki T, et al. Anti-glomerular basement membrane antibody-induced glomerulonephritis with periglomerular granulomatous reaction and massive renal eosinophilic infiltration. *Am J Kidney Dis* 2003;42:E28.
58. Magil AB, Wadsworth LD. Monocyte involvement in glomerular crescents: a histochemical and ultrastructural study. *Lab Invest* 1982;47:160.
59. Ferrario F, Tadros MT, Napodano P, et al. Critical re-evaluation of 41 cases of "idiopathic" crescentic glomerulonephritis. *Clin Nephrol* 1994;41:1.
60. Ferrario F, Rastaldi MP. Necrotizing-crescentic glomerulonephritis in ANCA-associated vasculitis: the role of monocytes. *Nephrol Dial Transplant* 1999;14:1627–1631.
61. Bajema IM, Hagen EC, Ferrario F, et al. Renal granulomas in systemic vasculitis. EC/BCR Project for ANCA-Assay Standardization. *Clin Nephrol* 1997;48:16.
62. Andres G, Brentjens J, Kohli R, et al. Histology of human tubulo-interstitial nephritis associated with antibodies to renal basement membranes. *Kidney Int* 1978;13:480.
63. Stachura I, Si L, Whiteside TL. Mononuclear-cell subsets in human idiopathic crescentic glomerulonephritis (ICGN): analysis in tissue sections with monoclonal antibodies. *J Clin Immunol* 1984;4:202.
64. Lombard CM, Colby TV, Elliott CG. Surgical pathology of the lung in anti-basement membrane antibody-associated Goodpasture's syndrome. *Hum Pathol* 1989;20:445.
65. Poskitt TR. Immunologic and electron microscopic studies in Goodpasture's syndrome. *Am J Med* 1970;49:250.
66. Qu Z, Cui Z, Liu G, et al. The distribution of IgG subclass deposition on renal tissues from patients with anti-glomerular basement membrane disease. *BMC Immunol* 2013;14:19.
67. Lehman DH, Wilson CB, Dixon FJ. Extraglomerular immuno-globulin deposits in human nephritis. *Am J Med* 1975;58:765.
68. Mahler M, Radice A, Sinico RA, et al. Performance evaluation of a novel chemiluminescence assay for detection of anti-GBM antibodies: an international multicenter study. *Nephrol Dial Transplant* 2012;27:243.
69. Jia XY, Qu Z, Cui Z, et al. Circulating anti-glomerular basement membrane autoantibodies against α3(IV)NC1 undetectable by commercially available enzyme-linked immunosorbent assays. *Nephrology (Carlton)* 2012;17:160.
70. Border WA, Baehler RW, Bhathena D, et al. IgA antibasement membrane nephritis with pulmonary hemorrhage. *Ann Intern Med* 1979;91:21.
71. Gris P, Pirson Y, Hamels J, et al. Antiglomerular basement membrane nephritis induced by IgA1 antibodies. *Nephron* 1991;58:418.
72. Borza DB, Chedid MF, Colon S, et al. Recurrent Goodpasture's disease secondary to a monoclonal IgA1-kappa antibody autoreactive with the alpha1/alpha2 chains of type IV collagen. *Am J Kidney Dis* 2005;45:397.
73. Shaer AJ, Stewart LR, Cheek DE, et al. IgA antiglomerular basement membrane nephritis associated with Crohn's disease: a case report and review of glomerulonephritis in inflammatory bowel disease. *Am J Kidney Dis* 2003;41:1097.
74. Ho J, Gibson IW, Zacharias J, et al. Antigenic heterogeneity of IgA anti-GBM disease: new renal targets of IgA autoantibodies. *Am J Kidney Dis* 2008;52:761.
75. Klassen J, Elwood C, Grossberg AL, et al. Evolution of membranous nephropathy into anti-glomerular-basement-membrane glomerulonephritis. *N Engl J Med* 1974;290:1340.
76. Moorthy AV, Zimmerman SW, Burkholder PM, et al. Association of crescentic glomerulonephritis with membranous glomerulonephropathy: a report of three cases. *Clin Nephrol* 1976;6:319.
77. Jennette JC, Lamanna RW, Burnette JP, et al. Concurrent antiglomerular basement membrane antibody and immune complex mediated glomerulonephritis. *Am J Clin Pathol* 1982;78:381.
78. Kurki P, Helve T, von Bonsdorff M, et al. Transformation of membranous glomerulonephritis into crescentic glomerulonephritis with glomerular basement membrane antibodies: serial determinations of anti-GBM before the transformation. *Nephron* 1984;38:134.
79. Pettersson E, Tornroth T, Miettinen A. Simultaneous anti-glomerular basement membrane and membranous glomerulonephritis: case report and literature review. *Clin Immunol Immunopathol* 1984;31:171.
80. Imtiaz S, Alswaida A, Rehman H, et al. Transformation of membranous into anti-GBM nephritis. *Indian J Nephrol* 2012;22:370–373.
81. Fivush B, Melvin T, Solez K, et al. Idiopathic linear glomerular IgA deposition. *Arch Pathol Lab Med* 1986;110:1189.
82. Koffler D, Sandson J, Carr R, et al. Immunologic studies concerning the pulmonary lesions in Goodpasture's syndrome. *Am J Pathol* 1969;54:293.
83. Bonsib SM. Glomerular basement membrane necrosis and crescent organization. *Kidney Int* 1988;33:966.
84. Boucher A, Droz D, Adafer E, et al. Relationship between the integrity of Bowman's capsule and the composition of cellular crescents in human crescentic glomerulonephritis. *Lab Invest* 1987;56:526.
85. Morita T, Suzuki Y, Churg J. Structure and development of the glomerular crescent. *Am J Pathol* 1973;72:349.
86. Bohman SO, Olsen S, Petersen VP. Glomerular ultrastructure in extracapillary glomerulonephritis. *Acta Pathol Microbiol Scand A* 1974;82(Suppl 249):29.
87. Gabbert H, Thoenes W. Formation of basement membrane in extracapillary proliferates in rapidly progressive glomerulonephritis. *Virchows Arch B Cell Pathol* 1977;25:265.
88. Hoyer JR, Michael AF, Hoyer LW. Immunofluorescent localization of antihemophilic factor antigen and fibrinogen in human renal diseases. *J Clin Invest* 1974;53:1375.
89. Magil AB. Histogenesis of glomerular crescents: immunohistochemical demonstration of cytokeratin in crescent cells. *Am J Pathol* 1985;120:222.
90. Jennette JC, Hipp CG. The epithelial antigen phenotype of glomerular crescent cells. *Am J Clin Pathol* 1986;86:274.
91. Harrison DJ, MacDonald MK. The origin of cells in the glomerular crescent investigated by the use of monoclonal antibodies. *Histopathology* 1986;10:945.
92. Guettier C, Nochy D, Jacquot C, et al. Immunohistochemical demonstration of parietal epithelial cells and macrophages in human proliferative extra-capillary lesions. *Virchows Arch A Pathol Anat Histopathol* 1986;409:739.
93. Hancock WW, Atkins RC. Cellular composition of crescents in human rapidly progressive glomerulonephritis identified using monoclonal antibodies. *Am J Nephrol* 1984;4:177.
94. Bhathena DB, Migdal SD, Julian BA, et al. Morphologic and immunohistochemical observations in granulomatous glomerulonephritis. *Am J Pathol* 1987;126:581.
95. Rich AR. The pathology and pathogenesis of experimental anaphylactic glomerulonephritis in relation to human acute glomerulonephritis. *Bull Johns Hopkins Hosp* 1956;98:120.
96. Naish P, Penn GB, Evans DJ, et al. The effect of defibrination on nephrotoxic serum nephritis in rabbits. *Clin Sci* 1972;42:643.
97. Tipping PG, Thomson NM, Holdsworth SR. A comparison of fibrinolytic and defibrinating agents in established experimental glomerulonephritis. *Br J Exp Pathol* 1986;67:481.

98. Zoja C, Corna D, Macconi D, et al. Tissue plasminogen activator therapy of rabbit nephrotoxic nephritis. *Lab Invest* 1990;62:34.
99. McCluskey RT, Andres GA. Does t-PA have a role in the treatment of crescentic glomerulonephritis? *Lab Invest* 1990;62:1.
100. Brentjens JR. Glomerular procoagulant activity and glomerulonephritis. *Lab Invest* 1987;57:107.
101. Wiggins RC, Glatfelter A, Brukman J. Procoagulant activity in glomeruli and urine of rabbits with nephrotoxic nephritis. *Lab Invest* 1985;53:156.
102. Tipping PG, Dowling JP, Holdsworth SR. Glomerular procoagulant activity in human proliferative glomerulonephritis. *J Clin Invest* 1988;81:119.
103. Tipping PG, Erlich JH, Apostolopoulos J, et al. Glomerular tissue factor expression in crescentic glomerulonephritis: correlations between antigen, activity, and mRNA. *Am J Pathol* 1995;147:1736.
104. Grandaliano G, Gesualdo L, Ranieri E, et al. Tissue factor, plasminogen activator inhibitor-1, and thrombin receptor expression in human crescentic glomerulonephritis. *Am J Kidney Dis* 2000;35:726.
105. Silva FG, Hoyer JR, Pirani CL. Sequential studies of glomerular crescent formation in rats with antiglomerular basement membrane-induced glomerulonephritis and the role of coagulation factors. *Lab Invest* 1984;51:404.
106. He CJ, Rondeau E, Medcalf RL, et al. Thrombin increases proliferation and decreases fibrinolytic activity of kidney glomerular epithelial cells. *J Cell Physiol* 1991;146:131.
107. Adler S, Chen X, Eng B. Control of rat glomerular epithelial cell growth in vitro. *Kidney Int* 1990;37:1048.
108. Matsumoto K, Dowling J, Atkins RC. Production of interleukin 1 in glomerular cell cultures from patients with rapidly progressive crescentic glomerulonephritis. *Am J Nephrol* 1988;8:463.
109. Li HL, Hancock WW, Dowling JP, et al. Activated (IL-2R+) intraglomerular mononuclear cells in crescentic glomerulonephritis. *Kidney Int* 1991;39:793.
110. Lan HY, Nikolic-Paterson DJ, Zarama M, et al. Suppression of experimental crescentic glomerulonephritis by the interleukin-1 receptor antagonist. *Kidney Int* 1993;43:479.
111. Lan HY, Nikolic-Paterson DJ, Mu W, et al. Interleukin-1 receptor antagonist halts the progression of established crescentic glomerulonephritis in the rat. *Kidney Int* 1995;47:1303.
112. Baraldi A, Zambruno G, Furci L, et al. Beta 1 and beta 3 integrin upregulation in rapidly progressive glomerulonephritis. *Nephrol Dial Transplant* 1995;10:1155.
113. Patey N, Lesavre P, Halbwachs-Mecarelli L, et al. Adhesion molecules in human crescentic glomerulonephritis. *J Pathol* 1996;179:414.
114. Lan HY, Nikolic-Paterson DJ, Atkins RC. Involvement of activated perglomerular leukocytes in the rupture of Bowman's capsule and glomerular crescent progression in experimental glomerulonephritis. *Lab Invest* 1992;67:743.
115. Halliwell B, Hoult JR, Blake DR. Oxidants, inflammation, and anti-inflammatory drugs. *FASEB J* 1988;2:2867.
116. Jennette JC, Falk RJ. Acute renal failure secondary to leukocyte-mediated acute glomerular injury. *Ren Fail* 1992;14:395.
117. Sady SP, Goyal M, Thomas PE, et al. Fibronectin mRNA in the developing glomerular crescent in rabbit antiglomerular basement membrane disease. *J Am Soc Nephrol* 1995;5:2087.
118. Shimizu A, Masuda Y, Kitamura H, et al. Apoptosis in progressive crescentic glomerulonephritis. *Lab Invest* 1996;74:941.
119. Yoshioka K, Takemura T, Iseki T, et al. Cellular and non-cellular compositions of crescents in human glomerulonephritis. *Kidney Int* 1987;32:284.
120. Morel-Maroger Striker L, Killen PD, Chi E, et al. The composition of glomerulosclerosis. I. Studies in focal sclerosis, crescentic glomerulonephritis, and membranoproliferative glomerulonephritis. *Lab Invest* 1984;51:181.
121. Yoshioka K, Tohda M, Takemura T, et al. Distribution of type I collagen in human kidney diseases in comparison with type III collagen. *J Pathol* 1990;162:141.
122. Noh JW, Wiggins R, Phan SH. Urine transforming growth factor-beta activity is related to the degree of scarring in crescentic nephritis in the rabbit. *Nephron* 1993;63:73.
123. Phan SH, Wolber F, Wiggins R. Fibronectin is the major fibroblast chemotactin in crescentic nephritis. *J Am Soc Nephrol* 1992;3:576.
124. Germuth FG Jr, Choi IJ, Taylor JJ, et al. Antibasement membrane disease. I. The glomerular lesions of Goodpasture's disease and experimental disease in sheep. *Johns Hopkins Med J* 1972;131:367.
125. Thompson EM, Hughes J, Van Noorden S, et al. Expression of the multifunctional extracellular matrix protein thrombospondin in crescentic glomerulonephritis. *J Pathol* 1996;178:89.
126. Han Y, Ma FY, Tesch GH, et al. Role of macrophages in the fibrotic phase of rat crescentic glomerulonephritis. *Am J Physiol Renal Physiol* 2013;304:F1043–F1053.
127. Steblay RW. Glomerulonephritis induced in sheep by injections of heterologous glomerular basement membrane and Freund's complete adjuvant. *J Exp Med* 1962;116:253.
128. Ohnuki T. Crescentic glomerulonephritis induced in the goat by immunization with homologous or heterologous glomerular basement membrane antigen. *Acta Pathol Jpn* 1975;25:319.
129. Teruel JL, Liano F, Mampaso F, et al. Allograft antiglomerular basement membrane glomerulonephritis in a patient with Alport's syndrome. *Nephron* 1987;46:43.
130. Shah B, First MR, Mendoza NC, et al. Alport's syndrome: risk of glomerulonephritis induced by anti-glomerular-basement-membrane antibody after renal transplantation. *Nephron* 1988;50:34.
131. Goldman M, Depierreux M, De Pauw L, et al. Failure of two subsequent renal grafts by anti-GBM glomerulonephritis in Alport's syndrome: case report and review of the literature. *Transpl Int* 1990;3:82.
132. Oliver TB, Gouldesbrough DR, Swainson CP. Acute crescentic glomerulonephritis associated with antiglomerular basement membrane antibody in Alport's syndrome after second transplantation. *Nephrol Dial Transplant* 1991;6:893.
133. Wieslander J, Barr JF, Butkowski RJ, et al. Goodpasture antigen of the glomerular basement membrane: localization to noncollagenous regions of type IV collagen. *Proc Natl Acad Sci U S A* 1984;81:3838.
134. Saus J, Wieslander J, Langeveld JP, et al. Identification of the Goodpasture antigen as the alpha 3(IV) chain of collagen IV. *J Biol Chem* 1988;263:13374.
135. Hudson BG, Kalluri R, Gunwar S, et al. Molecular characteristics of the Goodpasture autoantigen. *Kidney Int* 1993;43:135.
136. Hellmark T, Johansson C, Wieslander J. Characterization of anti-GBM antibodies involved in Goodpasture's syndrome. *Kidney Int* 1994;46:823.
137. Kalluri R, Sun MJ, Hudson BG, et al. The Goodpasture autoantigen. Structural delineation of two immunologically privileged epitopes on alpha3(IV) chain of type IV collagen. *J Biol Chem* 1996;271:9062.
138. Cashman SJ, Pusey CD, Evans DJ. Extraglomerular distribution of immunoreactive Goodpasture antigen. *J Pathol* 1988;155:61.
139. Jia XY, Cui Z, Yang R, et al. Antibodies against linear epitopes on the Goodpasture autoantigen and kidney injury. *Clin J Am Soc Nephrol* 2012;7:926.
140. Churchill DN, Fine A, Gault MH. Association between hydrocarbon exposure and glomerulonephritis: an appraisal of the evidence. *Nephron* 1983;33:169.
141. Donaghy M, Rees AJ. Cigarette smoking and lung haemorrhage in glomerulonephritis caused by autoantibodies to glomerular basement membrane. *Lancet* 1983;2:1390.
142. Iwamoto I, Yonekawa S, Takeda T, et al. Anti-glomerular basement membrane nephritis after extracorporeal shock wave lithotripsy. *Am J Nephrol* 1998;18:534.
143. Xenocostas A, Jothy S, Collins B, et al. Anti-glomerular basement membrane glomerulonephritis after extracorporeal shock wave lithotripsy. *Am J Kidney Dis* 1999;33:128.
144. Cui Z, Zhao MH, Segelmark M, et al. Natural autoantibodies to myeloperoxidase, proteinase 3, and the glomerular basement membrane are present in normal individuals. *Kidney Int* 2010;78:590.
145. Olson SW, Arbogast CB, Baker TP, et al. Asymptomatic autoantibodies associate with future anti-glomerular basement membrane disease. *J Am Soc Nephrol* 2011;22:1946.
146. Huey B, McCormick K, Capper J, et al. Associations of HLA-DR and HLA-DQ types with anti-GBM nephritis by sequence-specific oligonucleotide probe hybridization. *Kidney Int* 1993;44:307.
147. Kitagawa W, Imai H, Komatsuda A, et al. The HLA-DRB1*1501 allele is prevalent among Japanese patients with anti-glomerular basement membrane antibody-mediated disease. *Nephrol Dial Transplant* 2008;23:3126–3129.

148. Luo H, Chen M, Cui Z, et al. The association of HLA-DQB1, -DQA1 and -DPB1 alleles with anti-glomerular basement membrane (GBM) disease in Chinese patients. *BMC Nephrol* 2011;12:21.
149. Bolton WK, Innes DJ Jr, Sturgill BC, et al. T-cells and macrophages in rapidly progressive glomerulonephritis: clinicopathologic correlations. *Kidney Int* 1987;32:869.
150. Reynolds J, Tam FW, Chandraker A, et al. CD28-B7 blockade prevents the development of experimental autoimmune glomerulonephritis. *J Clin Invest* 2000;105:643.
151. Salama AD, Chaudhry AN, Ryan JJ, et al. In Goodpasture's disease, CD4(+) T cells escape thymic deletion and are reactive with the autoantigen alpha3(IV)NC1. *J Am Soc Nephrol* 2001;12:1908.
152. Wu J, Hicks J, Borillo J, et al. CD4(+) T cells specific to a glomerular basement membrane antigen mediate glomerulonephritis. *J Clin Invest* 2002;109:517.
153. Wu J, Borillo J, Glass WF, et al. T-cell epitope of alpha3 chain of type IV collagen induces severe glomerulonephritis. *Kidney Int* 2003;64:1292.
154. Hopfer H, Maron R, Butzmann U, et al. The importance of cell-mediated immunity in the course and severity of autoimmune anti-glomerular basement membrane disease in mice. *FASEB J* 2003;17:860.
155. Kalluri R, Danoff TM, Okada H, et al. Susceptibility to anti-glomerular basement membrane disease and Goodpasture's syndrome is linked to MHC class II genes and the emergence of T cell-mediated immunity in mice. *J Clin Invest* 1997;100:2263.
156. Salama AD, Chaudhry AN, Holthaus KA, et al. Regulation by CD25+ lymphocytes of autoantigen-specific T-cell responses in Goodpasture's (anti-GBM) disease. *Kidney Int* 2003;64:1685.
157. Wolf D, Hochegger K, Wolf AM, et al. CD4+ CD25+ regulatory T cells inhibit experimental anti-glomerular basement membrane glomerulonephritis in mice. *J Am Soc Nephrol* 2005;16:1360.
158. Cochrane CG, Unanue ER, Dixon FJ. A role of polymorphonuclear leukocytes and complement in nephrotoxic nephritis. *J Exp Med* 1965;122:99.
159. Unanue ER, Dixon FJ. Experimental glomerulonephritis. V. Studies on the interaction of nephrotoxic antibodies with tissue of the rat. *J Exp Med* 1965;121:697.
160. Assmann KJ, Tangelder MM, Lange WP, et al. Anti-GBM nephritis in the mouse: severe proteinuria in the heterologous phase. *Virchows Arch A Pathol Anat Histopathol* 1985;406:285.
161. Sheerin NS, Springall T, Carroll MC, et al. Protection against anti-glomerular basement membrane (GBM)-mediated nephritis in C3- and C4-deficient mice. *Clin Exp Immunol* 1997;110:403.
162. Suzuki Y, Shirato I, Okumura K, et al. Distinct contribution of Fc receptors and angiotensin II-dependent pathways in anti-GBM glomerulonephritis. *Kidney Int* 1998;54:1166.
163. Radeke HH, Janssen-Graalfs I, Sowa EN, et al. Opposite regulation of type II and III receptors for immunoglobulin G in mouse glomerular mesangial cells and in the induction of anti-glomerular basement membrane (GBM) nephritis. *J Biol Chem* 2002;277:27535.
164. Sharp PE, Martin-Ramirez J, Mangsbo SM, et al. FcγRIIb on myeloid cells and intrinsic renal cells rather than B cells protects from nephrotoxic nephritis. *J Immunol* 2013;190:340–348.
165. Sado Y, Naito I, Okigaki T. Transfer of anti-glomerular basement membrane antibody-induced glomerulonephritis in inbred rats with isologous antibodies from the urine of nephritic rats. *J Pathol* 1989;158:325.
166. Sado Y, Okigaki T, Takamiya H, et al. Experimental autoimmune glomerulonephritis with pulmonary hemorrhage in rats: the dose-effect relationship of the nephritogenic antigen from bovine glomerular basement membrane. *J Clin Lab Immunol* 1984;15:199.
167. Kohda T, Okada S, Hayashi A, et al. High nephritogenicity of monoclonal antibodies belonging to IgG2a and IgG2b subclasses in rat anti-GBM nephritis. *Kidney Int* 2004;66:177.
168. Ma R, Cui Z, Liao YH, et al. Complement activation contributes to the injury and outcome of kidney in human anti-glomerular basement membrane disease. *J Clin Immunol* 2013;33:172.
169. Niles JL, Bottinger EP, Saurina GR, et al. The syndrome of lung hemorrhage and nephritis is usually an ANCA-associated condition. *Arch Intern Med* 1996;156:440.
170. Southwest Pediatric Nephrology Study Group. A clinico-pathologic study of crescentic glomerulonephritis in 50 children. *Kidney Int* 1985;27:450.
171. Johnson JP, Moore J Jr, Wilson CB, et al. Therapy of anti-glomerular basement membrane antibody disease: analysis of prognostic significance of clinical, pathologic and treatment factors. *Medicine* 1985;64:219.
172. Couser WG. Rapidly progressive glomerulonephritis: classification, pathogenetic mechanisms, and therapy. *Am J Kidney Dis* 1988;11:449.
173. Levy JB, Turner AN, Rees AJ, et al. Long-term outcome of anti-glomerular basement membrane antibody disease treated with plasma exchange and immunosuppression. *Ann Intern Med* 2001;134:1033.
174. Shah Y, Mohiuddin A, Sluman C, et al. Rituximab in anti-glomerular basement membrane disease. *QJM* 2012;105:195.
175. Dahlberg PJ, Kurtz SB, Donadio JV, et al. Recurrent Goodpasture's syndrome. *Mayo Clin Proc* 1978;53:533.
176. Wu MJ, Moorthy AV, Beirne GJ. Relapse in anti-glomerular basement membrane antibody mediated crescentic glomerulonephritis. *Clin Nephrol* 1980;13:97.
177. Klasa RJ, Abboud RT, Ballon HS, et al. Goodpasture's syndrome: Recurrence after a five-year remission: case report and review of the literature. *Am J Med* 1988;84:751.
178. Levy JB, Lachmann RH, Pusey CD. Recurrent Goodpasture's disease. *Am J Kidney Dis* 1996;27:573.
179. Almkuist RD, Buckalew VM Jr, Hirszel P, et al. Recurrence of anti-glomerular basement membrane antibody mediated glomerulonephritis in an isograft. *Clin Immunol Immunopathol* 1981;18:54.
180. Herody M, Bobrie G, Gouarin C, et al. Anti-GBM disease: predictive value of clinical, histological and serological data. *Clin Nephrol* 1993;40:249.
181. Hind CR, Paraskevakou H, Lockwood CM, et al. Prognosis after immunosuppression of patients with crescentic nephritis requiring dialysis. *Lancet* 1983;1:263.

CHAPTER 16

J. Charles Jennette • David B. Thomas

# Pauci-Immune and Antineutrophil Cytoplasmic Autoantibody–Mediated Crescentic Glomerulonephritis and Vasculitis



A landmark publication in 1979 by Stilmant et al. (1) reported that many if not most patients with crescentic glomerulonephritis did not have evidence for anti–glomerular basement membrane (anti-GBM) antibodies or immune complexes in glomeruli. Of 46 patients with crescentic glomerulonephritis, 16 (35%) had no substantial glomerular immunoglobulin deposits, 10 (22%) had linear IgG deposits indicative of anti-GBM disease, 19 (41%) had immune complex disease, and 1 had malignant hypertension (1). This category of crescentic glomerulonephritis with no evidence for mediation by anti-GBM antibodies or deposited immune complexes has been called *idiopathic crescentic glomerulonephritis* or *primary crescentic glomerulonephritis*; however, the most widely used term currently is *pauci-immune crescentic glomerulonephritis*, which is based on the paucity of glomerular staining for immunoglobulins. Pauci-immune crescentic glomerulonephritis occurs as a renal-limited disease and as a component of systemic necrotizing small-vessel vasculitis (2–8). In fact, most patients with pauci-immune crescentic glomerulonephritis have at least some constitutional signs and symptoms that raise the possibility of systemic vasculitis, and approximately 75% have overt evidence for systemic vasculitis. Some have advocated calling it *renal-limited vasculitis* (RLV) because the glomerular lesion in patients with necrotizing small-vessel vasculitis, such as microscopic polyangiitis (MPA) and granulomatosis with polyangiitis (GPA; Wegener granulomatosis), is indistinguishable from that seen in patients with pauci-immune crescentic glomerulonephritis in the absence of systemic vasculitis (4,5).

In 1988, Falk and Jennette (6) demonstrated that most patients with pauci-immune crescentic glomerulonephritis have antineutrophil cytoplasmic autoantibodies (ANCA), including patients with and without systemic vasculitis (6). This has been confirmed by many investigators (2,5,7–13). Approximately 85% to 95% of patients with active untreated pauci-immune crescentic glomerulonephritis are found to be ANCA positive (11,13–20). Crescentic glomerulonephritis that is not associated with ANCA and does not have evidence for immune complex or anti-GBM mediation is rare and accounts for no more than 5% of all crescentic glomerulonephritis (5,21).

Pauci-immune crescentic glomerulonephritis (i.e., ANCA crescentic glomerulonephritis) is the most common cause of crescentic glomerulonephritis (i.e., with crescents in 50% or more of glomeruli) (Fig. 16.1) (7,21,22). Pauci-immune crescentic glomerulonephritis is most prevalent in older patients. For example, pauci-immune crescentic glomerulonephritis was the category of crescentic glomerulonephritis found in 79% (201 of 256) of patients older than 60 years of age, compared with 48% (145 of 303) of patients 21 to 60 years old and 42% (31 of 73) of patients younger than 21 years of age (21). Most patients with pauci-immune crescentic glomerulonephritis are ANCA positive, and approximately 90% of ANCA-positive patients who have glomerulonephritis have crescent formation (21). The pathologic and clinical features of disease, including the association with systemic vasculitis, are similar in patients with pauci-immune crescentic glomerulonephritis who are

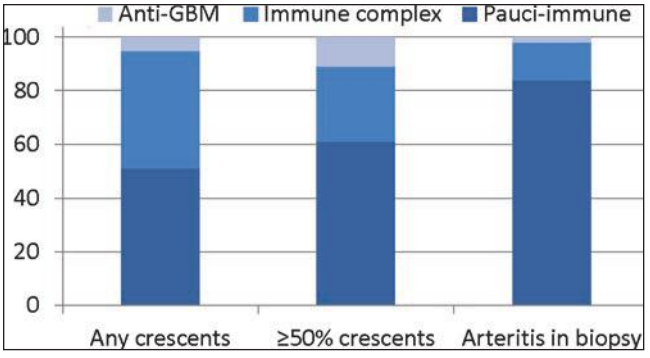



**FIGURE 16.1 Frequency of immunopathologic categories of glomerulonephritis in native kidney biopsies evaluated by immunofluorescence microscopy in the University of North Carolina Nephropathology Laboratory.** Data are derived from 540 patients with any crescents, 195 patients with ≥50% crescents, and 37 patients with arteritis in the biopsy (137). The anti-GBM patient with arteritis was ANCA positive.

ANCA positive compared with those who are ANCA negative (23). ANCA-associated pauci-immune crescentic glomerulonephritis is the most common cause for the renal component of pulmonary-renal vasculitic syndrome (24,25). For example, in a study of 88 patients with pulmonary-renal syndrome by Niles et al. (24), 48 (55%) had ANCA, 6 (7%) had anti-GBM antibodies, and 7 (8%) had both. Thus, 63% of patients with pulmonary-renal syndrome had ANCA.

The systemic vasculitides that may be accompanied by pauci-immune crescentic glomerulonephritis include granulomatosis with polyangiitis (GPA, Wegener granulomatosis), microscopic polyangiitis (MPA), and eosinophilic granulomatosis with polyangiitis (EGPA, Churg-Strauss syndrome) (3,22,26,27). In a patient with pauci-immune crescentic glomerulonephritis, a diagnosis of GPA is appropriate when there is evidence for necrotizing granulomatous inflammation but no asthma. A diagnosis of EGPA is appropriate when there is a history of asthma and eosinophilia and there is granulomatous pulmonary disease. And a diagnosis of MPA is appropriate when there is systemic necrotizing small-vessel vasculitis with no evidence for granulomatous inflammation or asthma (Table 16.1) (26). Clinical or pathologic evidence of renal disease is seen in approximately 90% of patients with MPA, 80% of patients with GPA, and 45% of patients with EGPA (Table 16.2) (28–36). EGPA syndrome has the lowest incidence of renal disease, but, when present, it is pathologically indistinguishable from the renal injury caused by MPA and GPA (22,37,38). The proportion of patients with renal involvement varies among cohorts in part based on the subspecialty interests of the investigators, with cohorts studied by nephrologists having a higher frequency of renal disease than those studied by pulmonologists and otolaryngologists.

Godman and Churg (39) recognized the relatedness of MPA, GPA, and EGPA in 1954, and this conclusion is supported by the association of these three disorders with ANCA (13–20). MPA, GPA, and EGPA also share pathologically indistinguishable pauci-immune systemic vasculitis that involves many different vessels, such as alveolar capillaries, dermal venules, sinus mucosal venules, and arterioles and arteries in peripheral nerves, skeletal muscles, and most viscera (22,26,27). The major pathologic difference in the kidneys is the occurrence of interstitial granulomatous inflammation in GPA and EGPA; however, identification of interstitial granulomatous inflammation in renal biopsy specimens from patients with GPA or EGPA is rare.

## CLINICAL PRESENTATION

### Signs and Symptoms

Patients with pauci-immune crescentic glomerulonephritis usually present with rapidly progressive glomerulonephritis with hematuria, proteinuria, and elevated serum creatinine (11,21,39–54). These patients have the typical necrotizing and crescentic glomerulonephritis that is classically associated with ANCA disease. A smaller subset of patients have smoldering disease and on renal biopsy have glomerular sclerosis either alone or accompanied by focal active lesions with necrosis and crescents (12).

**TABLE 16.1 Definitions of ANCA-associated vasculitis, microscopic polyangiitis, granulomatosis with polyangiitis, and eosinophilic granulomatosis with polyangiitis based on the 2012 Chapel Hill Consensus Conference on the Nomenclature of Systemic Vasculitis**

| | |
|---|---|
| ANCA-associated vasculitis (AAV) | Necrotizing vasculitis, with few or no immune deposits, predominantly affecting small vessels (i.e., capillaries, venules, arterioles, and small arteries), associated with MPO-ANCA or PR3-ANCA. Not all patients have ANCA. Add a prefix indicating ANCA reactivity, for example, PR3-ANCA, MPO-ANCA, ANCA-negative. |
| Microscopic polyangiitis (MPA) | Necrotizing vasculitis, with few or no immune deposits, predominantly affecting small vessels (i.e., capillaries, venules, or arterioles). Necrotizing arteritis involving small and medium arteries may be present. Necrotizing glomerulonephritis is very common. Pulmonary capillaritis often occurs. Granulomatous inflammation is absent. |
| Granulomatosis with polyangiitis (GPA) | Necrotizing granulomatous inflammation usually involving the upper and lower respiratory tract and necrotizing vasculitis affecting predominantly small to small vessels (e.g., capillaries, venules, arterioles, arteries, and veins). Necrotizing glomerulonephritis is common. |
| Eosinophilic granulomatosis with polyangiitis (EGPA) | Eosinophil-rich and necrotizing granulomatous inflammation often involving the respiratory tract and necrotizing vasculitis predominantly affecting small to medium vessels and associated with asthma and eosinophilia. ANCA is more frequent when glomerulonephritis is present. |

Modified from Jennette JC, Falk RJ, Bacon PA, et al. 2012 Revised International Chapel Hill Consensus Conference Nomenclature of Vasculitides. *Arthritis Rheum* 2013;65:1–11.

**TABLE 16.2 Comparison of approximate frequency of manifestations of microscopic polyangiitis to several other forms of small-vessel vasculitis**

| | MPA (%) | GPA (%) | EGPA (%) | IgA vasculitis (HSP) (%) | Cryoglobulinemic vasculitis (%) |
|---|---|---|---|---|---|
| Renal | 90 | 80 | 45 | 50 | 55 |
| Pulmonary | 50 | 90 | 70 | <5 | <5 |
| Cutaneous | 40 | 40 | 60 | 90 | 90 |
| ENT | 35 | 90 | 50 | <5 | <5 |
| Musculoskeletal | 60 | 60 | 50 | 75 | 70 |
| Neurologic | 30 | 50 | 70 | 10 | 40 |
| Gastrointestinal | 50 | 50 | 50 | 60 | 30 |

MPA, microscopic polyangiitis; GPA, granulomatosis with polyangiitis; EGPA, eosinophilic granulomatosis with polyangiitis; HSP, Henoch-Schönlein purpura; ENT, ear, nose, and throat.

From Jennette JC, Falk RJ. Small-vessel vasculitis. *N Engl J Med* 1997;337:1512.

At the time of biopsy in a cohort of over 300 pauci-immune crescentic glomerulonephritis patients evaluated in the University of North Carolina Nephropathology Laboratory (21), the mean age was 56 ± 20 years with a range of 2 to 92, male:female ratio was 1.0:0.9, mean serum creatinine was 6.5 ± 4.0 with a range of 0.8 to 22.1 mg/dL, and proteinuria was 1.94 ± 2.95 with a range of 0.11 to 18.00 g/dL. Patients with pauci-immune crescentic glomerulonephritis had less severe clinical and pathologic manifestations of disease than patients with anti-GBM crescentic glomerulonephritis but more severe disease than patients with immune complex crescentic glomerulonephritis (21).

Approximately 75% of patients with ANCA glomerulonephritis have manifestations of systemic small-vessel vasculitis at the time of initial presentation (44). Many patients report a recent "flu-like" illness prior to the onset of symptoms of renal disease or systemic vasculitis (44). Patients with ANCA disease who initially manifest only crescentic glomerulonephritis may later have manifestations of systemic small-vessel vasculitis. For example, Woodworth et al. (51) reviewed 19 patients who initially presented with crescentic glomerulonephritis alone and developed features of GPA 4 to 78 months after renal biopsy.

MPA, GPA, and EGPA share certain clinical features of small-vessel vasculitis, but each also has distinctive characteristics (28,33,34,44,52,53). Shared features include fever, arthralgias, myalgias, purpura, and peripheral neuropathy. These features occur at different frequencies in the different ANCA small-vessel vasculitides (see Table 16.2) (22). Cutaneous vasculitic lesions, especially palpable purpura and nodules, occur in approximately 40% of patients with MPA and GPA and in approximately 60% of patients with EGPA. Cutaneous nodules are caused by subcutaneous or dermal arteritis or granulomatous inflammation. Cutaneous nodules caused by granulomatous inflammation are more common in EGPA than in GPA. Peripheral neuropathy caused by vasculitis in epineural arterioles and arteries occurs in approximately 30% of patients with MPA, in 50% of patients with GPA, and in 70% of patients with EGPA. Clinical manifestations of vasculitic gastrointestinal involvement, such as abdominal pain, occur in approximately one half of the patients with each of the three categories of pauci-immune small-vessel vasculitis.

EGPA is distinguished from the other two pauci-immune small-vessel vasculitides by a history of asthma or severe allergic rhinitis (rhinosinusitis and/or polyposis) and blood eosinophilia (26,27,37,38,40–42,55,56). EGPA has an incidence of 1 to 5 per million compared to 1 to 10 per 100,000 for the other ANCA diseases (41). The vasculitic phase of the disease is typically preceded by eosinophilic inflammatory disease involving the lungs (eosinophilic pneumonia) or gut (eosinophilic gastroenteritis). The time interval between the onset of asthma and the onset of vasculitis varies from a few months to 30 years, but averages 3 to 4 years (42). The heart may be involved, and heart failure is the most common cause of death (56). Pulmonary, neural, and cutaneous disease are common, although the pulmonary involvement is usually eosinophilic pneumonia and granulomatous inflammation rather than capillaritis (41). Renal disease is usually mild, and it is much less frequent than in GPA and MPA (38). However, depending on the patient cohort, the frequency of renal involvement varies from 10% to 80% (40–42). The high frequency probably resulted from biased referrals to nephrology centers.

The typical histologic glomerular lesion is a pauci-immune necrotizing glomerulonephritis identical to the injury in patients with MPA or GPA. Extravascular granulomatous inflammation and eosinophilic infiltrates were uncommon (40). Pathologically, the glomerulonephritis in patients with EGPA tends to be less severe, but severe crescentic disease can occur (38). GPA usually has major involvement of the upper or lower respiratory tract or both by necrotizing granulomatous inflammation, with or without identifiable vasculitis (31,33,34,39). Destructive lesions in the cartilage and bone of the nose may cause collapse with saddle nose deformity. Acute and chronic pansinusitis is common, sometimes with extensive destruction of soft tissue and bone. Ocular and ear inflammation may occur. Subglottic stenosis may develop secondary to inflammation and scarring. Pulmonary involvement often results in radiographic evidence of pulmonary nodules, which may cavitate. These nodules are caused by necrotizing granulomatous inflammation. Pulmonary hemorrhage from the granulomatous inflammation, pulmonary arteritis, or alveolar capillaritis can be a major problem. Extrarenal manifestations may occur before the onset of nephritis or vice versa.

MPA shares pathologic and clinical vasculitic features with GPA and EGPA (see Table 16.2), but it lacks the granulomatous inflammation of GPA and the prodromal asthma of EGPA (2,22,44,52). Upper respiratory tract disease occurs in about

one third of patients with MPA, and pulmonary involvement occurs in about one half, although, by definition, it is caused by vasculitis alone (usually alveolar capillaritis) rather than by granulomatous inflammation. Thus, clinical signs and symptoms of respiratory tract disease alone are not adequate for distinguishing between MPA and GPA. In a given patient, it may be difficult or impossible to confirm or rule out granulomatous inflammation; thus, it is not always possible to distinguish confidently between MPA and GPA. This problem is offset by the knowledge that the treatment of both expressions of small-vessel vasculitis is similar in the presence of active major organ damage, such as crescentic glomerulonephritis or pulmonary capillaritis; therefore, if a diagnosis of ANCA-associated pauci-immune small-vessel vasculitis can be made, treatment can proceed even if it is not clear whether the patient has MPA or GPA. For clinical management, including selection of treatment regimen and prognostication, in addition to diagnostic classification of patients with ANCA disease based on clinicopathologic features into MPA, GPA, EGPA, and renal-limited disease, the ANCA antigen specificity should be included in the diagnosis (e.g., MPO-ANCA MPA, PR3-ANCA MPA, or ANCA-negative MPA) because the ANCA specificity correlates as well or better than the clinicopathologic category with clinical outcomes, such as response to treatment, likelihood of relapse, renal survival, and patient survival (27,50,57). In addition, a genome-wide association study (GWAS) revealed the strongest genetic associations with antigenic specificity of ANCA, not with the clinicopathologic syndrome (57). PR3-ANCA was associated with HLA-DP and the genes encoding α(1)-antitrypsin (SERPINA1) and proteinase 3 (PRTN3); and MPO-ANCA was associated with HLA-DQ (57).

Relapsing polychondritis is a rare presenting feature of patients with pauci-immune crescentic glomerulonephritis (58). At least some patients with relapsing polychondritis have ANCA (59), but the relationship of ANCA to crescentic glomerulonephritis in such patients has not been clarified. Other patients with crescentic glomerulonephritis and relapsing polychondritis have evidence for immune complex mediation of the glomerulonephritis (59). In some patients, relapsing polychondritis is a manifestation of MPA, along with pauci-immune crescentic glomerulonephritis and ANCA (60).

A minority of patients with ANCA glomerulonephritis give a history of exposure to a drug that is known to induce ANCA and pauci-immune crescentic glomerulonephritis (61). This has been reported most often with propylthiouracil (62–64), penicillamine (65,66), hydralazine (67), and minocycline (68). Because penicillamine can induce membranous glomerulopathy, some patients with penicillamine-induced ANCA and crescentic glomerulonephritis also have concurrent membranous glomerulopathy (69). Cocaine cut with levamisole, an antihelminthic, can induce ANCA vasculitis characterized by extremely high titers of MPO-ANCA, PR3-ANCA, and ANCA specific for elastase (70,71). Dual positivity for MPO-ANCA and PR3-ANCA should raise the possibility of cocaine-induced disease. Cocaine/levamisole-induced vasculitis has frequent cutaneous leukocytoclastic angiitis and upper respiratory tract involvement, but renal and lung involvement are uncommon (70). However, McGrath et al. (71) identified proteinuria, hematuria, or RBC casts in 8 of 30 patients with cocaine-induced ANCA vasculitis, two of whom had severe glomerulonephritis.

Patients with ESRD caused by ANCA disease have patient and renal transplant survival rates comparable to control groups (72). Recurrence rate is very low although patients with GPA have a higher relapse rate. In the published medical literature, the recurrence rate ranges between 0.08 and 0.006 per patient per year (72). Positive ANCA serology at the time of transplantation does not increase the risk of recurrent disease in the transplant (73,74).

## ANCA Serology

Pauci-immune crescentic glomerulonephritis is almost synonymous with ANCA crescentic glomerulonephritis, because 85% to 90% of pauci-immune crescentic glomerulonephritis occurs in ANCA-positive patients and 80% to 90% of the crescentic glomerulonephritis that occurs in ANCA-positive patients is pauci-immune (5,7–11,13,21,75,76). Serologic detection of ANCA is a useful diagnostic marker not only for renal-limited pauci-immune crescentic glomerulonephritis but also for MPA and GPA, and to a lesser extent EGPA. As with all serologic markers, however, ANCA are not absolutely specific or sensitive and thus must be interpreted in light of the other available data. Approximately 90% to 95% of patients with active untreated GPA or MPA, and approximately 45% of patients with EGPA, are ANCA positive (30). Thus, ANCA serology is least sensitive for EGPA; however, three quarters or more of EGPA patients with evidence for glomerulonephritis have ANCA (77).

ANCA are specific for proteins in the cytoplasmic granules of neutrophils and the lysosomes of monocytes (6,14,17,18,78). Davies et al. (79) first reported ANCA in the serum of patients with systemic small-vessel vasculitis and pauci-immune necrotizing glomerulonephritis. This association was confirmed by Hall et al. (80) in 1984. ANCA did not receive much attention until 1985 when a collaborative European group reported a strong association between ANCA and active GPA (81). Subsequent investigations revealed that ANCA also are associated with MPA, EGPA, renal-limited pauci-immune crescentic glomerulonephritis, and certain forms of drug-induced vasculitis (2,6,9,63,82–84).

Although ANCA are far more frequent in pauci-immune small-vessel vasculitis and glomerulonephritis, ANCA are identified in the serum of approximately one third of patients with anti-GBM disease (21,85–90). Compared with patients with anti-GBM alone, patients with both autoantibodies are older, often have features of systemic small-vessel vasculitis, and have a better prognosis for renal survival with treatment (85). The concurrent anti-GBM and ANCA specificity of serum is the result of two different antibody populations rather than one cross-reacting population (88,91), and the antigen specificities of anti-GBM antibodies are the same in patients with anti-GBM antibodies alone compared to those with ANCA and anti-GBM antibodies (91).

Two major antigen specificities for ANCA are seen in patients with pauci-immune crescentic glomerulonephritis and small-vessel vasculitis. These two specificities cause two staining patterns, cytoplasmic (c-ANCA) and perinuclear (p-ANCA), when ANCA are detected by indirect immunofluorescence microscopy using alcohol-fixed neutrophils as substrate. The perinuclear distribution of antigen is an artifact of substrate preparation that results from redistribution of the antigen from the cytoplasm to the nucleus during substrate preparation (92).

More precise determination of antigen specificity is accomplished by enzyme immunoassay. The most common cANCA specificity is for proteinase 3 (PR3-ANCA) (93–95), and the most common p-ANCA specificity is for myeloperoxidase (MPO-ANCA) (6). p-ANCA that do not have specificity for MPO are associated with various inflammatory diseases, such as ulcerative colitis, sclerosing cholangitis, autoimmune hepatitis, rheumatoid arthritis, and Felty syndrome (18). Thus, a positive p-ANCA result in this context is a "false-positive" result with respect to pauci-immune crescentic glomerulonephritis even though it is an analytically true positive result and is a true positive result for inflammatory bowel disease. Because of this and other instances in which a positive indirect immunofluorescence microscopy assay (IFA) result does not correspond to a positive MPO-ANCA or PR3-ANCA, an international consensus conference recommendation is to perform both IFA for p-ANCA and c-ANCA and enzyme-linked immunosorbent assay (ELISA) for MPO-ANCA and PR3-ANCA when testing for the presence of pauci-immune crescentic glomerulonephritis or small-vessel vasculitis (96). When both a positive IFA and positive ELISA are required to conclude that the ANCA serology is positive, this positive result has a sensitivity of approximately 81% and a specificity of approximately 96% for pauci-immune crescentic glomerulonephritis (with or without systemic vasculitis) (16). Figure 16.2 shows the calculated predictive value of an ANCA serology result with this sensitivity and specificity. As with all diagnostic testing, the positive and negative predictive values of the test are dependent on the pretest likelihood of the disease, which in turn is determined by the prevalence of the disease in patients with the signs and symptoms of the patient being tested. As noted in Figure 16.1, pauci-immune crescentic glomerulonephritis has a prevalence of approximately 50% in all patients with crescents and an even higher prevalence when there are more than 50% of glomeruli with crescents. Assuming a prevalence (pretest likelihood) of 50%, a positive ANCA result in a patient with signs and symptoms of rapidly progressive glomerulonephritis would yield a posttest likelihood of the disease (positive predictive value) of 95%, and a negative result would yield a posttest likelihood of no disease (negative predictive value) of 85%. The negative predictive value in this instance is very close to the percentage of all patients with pauci-immune crescentic glomerulonephritis who in fact are ANCA negative using first-generation ANCA immunoassays. When there is low pretest likelihood of pauci-immune crescentic glomerulonephritis, for example, in patients with hematuria and proteinuria but no renal insufficiency, the major value of ANCA testing is to rule out ANCA disease or to increase the suspicion for ANCA disease to a level that will prompt more extensive and rapid diagnostic evaluation (e.g., renal biopsy). In this setting, a negative result provides a negative predictive value mathematically at essentially 100%. Although a positive result in this setting has a very low positive predictive value that certainly would not warrant immediate institution of immunosuppressive therapy, it does increase the likelihood of this life-threatening disease to a level that would warrant more careful and timely diagnostic evaluation.

The antigen specificity of ANCA correlates to a degree with the category of pauci-immune small-vessel vasculitis, although overlap is so great that antigen specificity alone cannot be used to differentiate between them. Patients with

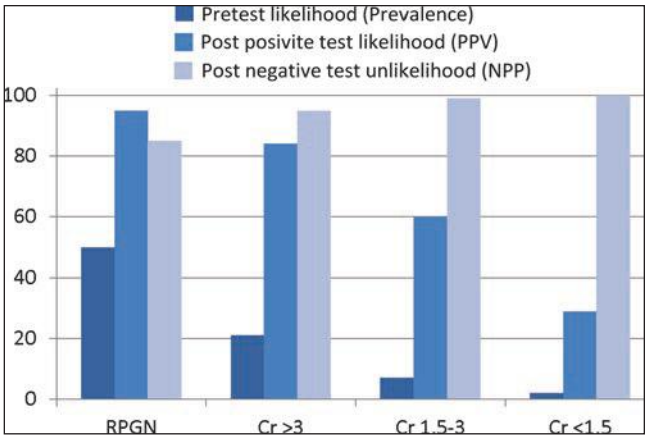



**FIGURE 16.2 Predictive value of ANCA serology for pauci-immune crescentic glomerulonephritis in adults with clinical evidence for glomerulonephritis (e.g., dysmorphic hematuria and proteinuria) and different degrees of renal insufficiency.** In a patient with RPGN, the pretest likelihood of pauci-immune crescentic glomerulonephritis is approximately 50%, increases to 95% with a positive result, and falls to 15% with a negative result. By comparison, in a nephritic adult with normal renal function, the pretest likelihood of ANCA disease of less than 5% increases to only approximately 30% with a positive result but is essentially 0% with a negative result; however, the positive result increases the likelihood enough to warrant expeditious further evaluation including renal biopsy. RPGN, rapidly progressive glomerulonephritis with rapid loss of GFR and overt active urinary finds of glomerulonephritis; PPV, positive predictive value; NPV, negative predictive value. (Data derived from Lim LC, Taylor JG III, Schmitz JL, et al. Diagnostic usefulness of antineutrophil cytoplasmic autoantibody serology: Comparative evaluation of commercial indirect fluorescent antibody kits and enzyme immunoassay kits. *Am J Clin Pathol* 1999;111:363.)

active GPA usually have PR3-ANCA. In North America and Europe, patients with MPA have slightly more MPO-ANCA than PR3-ANCA, and patients with EGPA and renal-limited pauci-immune crescentic glomerulonephritis have predominantly MPO-ANCA (Fig. 16.3). ANCA specificity correlates with clinical symptoms independent of the clinicopathologic variants, for example, 90% of patients with ANCA who have destructive upper respiratory tract disease (e.g., saddle nose deformity) have PR3-ANCA, whereas most patients with exclusively or predominantly renal involvement have MPO-ANCA (50). Dual positivity for both MPO-ANCA and PR3-ANCA is rare (13) except in the setting of drug-induced ANCA (71). It is extremely important to note that some patients with otherwise absolutely classic disease have pauci-immune crescentic glomerulonephritis, MPA, GPA, or EGPA, but are ANCA negative. Thus, a negative ANCA result by no means rules out these diseases, and patients who are ANCA negative have the same prognosis and should receive the same treatment as ANCA-positive patients (23).

Roth et al. (97) studied the epitope specificity of MPO-ANCA IgG using a highly sensitive assay. They detected low titer MPO-ANCA natural autoantibodies in healthy controls directed against a limited set of epitopes. In patients with MPO-ANCA disease, autoantibodies against greater than 20 MPO epitopes were identified. MPO-ANCA IgG in patients

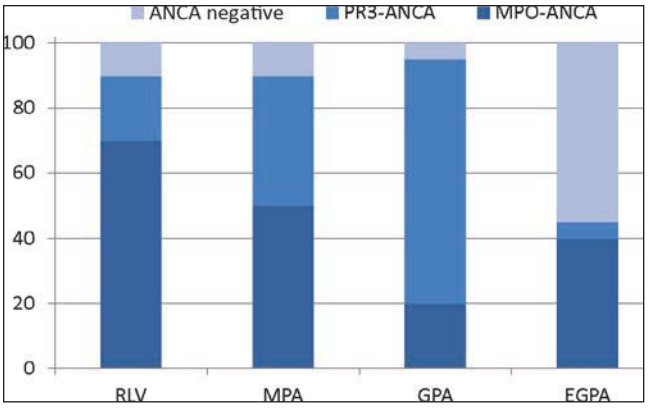



**FIGURE 16.3 Approximate frequency of MPO-ANCA and PR3-ANCA in patients with active untreated GPA, MPA, EGPA, or renal-limited pauci-immune crescentic glomerulonephritis (RLV).** GPA patients have a lower frequency of ANCA-positivity if there is no renal involvement. EGPA patients are greater than 75% ANCA-positive when glomerulonephritic is present. These data are for patients in North America and Europe. Patients in Asia have a marked predominance of MPO-ANCA in all clinicopathologic variants. MPA, microscopic polyangiitis; GPA, granulomatosis with polyangiitis; EGPA, eosinophilic granulomatosis with polyangiitis.

with active disease reacted with a restricted set of epitopes that were not recognized by IgG from patients in remission. Interestingly, this assay method detected MPO-ANCA in ANCA-negative disease that reacted against a sole linear MPO epitope. This reactivity could only be detected using IgG rather than serum because serum contains a ceruloplasmin fragment that binds to MPO and blocks detection of these autoantibodies by routine serologic methods (97). Thus, some ANCA-negative patients appear to in fact have MPO-ANCA that is not detected by first-generation clinical ANCA assays; however, this experimental observation has not been confirmed, and an assay to detect these masked ANCA is not commercially available at this time.

Kain et al. (98,99) have reported that lysosomal membrane protein 2 (LAMP2) is another major ANCA autoantigen in addition to MPO and PR3 in patients with pauci-immune small-vessel vasculitis and glomerulonephritis. LAMP2-ANCA were detected in patients with either MPO-ANCA, PR3-ANCA, or ANCA-negative pauci-immune crescentic glomerulonephritis. LAMP2 is homologous to a bacterial adhesin called FimH, and LAMP2-ANCA have been hypothesized to arise through molecular mimicry caused by infection with fimbriated Gram-negative bacteria. To support this hypothesis, Kain et al. reported induction of crescentic glomerulonephritis in rabbits immunized with FimH. However, our research group has not been able to reproduce these results (100).

## PATHOLOGIC FINDINGS

### Gross Pathology

In patients with severe disease, the kidneys are normal or slightly increased in size. When acute pauci-immune crescentic glomerulonephritis is extensive and severe, the subcapsular surface and cut surfaces have scattered small red dots caused by blood within the Bowman spaces and in tubular lumens. Slightly larger irregular petechial lesions may be present and represent hemorrhage from necrotic glomeruli and small interlobular arteries. Occasionally, pale or hemorrhagic nodular inflammatory lesions can be discerned in arcuate arteries and interlobar arteries. However, grossly discernible nodular and aneurysmal lesions in interlobar and larger arteries are rare in the pauci-immune small-vessel vasculitis, unlike the higher frequency of such lesions in polyarteritis nodosa and Kawasaki disease (see Chapter 17). When grossly identifiable arterial lesions are present, they are usually in the arcuate and interlobar arteries and may manifest as pale nodules or foci of hemorrhage. One may see cortical infarcts, which are usually small. In Heptinstall' series (101) of 18 patients with systemic arteritis and glomerulonephritis, which was consistent with MPA, 16 patients had arteritis in the kidney at postmortem examination, 4 had grossly identifiable aneurysms, and 7 had infarcts. Therefore, the presence of grossly identifiable aneurysms does not differentiate between the pauci-immune small-vessel vasculitides and polyarteritis nodosa, although such aneurysms are much more frequent in the latter.

A few patients, especially those with GPA, have focal hemorrhagic papillary necrosis that is caused by leukocytoclastic angiitis affecting the medullary vasa recta (3,102,103). The lesions usually appear as fan-shaped hemorrhagic areas in the inner medulla. Watanabe et al. (102) observed papillary necrosis in 5 of 23 autopsies of patients with GPA.

Grossly apparent granulomatous inflammation is rare in the kidneys of patients with GPA. It causes irregular pale to hemorrhagic zones that may involve the cortex or medulla.

Hydronephrosis caused by ureteral obstruction from vasculitic and granulomatous lesions is a rare complication of GPA (104,105). Ureteral involvement by granulomatous inflammation has been a rare problem in renal transplants in patients with GPA (106). Even when ureteral obstruction is not present, careful examination of the ureter may reveal areas of hemorrhage or nodularity that prove to be vasculitic or granulomatous lesions when they are examined microscopically.

Pauci-immune small-vessel vasculitis can affect any organ of the body and may produce gross lesions in these organs. Favored sites, in addition to the kidney, include the skin, respiratory tract, gastrointestinal tract, skeletal muscle, and peripheral nerves. Small foci of hemorrhage at sites of vascular rupture caused by necrotizing inflammation are the most frequent manifestation of vasculitis. A variation on this theme is the more extensive pulmonary hemorrhage that can result from alveolar capillaritis. Grossly, the granulomatous inflammation of GPA and EGPA, which most often affects the respiratory tract, typically produces irregular pale zones with varying degrees of hemorrhage. Granulomatous inflammation in the lungs may produce cavities, and granulomatous inflammation in the nose may cause perforation of the nasal septum or collapse of the bridge of the nose.

### Light Microscopy

#### Glomeruli

The hallmark histologic lesions of acute pauci-immune ANCA glomerulonephritis are crescents and fibrinoid necrosis (Figs. 16.4 to 16.7), which occur at the same frequency irrespective

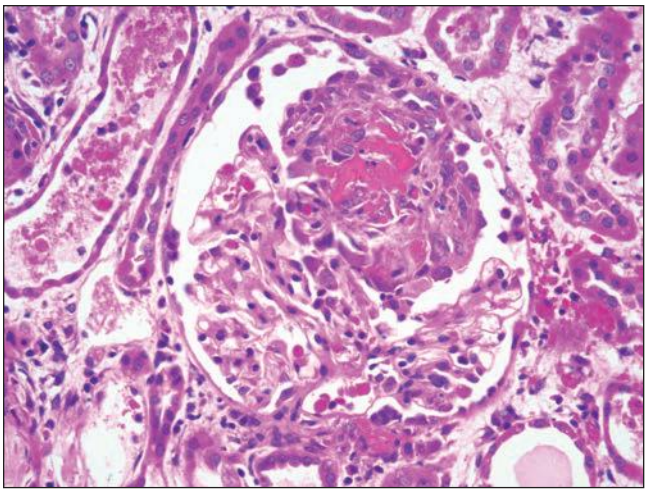


**FIGURE 16.4 Segmental fibrinoid necrosis that is deeply acidophilic surrounded by early crescent formation.** Note that the nonnecrotic segments are relatively unremarkable with thin capillary walls and no hypercellularity. Also note the acute tubulointerstitial changes including interstitial edema and tubular epithelial cell simplification (e.g., of the tubule in the upper left that has flat epithelium and cellular debris in the lumen). (H&E.)

of the presence or absence of associated systemic vasculitis (2,3,11,12,21,22,36,70,71,107). The glomerular inflammation is accompanied by proportional nonspecific tubulointerstitial inflammatory lesions (107). In an analysis of 45 ANCA-positive patients with glomerulonephritis and systemic small-vessel vasculitis, renal biopsies demonstrated crescents in 93% of patients and glomerular necrosis in 98% (2). In a study of 32 renal biopsies from patients with MPA, Savage et al. (36) identified glomerular segmental necrosis in 100% and crescent formation in 88%. Likewise, in a study of 20 renal biopsies in MPA by D'Agati et al. (108), 80% of 20 specimens had segmental glomerular necrosis, and 85% had crescents. The severity of acute lesions ranges from focal segmental fibrinoid necrosis and crescent formation affecting less than 10% of glomeruli to severe diffuse necrotizing and crescentic glomerulonephritis with global necrosis of virtually all glomeruli. On average in a given renal biopsy specimen, 45% to 55% of glomeruli have crescents, and 20% to 25% of glomeruli have fibrinoid necrosis (12). The fibrinoid necrosis usually is accompanied by crescent formation. For example, Hauer et al. (109) identified fibrinoid necrosis in the absence of extracapillary hypercellularity in only 1 of 87 glomeruli from renal biopsy specimens of pauci-immune crescentic glomerulonephritis that were examined by serial section. Eisenberger et al. (23) compared glomerular crescents, necrosis, and sclerosis in patients with ANCA-negative pauci-immune glomerulonephritis and patients with ANCA-positive pauci-immune glomerulonephritis and found no significant difference, which corresponds to our experience.

The extent of crescent formation is not different between patients with PR3-ANCA versus MPO-ANCA. Jennette (21) observed a mean of 48% glomerular involvement with crescents in patients with MPO-ANCA ($n$ = 102) versus 46% with PR3-ANCA ($n$ = 52), Hauer et al. (12) observed 46% with MPO-ANCA ($n$ = 58) versus 50% with PR3-ANCA ($n$ = 63), and Vizjak et al. (11) observed 44% with MPO-ANCA ($n$ = 74) versus 39% with PR3-ANCA ($n$ = 55).

Fibrinoid necrosis is fuchsinophilic (red) with trichrome staining (Figs. 16.5A and 16.6A), a feature that distinguishes it from the sclerosis (blue or green) that develops at sites of scarring caused by fibrinoid necrosis. Special stains for basement membranes, such as Jones silver methenamine (Fig. 16.6B) and periodic acid–Schiff (PAS) (Fig. 16.6C), reveal disruption of the GBM, and often the Bowman capsule as well, in the areas of fibrinoid necrosis. Fibrin thrombi often are present in glomerular capillaries adjacent to necrotic zones (110) and have the same tinctorial properties as the fibrinoid material in necrotic segments and the fibrin between crescent cells. Foci

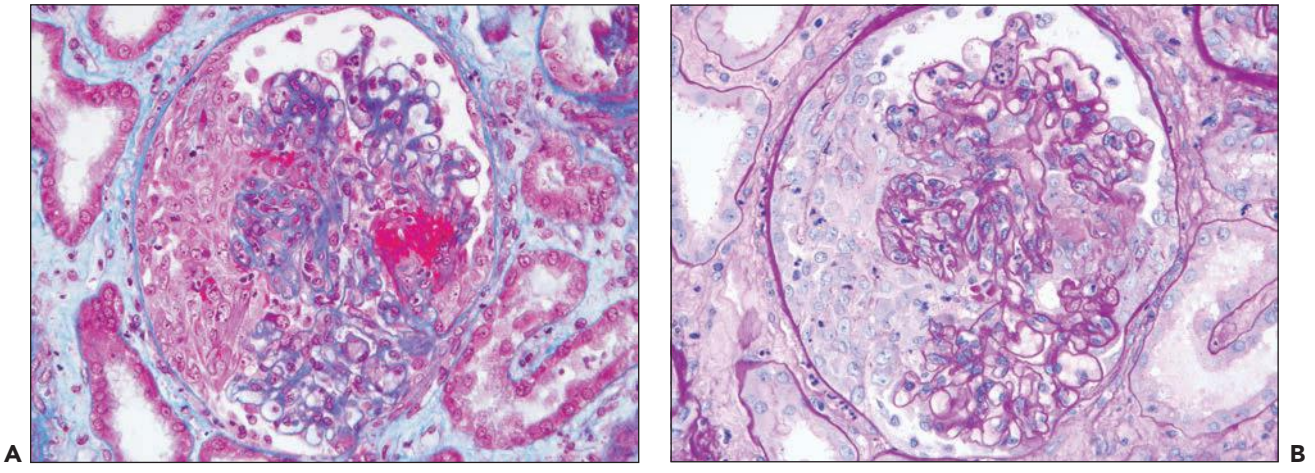



**FIGURE 16.5** Glomerulus from a patient with MPO-ANCA renal-limited pauci-immune crescentic glomerulonephritis stained with Masson trichrome **(A)** and PAS **(B)** showing a well-formed cellular crescent on the left and a well-defined irregular focus of segmental fibrinoid necrosis on the right that stains red with the Masson trichrome stain **(A)**. Most capillary lumens are patent, but there are a few neutrophils in some capillaries, for example, at the top of the tuft in **(B)**. Note also the edema in the periglomerular interstitium.

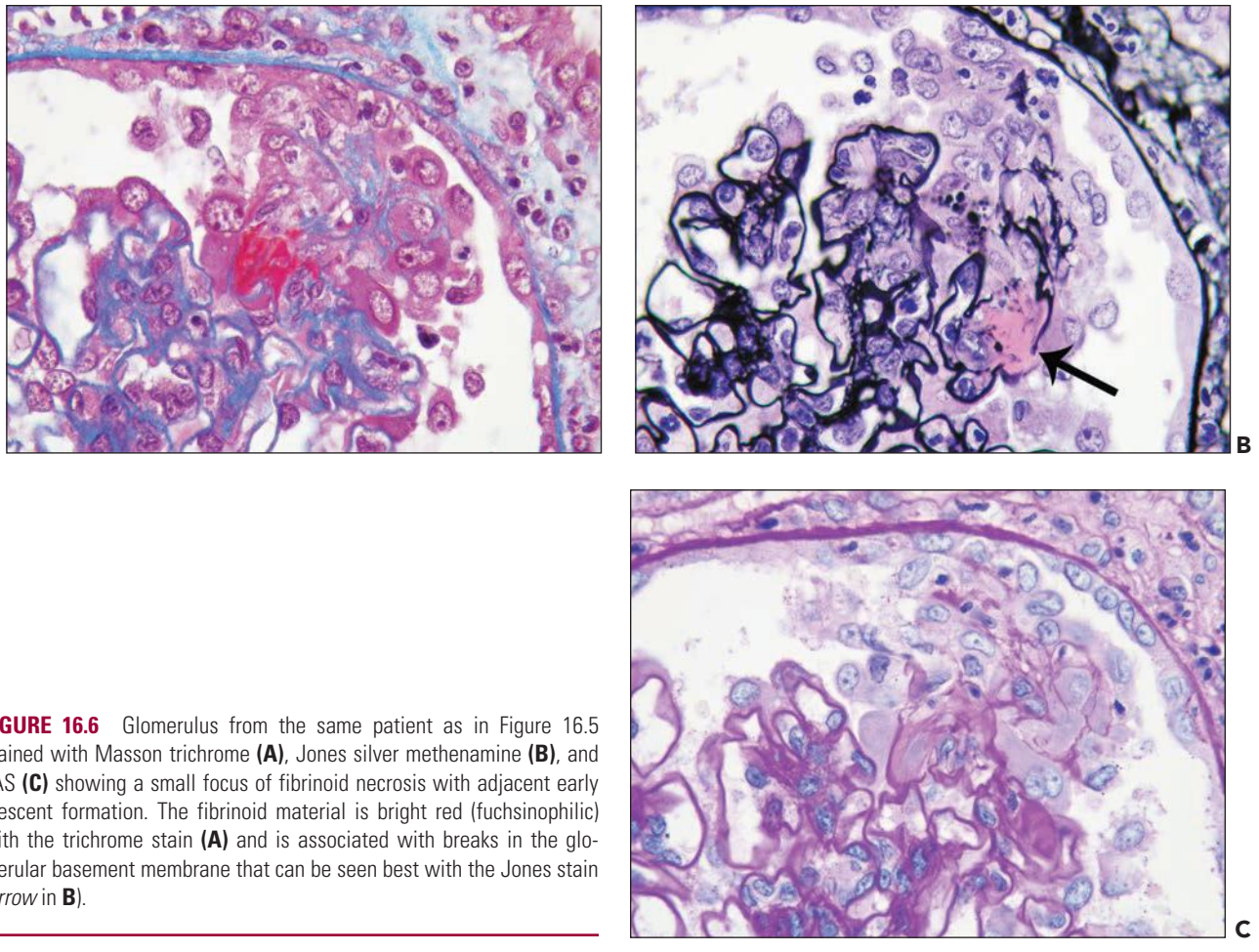



**FIGURE 16.6** Glomerulus from the same patient as in Figure 16.5 stained with Masson trichrome **(A)**, Jones silver methenamine **(B)**, and PAS **(C)** showing a small focus of fibrinoid necrosis with adjacent early crescent formation. The fibrinoid material is bright red (fuchsinophilic) with the trichrome stain **(A)** and is associated with breaks in the glomerular basement membrane that can be seen best with the Jones stain (*arrow* in **B**).

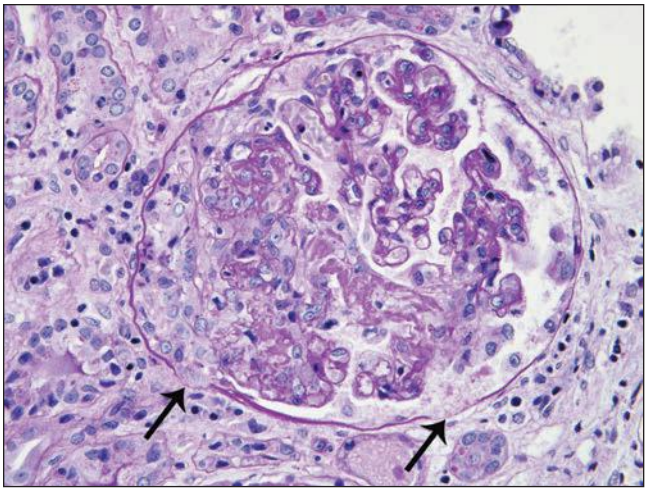


**FIGURE 16.7 Glomerulus from a patient with microscopic polyangiitis showing segmental fibrinoid necrosis with an adjacent relatively small cellular crescent.** The PAS stain allows demarcation of focal dissolution of Bowman capsule basement membrane (*arrows*). Glomerular basement membranes and mesangial matrix have been destroyed in the area of fibrinoid necrosis. Note the periglomerular tubulointerstitial inflammation and edema.

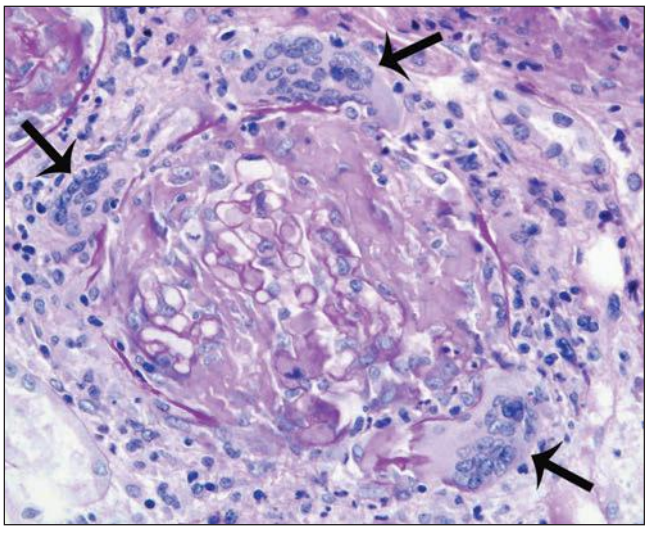


**FIGURE 16.8** Glomerulus from a child with cANCA GPA showing severe fibrinoid necrosis of the glomerular tuft, extensive disruption of Bowman capsule basement membrane, and prominent periglomerular granulomatous inflammation with multinucleated giant cells (*arrows*). (PAS stain.)

of fibrinoid necrosis in pauci-immune crescentic glomerulonephritis often contain neutrophil granule constituents indicating that there has been extensive neutrophil activation and degranulation at these sites. For example, using double immunohistochemical staining procedures, Bajema et al. (111) identified PR3, MPO, elastase, and lactoferrin at sites of glomerular fibrinoid necrosis.

Crescent formation appears to begin adjacent to foci of segmental necrosis (Figs. 16.4, 16.6, and 16.7) (109), but it can extend to surround most, if not all, of the tuft forming a circumferential crescent. Breaks in the Bowman capsule, which are identified best with special stains such as PAS or Jones silver methenamine, are common in pauci-immune crescentic glomerulonephritis (75,76) (see Fig. 16.7). There may be extensive dissolution of the Bowman capsule with severe necrotizing glomerular injury, resulting in continuity between the glomerular inflammation and pronounced periglomerular inflammation (Fig. 16.8). This extremely lytic necrosis is similar to focal lytic lesions in many other small vessels in ANCA disease (112). Periglomerular interstitial infiltrates of leukocytes sometimes have a granulomatous appearance (11,113). Vizjak et al. (11) observed periglomerular granulomatous inflammation more often in MPO-ANCA disease than in PR3-ANCA disease, which indicates that this reaction is not related to the type of granulomatous inflammation that corresponds to GPA. Hauer et al. (12) observed no difference in the extent of periglomerular granulomatous inflammation in patients with GPA versus MPA or in patients with PR3-ANCA versus MPO-ANCA. Occasional specimens have multinucleated giant cells in the glomerular or periglomerular infiltrates. The multinucleated giant cells seem to localize preferentially to sites of fragmentation of the Bowman capsule. Which is cause and which is effect, if either, is unknown. When there are only a few residual fragments of glomerular structure remaining, care must be taken not to mistake this destructive glomerular inflammation for purely interstitial necrotizing granulomatous inflammation. This may require examination of the specimen at multiple levels of section with special stains that highlight residual fragments of GBM and the Bowman capsule.

Periglomerular granulomatous inflammation probably is not specific for pauci-immune crescentic glomerulonephritis or ANCA glomerulonephritis, but this is controversial. Some investigators have reported periglomerular granulomatous inflammation only in patients with ANCA. For example, Rutgers et al. (89) identified periglomerular granulomas in 11% of patients with MPO-ANCA, 40% of patients with concurrent MPO-ANCA and anti-GBM, and in 0% of 13 patients with anti-GBM but no ANCA. However, periglomerular granulomas have been reported in anti-GBM glomerulonephritis without ANCA by other investigators (114,115). We also have observed periglomerular granulomas in specimens from patients with anti-GBM but no ANCA.

In the acute phase, glomeruli may have total destruction of some segments by fibrinoid necrosis, whereas adjacent segments are histologically unremarkable or contain only a few neutrophils (see Figs. 16.4 to 16.6). Occasional specimens with acute lesions will have numerous neutrophils in capillary lumens adjacent to the foci of segmental necrosis (Fig. 16.9). A similar clustering of neutrophils at sites of glomerular necrosis has been observed in a mouse model of pauci-immune crescentic glomerulonephritis caused by anti-MPO ANCA (116).

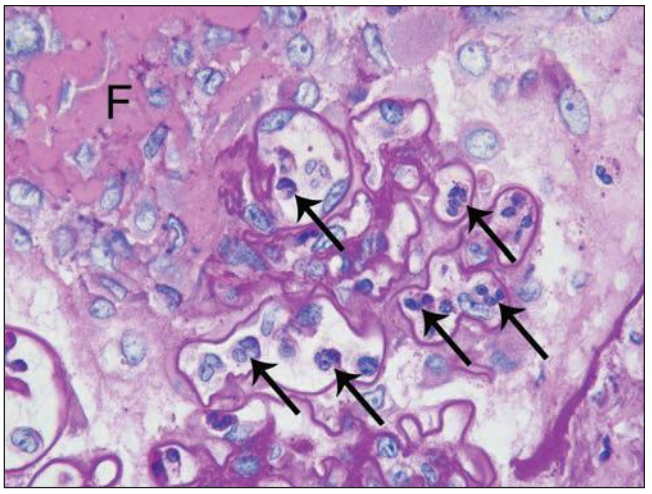



**FIGURE 16.9** High magnification of a glomerular segment from the same specimen as in Figure 16.5 showing prominent influx and margination of neutrophils (*arrows*) and a site of necrosis with adjacent fibrinous material (*F*) spilling into the Bowman space where it is associated with cells that probably include disrupted epithelial cells and infiltrating monocytes and macrophages. (PAS stain.)

Within days, mononuclear leukocytes become predominant in the necrotizing lesions, and collagenous matrix progressively replaces the fibrinoid material. Thus, at the time of biopsy, macrophages often are the predominant cell types in glomeruli and are accompanied by varying numbers of neutrophils and T lymphocytes (117,118). Glomerular lesions in various phases of evolution often are present in the same renal biopsy specimens indicating recurring episodes of acute glomerular injury. In some specimens, a careful search of multiple levels may be required to identify an active necrotizing lesion among many glomeruli with more advanced lesions. A few specimens from patients presenting to a nephrologist for the first time will have a marked dichotomy between focal chronic sclerotic lesions and focal acute necrotizing lesions, suggesting that the patient has had an earlier episode of active disease that remitted without treatment. This finding is more common in repeat biopsies from patients who have been treated for pauci-immune crescentic glomerulonephritis, enter remission, and later are rebiopsied because of a clinical relapse.

The finding of severe fibrinoid necrosis adjacent to relatively normal glomerular segments is similar to the features of acute anti-GBM glomerulonephritis, but contrasts with immune complex crescentic glomerulonephritis, which typically has more glomerular endocapillary hypercellularity, less glomerular necrosis, little or no disruption of the Bowman capsule, and less periglomerular inflammation (75,76). The histologic features of renal-limited pauci-immune crescentic glomerulonephritis are indistinguishable from those of pauci-immune crescentic glomerulonephritis that occurs as a component of systemic vasculitis (2,3,26,44).

When the histologic glomerular features of an immune complex glomerulonephritis, such as endocapillary hypercellularity or thick capillary walls, are observed in an ANCA-positive patient with glomerular fibrinoid necrosis or crescent formation, the possibility of concurrent immune complex and

ANCA disease must be considered. This will be revealed conclusively by immunofluorescence and electron microscopy. Likewise, if a renal biopsy specimen with confirmed immune complex disease has more extensive necrosis or crescent formation than would be expected given the type of immune complex disease, such as membranous glomerulopathy with exclusively subepithelial deposits or IgA nephropathy with exclusively mesangial deposits, the possibility of concurrent immune complex and ANCA disease must be considered, and the patients should be tested serologically for ANCA. Not surprisingly given their relative frequency, the immune complex diseases that have been reported most often in association with ANCA are membranous glomerulonephritis (119–123), IgA nephropathy (124–128), diabetic glomerulosclerosis (129), and lupus glomerulonephritis (122,130).

With time, the necrotic glomerular lesions of pauci-immune crescentic glomerulonephritis heal as segmental or global sclerosis (Fig. 16.10). In a study that compared initial renal biopsy specimens to follow-up biopsy specimens by Hauer et al. (131), the follow-up specimens had fewer cellular crescents (30% vs. 57%), less glomerular fibrinoid necrosis (8% vs. 22%), and more glomerular sclerosis (39% vs. 12%). The proportion of normal glomeruli did not change (30% in follow-up and 29% in initial biopsies), suggesting that no glomeruli were recruited into the disease process after therapy was initiated. The proportion of sclerotic glomeruli at the time of biopsy tends to be higher in MPO-ANCA glomerulonephritis than in PR3-ANCA glomerulonephritis. Hauer et al. (12) reported glomerular sclerosis in a mean of 25% of glomeruli in MPO-ANCA disease compared with 15% in PR3-ANCA disease ($P = 0.02$). Similarly, Vizjak et al. (11) reported global or segmental glomerular sclerosis in a mean of 37% of glomeruli in MPO-ANCA disease compared with 18% in PR3-ANCA disease ($P < 0.01$).

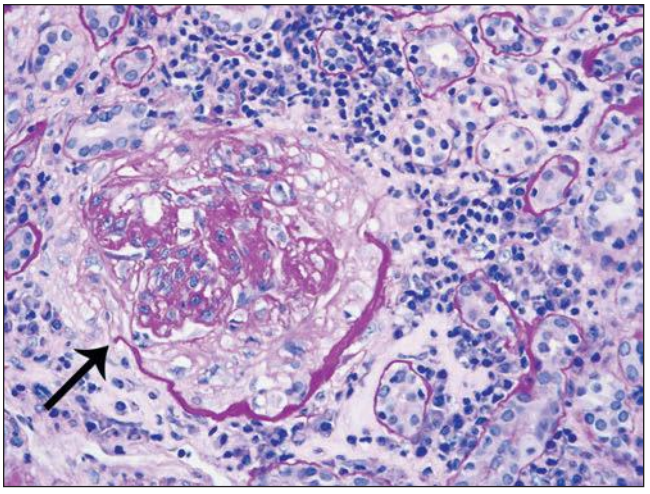


**FIGURE 16.10 Glomerulus from a patient with chronic pauci-immune crescentic glomerulonephritis showing global glomerular sclerosis and a fibrotic crescent.** There is extensive dissolution of the Bowman capsule (beginning at the *arrow*). Note that there is diffuse tubulointerstitial inflammation with interstitial influx of mononuclear leukocytes, focal tubulitis, tubular atrophy, and interstitial fibrosis. (PAS stain.)

Because there are no well-defined immune deposits to mark the initial pathogenic mechanism, the glomerular scarring may be confused with chronic injury caused by some other process, such as focal segmental glomerulosclerosis or arterionephrosclerosis. Special stains may reveal fragmented basement membranes embedded in the amorphous collagenous scar, a finding that attests to the necrotizing injury that preceded the scarring. As with other forms of crescentic glomerulonephritis, the crescents evolve from cellular to fibrocellular to fibrotic phases (discussed in detail in Chapter 15). The progressive fibrosis of crescents usually is accompanied by a comparable degree of progressive sclerosis of the underlying glomerular tuft.

Although glomerular necrosis and crescents are the most common and most distinctive histopathologic features of ANCA pauci-immune crescentic glomerulonephritis at the time of renal biopsy, a spectrum of acute and chronic lesions can be observed in renal tissue from patients with ANCA disease who undergo renal biopsy (11,12,132). In an analysis of 232 renal biopsy specimens with ANCA-positive pauci-immune renal disease who were evaluated at the University of North Carolina Nephropathology Laboratory, 75% had both glomerular necrosis and crescent formation, and this involved over 50% of glomeruli in 48% of specimens (132) (Fig. 16.11). Comparatively mild, possibly early, acute disease with focal necrosis but no crescents was observed in only 3% of specimens. Extensive crescent formation involving more than 50% of glomeruli in the absence of active necrosis was seen in 4%. Either focal (4%) or diffuse (12%) sclerosing glomerulonephritis with no necrosis and no cellular crescents was seen in 16% of specimens, presumably representing a chronic phase of disease. Many of the specimens with cellular crescents and necrosis also had minor populations of sclerotic glomeruli. Rare biopsy specimens from patients with clinical evidence for ANCA glomerulonephritis had no lesions identified by light microscopy (1%) or only tubulointerstitial inflammation (1%). These patients may have had focal ANCA glomerulonephritis that was not sampled in the renal biopsy specimen. However, Lockwood (133) proposed that some patients might have ANCA disease characterized by tubulointerstitial nephritis in the absence of glomerulonephritis.

ANCA pauci-immune crescentic glomerulonephritis can be classified based on the predominant pattern of injury at the time of biopsy. Several groups have investigated the predictive value of a classification system initially developed using specimens from European Vasculitis Study Group (EUVAS) patient cohorts (49). This classification system categorizes ANCA pauci-immune glomerulonephritis into 4 classes based on the histopathologic glomerular findings, that is, focal, crescentic, sclerotic, or mixed based on the criteria given below. Of 100 biopsies with ANCA pauci-immune glomerulonephritis, 13 were classified as sclerotic (≥50% globally sclerotic glomeruli), 16 as focal (≥50% normal glomeruli), 55 as crescentic (≥50% of glomeruli with cellular crescents), and 16 as mixed (greater than 50% of glomeruli with lesions but no predominance of crescentic or sclerotic glomeruli). Renal survival correlated with these classes. Renal survival at 1 year was 93% for the focal class, 84% for crescentic class, 69% for the mixed class, and 50% for the sclerotic class. Renal survival at 5 years was 93% in the focal class, 76% in crescentic class, 61% in mixed class, and 50% in sclerotic class. Several additional validations

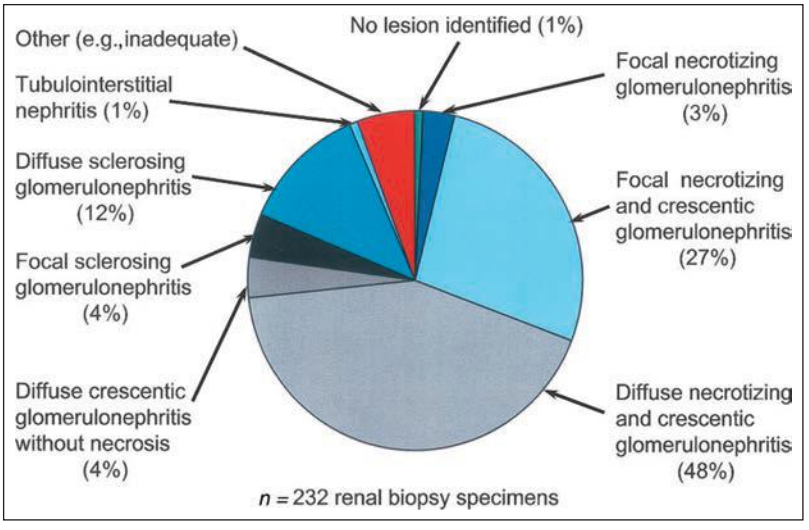



**FIGURE 16.11 Categorization of the pathologic findings in 232 renal biopsy specimens from ANCA-positive patients who underwent renal biopsy evaluation at the University of North Carolina Nephropathology Laboratory because of clinical evidence for renal disease.** Patients categorized as having necrotizing and crescentic glomerulonephritis often also had some degree of glomerular sclerosis. Patients categorized as having sclerosing glomerulonephritis had no necrosis, and any crescents were fibrocellular or fibrotic.

of the classification system have confirmed its utility with the focal class consistently having the best outcome and the sclerotic class the worst outcome (134–136).

## Renal Vessels Other Than Glomeruli

Arteries, arterioles, and medullary vasa recta should be carefully examined in a specimen that has pauci-immune crescentic glomerulonephritis because of the possibility of accompanying necrotizing small-vessel vasculitis that can cause renal arteritis, arteriolitis, and medullary angiitis (43,113,137). The interlobular arteries are the most commonly affected arteries, but any artery may be involved. Hilar arteriolar necrosis may be continuous with glomerular necrosis. If vasculitis is identified in addition to the pauci-immune crescentic glomerulonephritis, clinical, serologic, and other pathologic data should be evaluated to determine the appropriate clinicopathologic diagnosis for the vasculitis. The presence of intrarenal vasculitis increases the likelihood of systemic vasculitis, but a minority of patients will have renal extraglomerular vasculitis with no evidence for systemic vasculitis.

The characteristic acute arterial and arteriolar lesion in the kidney and elsewhere in pauci-immune small-vessel vasculitis is segmental fibrinoid necrosis with associated mural and perivascular infiltration of neutrophils or mononuclear leukocytes or both (Fig. 16.12) (2,3,11,43). The necrotizing arteritis of pauci-immune small-vessel vasculitis is histologically indistinguishable from the necrotizing arteritis of polyarteritis nodosa (see Chapter 17) (3,22). In the kidney, the presence of glomerulonephritis indicates that an arteritis is a component of a small-vessel vasculitis instead of a medium-sized vasculitis, such as polyarteritis nodosa (3,26). Jennette and Thomas (2) identified arteritis in 13% of renal biopsies from 45 patients with ANCA glomerulonephritis, Savage et al. (36) identified vasculitis in 19% of 32 renal biopsies from patients with MPA, and Vizjak et al. (11) identified active vasculitis in 23% of 55 patients with PR3-ANCA glomerulonephritis and 23% of 74 patients with MPO-ANCA glomerulonephritis. This is an underestimate of the frequency of arteritis because the lesions always are focal, and renal biopsy specimens provide only a very small sampling of renal arteries.

The acute arterial lesions have segmental fibrinoid necrosis with varying degrees of neutrophilic and mononuclear leukocyte infiltration. On hematoxylin and eosin (H&E)–stained sections, fibrinoid necrosis is deeply acidophilic, often with a coarsely fibrillary texture and ragged margins especially at the interface with the perivascular interstitium (see Fig. 16.12A and B). The fibrinoid necrosis is accentuated in red with a Masson trichrome stain (see Fig. 16.12C and D), a feature that facilitates differentiation from sclerosis, which stains blue or green with this stain (Figs. 16.13 and 16.14).

Initially, the intramural and perivascular infiltrate contains many neutrophils, often with karyorrhexis (leukocytoclasia). However, by the time lesions are examined pathologically, they often have predominantly mononuclear leukocytes (primarily macrophages and T cells), usually with admixed neutrophils and eosinophils. Numerous eosinophils may be present in the vascular and perivascular infiltrates of all types of pauci-immune small-vessel vasculitis and thus are not specifically diagnostic for EGPA. However, numerous eosinophils should at least heighten suspicion for EGPA. Thrombosis may be present at sites of vascular inflammation and necrosis. At sites of severe mural necrosis and thrombosis, determination of where thrombus ends and fibrinoid necrosis begins may not be possible.

The perivascular inflammatory infiltrate that occurs adjacent to necrotic segments of arteries may have a granulomatous appearance with a predominance of macrophages that sometimes form a palisade around the vessel and may contain multinucleated giant cells (Fig. 16.15). This perivascular inflammatory response is analogous to the periglomerular granulomatous response that occurs around some severely injured glomeruli in pauci-immune crescentic glomerulonephritis. Perivascular granulomatous inflammation is most frequent in patients with GPA but also may occur in patients with MPA or EGPA and thus is not as diagnostic for GPA as is granulomatous inflammation arising in the interstitium not adjacent to vasculitis or glomerulonephritis.

Over time, the acute necrotizing arterial lesions develop progressive sclerosis that evolves into segmental vascular scarring with or without associated mononuclear leukocytes, especially T cells and macrophages. With the initial progression to sclerosis, a Masson trichrome stain will reveal residual red (fuchsinophilic) fibrinoid material admixed with proliferating spindle cells (fibroblasts and myofibroblasts) and blue

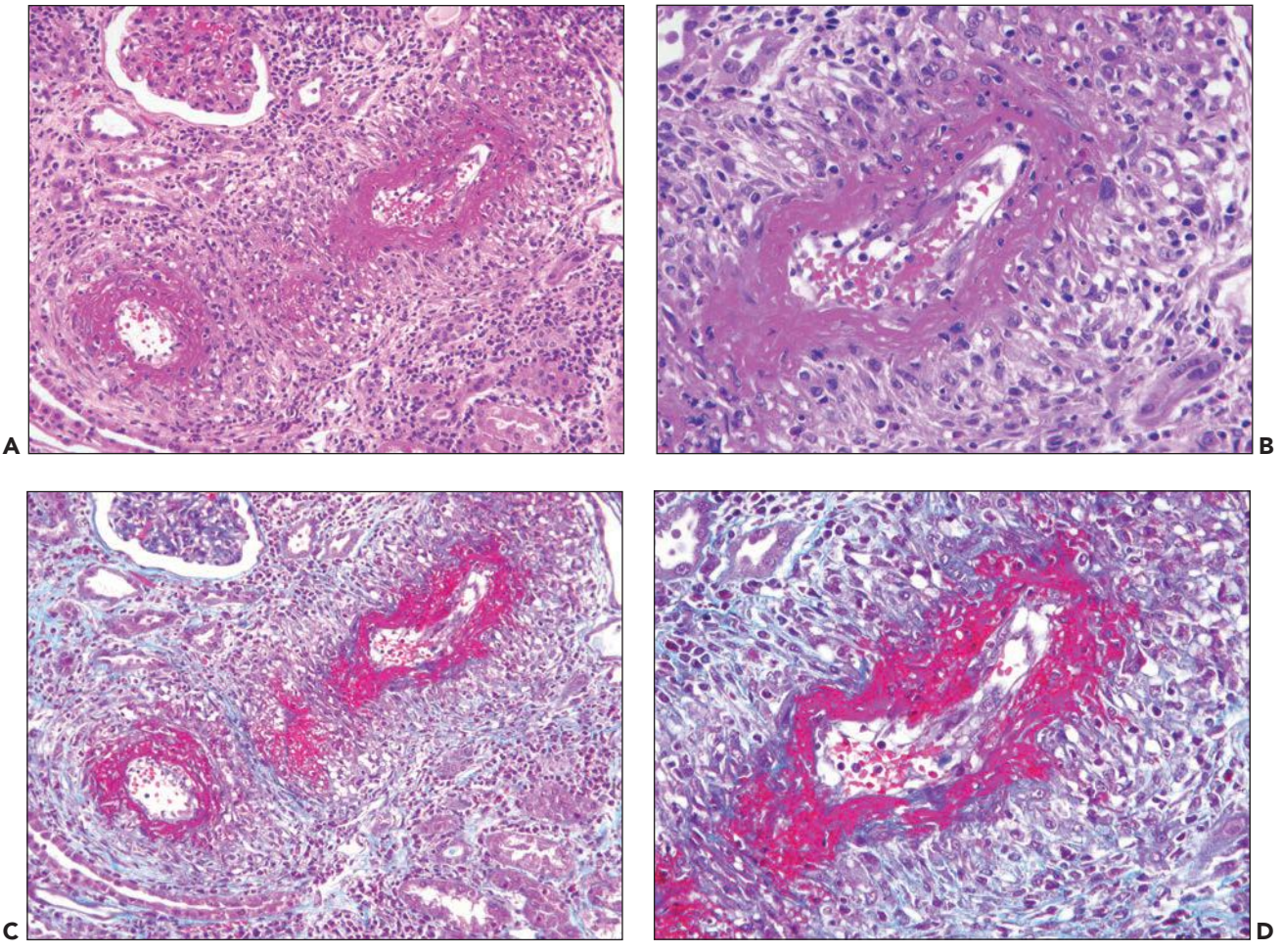



**FIGURE 16.12** Lower **(A)**- and higher **(B)**-magnification photomicrographs of a renal biopsy specimen from a patient with microscopic polyangiitis showing extensive necrotizing arteritis affecting an interlobular artery. The fibrinoid necrosis is deeply acidophilic in these H&E-stained sections. The muscularis of the artery is essentially totally destroyed. The perivascular infiltrate has a somewhat granulomatous appearance with vague palisading of macrophages adjacent to the necrosis. Note also the more diffuse tubulointerstitial inflammation farther away from the vessel. Lower **(C)**- and higher **(D)**-magnification photomicrographs of the same specimen with the fibrinoid necrosis highlighted in *red* by a Masson trichrome stain.

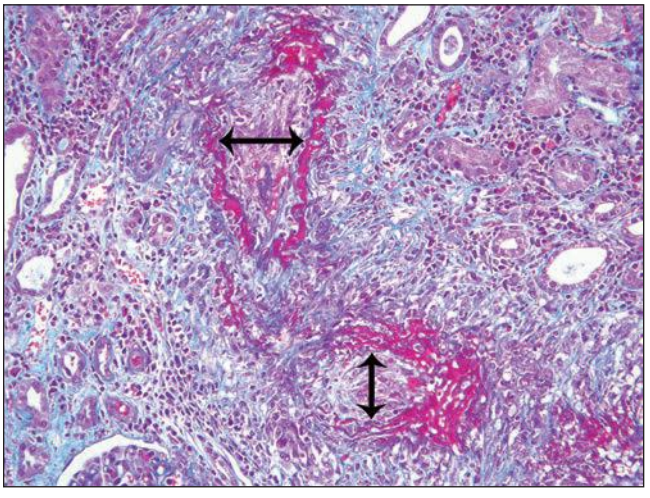


**FIGURE 16.13** Interlobular artery in a renal biopsy specimen from a patient with microscopic polyangiitis showing subacute arteritis with obliteration of the lumen by edematous intimal thickening (*double arrows*) and residual fibrinoid necrosis in the muscularis demarcated in red with this Masson trichrome stain. There is still a relatively intense perivascular inflammation composed predominantly of mononuclear leukocytes.

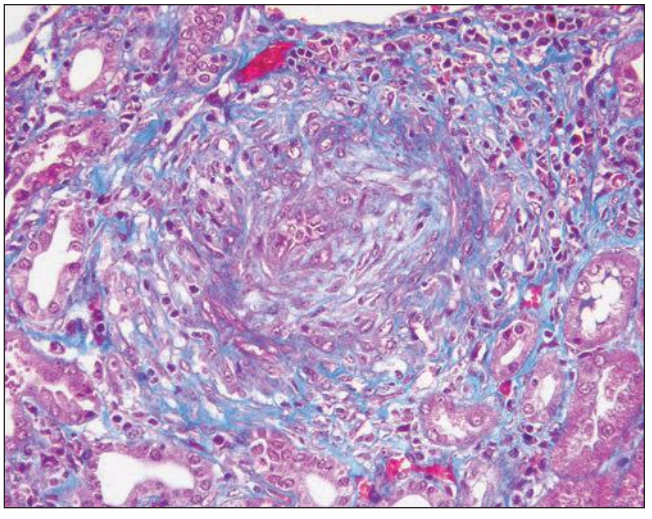


**FIGURE 16.14 Interlobular artery in a renal biopsy specimen from a patient with microscopic polyangiitis showing chronic arteritis with fibrosis of the intima and media.** This Masson trichrome stain reveals no residual fuchsinophilic fibrinoid necrosis. There is still a relatively mild perivascular inflammation composed predominantly of mononuclear leukocytes with some admixed eosinophils.

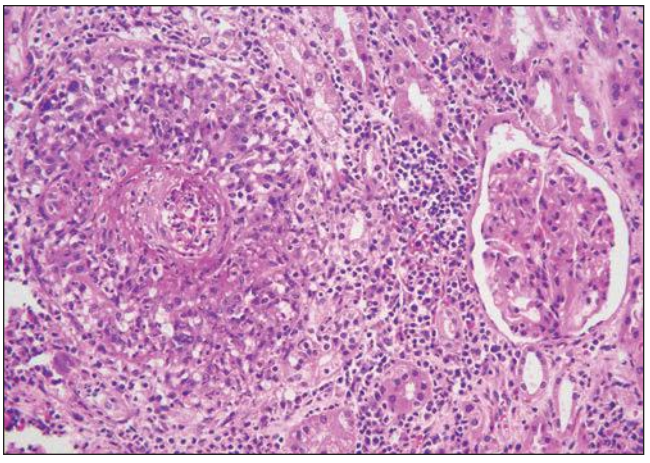


**FIGURE 16.15 Interlobular artery in a renal biopsy specimen from a patient with MPA showing necrotizing arteritis with a well-defined collar of granulomatous inflammation with palisading epithelioid macrophages.** The lumen and zone of fibrinoid necrosis contain some neutrophils. This patient was ANCA negative and had no respiratory tract disease and no evidence for extrarenal granulomatous inflammation.

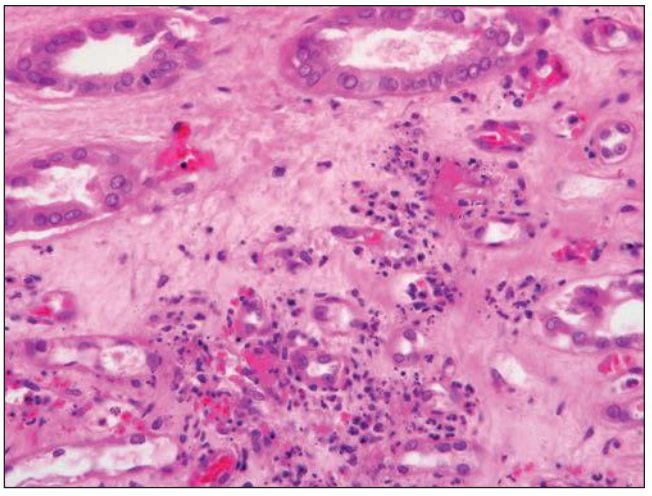


**FIGURE 16.16** Medullary angiitis in an autopsy specimen from a patient with GPA. Note the marked leukocytoclasia, which imparts an appearance reminiscent of dermal leukocytoclastic angiitis. (H&E stain.)

(or green) collagenous extracellular matrix (see Fig. 16.13). Eventually, the fibrinoid material disappears leaving behind a distorted vessel wall usually with a thickened intima and fibrotic muscularis (see Fig. 16.14). Vizjak et al. (11) identified lesions consistent with chronic scarring secondary to arteritis in 9% of 55 PR3-ANCA glomerulonephritis renal biopsy specimens and 12% of 74 MPO-ANCA specimens. In a patient with the sclerotic phase of pauci-immune crescentic glomerulonephritis, sclerotic lesions in arteries should be examined carefully for evidence of prior arterial inflammation. Asymmetrical scarring that extends into the muscularis and is associated with extensive disruption of the internal elastica is evidence for earlier active arteritis.

Patients with ANCA pauci-immune crescentic glomerulonephritis occasionally have a necrotizing, leukocytoclastic angiitis affecting the medullary vasa recta (Fig. 16.16) (2,102,103). Watanabe et al. (102) observed papillary necrosis with medullary angiitis in 5 of 23 autopsies of patients with GPA, all of whom had severe necrotizing and crescentic glomerulonephritis. This lesion is seen much less often in renal biopsy specimens. We have observed this lesion not only in patients with GPA but also MPA and pauci-immune crescentic glomerulonephritis with no evidence for systemic disease. If a renal biopsy specimen from an ANCA-positive patient contains only medulla and no cortex, the presence of medullary angiitis supports a diagnosis of small-vessel vasculitis. Because of the conspicuous neutrophils, at first glance, medullary angiitis can be mistaken for acute tubulointerstitial nephritis, especially acute pyelonephritis. The inflammation may spill into tubules, causing epithelial injury and luminal accumulation of neutrophils and cellular debris. However, careful examination reveals that most of the inflammation is centered on the peritubular vasa recta and is accompanied by focal interstitial hemorrhage. Leukocytoclasia usually is very conspicuous. This extensive neutrophil nuclear fragmentation is similar to the leukocytoclastic angiitis that often affects dermal venules in patients with ANCA small-vessel vasculitis. The severity of medullary angiitis does not correlate with the severity of glomerulonephritis or the presence of cortical vasculitis (103). Severe medullary angiitis results in ischemia and coagulative necrosis that may lead to sloughing of the necrotic papillary tip.

## Tubules and Interstitium

Interstitial edema, focal tubular epithelial flattening from acute simplification, and acute tubulointerstitial inflammation are common with severe acute pauci-immune crescentic glomerulonephritis (Fig. 16.17) (104). Tubular lumens may contain numerous dysmorphic red blood cells. Interstitial infiltration

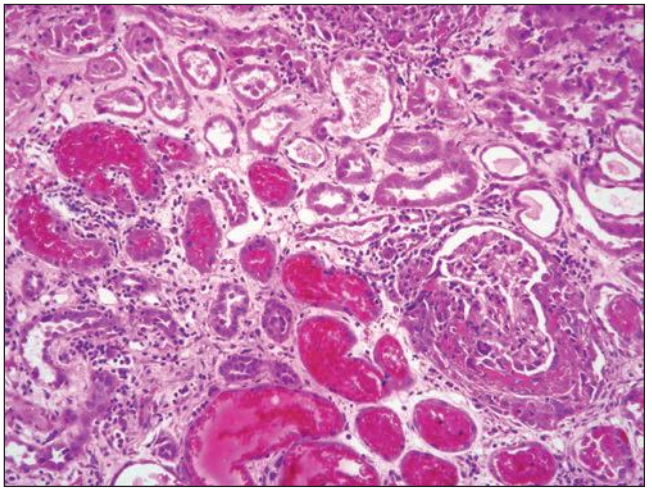


**FIGURE 16.17** Low magnification of a renal biopsy specimen from a patient with pauci-immune crescentic glomerulonephritis showing typical acute tubulointerstitial changes that include diffuse edema, slight leukocyte infiltration, focal simplification (flattening) of proximal tubular epithelial cells, and red blood cells in tubular lumens. Note also the glomerulus with segmental fibrinoid necrosis and crescent formation. (H&E stain.)

by leukocytes is common and is most pronounced adjacent to severely inflamed glomeruli or vessels. Tubulitis with lymphocytes infiltrating the epithelial side of tubular basement membranes may occur in areas of interstitial inflammation (104). Occasional interstitial infiltrates, usually with severe acute disease, contain conspicuous neutrophils. Interstitial eosinophils often occur in EGPA (38), but these are not specific because they also occur, although less frequently, in MPA, GPA, and renal-limited pauci-immune crescentic glomerulonephritis. Focal zones of tubular coagulative necrosis in a specimen with pauci-immune crescentic glomerulonephritis should raise the possibility of an accompanying vasculitis that has caused thrombosis and infarction.

Pauci-immune crescentic glomerulonephritis, especially as it progresses, is accompanied by interstitial leukocyte infiltration that has two patterns that usually coexist: tubulointerstitial and periglomerular. The tubulointerstitial pattern is not oriented around glomeruli but rather is associated with acute or chronic tubular changes such as tubulitis, simplification (acute) or atrophy (chronic). Tubular atrophy and tubulointerstitial inflammation always is accompanied by interstitial fibrosis. Interstitial leukocyte infiltration and interstitial fibrosis tend to be more pronounced with MPO-ANCA glomerulonephritis than with PR3-ANCA glomerulonephritis (11).

Periglomerular inflammation, as the name implies, is oriented adjacent to glomeruli. Periglomerular interstitial inflammation usually consists predominantly of lymphocytes, monocytes, and macrophages with varying numbers of admixed neutrophils and eosinophils (118). Periglomerular inflammation often is associated with breaks in the Bowman capsule (Fig. 16.18) (76,114). Most periglomerular infiltrates have a predominance of T lymphocytes (118). Macrophages, including epithelioid macrophages, may be conspicuous, and they may have a palisading orientation around the glomerulus. Multinucleated giant cells may be in the periglomerular infiltrates (see Fig. 16.8). When epithelioid macrophages, especially accompanied by multinucleated giant cells, are present, the periglomerular infiltrates have a granulomatous appearance. Granulomatous periglomerular inflammation is more common in GPA, but it also occurs in MPA, EGPA, and anti-GBM glomerulonephritis (114). Periglomerular granulomatous inflammation, however, is rare in glomerulonephritis other than ANCA glomerulonephritis, so its presence should raise the possibility of ANCA glomerulonephritis or ANCA small-vessel vasculitis.

Periglomerular granulomatous inflammation secondary to severe necrotizing glomerular injury should not be confused with the rare occurrence in patients with GPA and EGPA of interstitial necrotizing granulomatous inflammation that is not centered on glomeruli but rather is a component of the systemic necrotizing granulomatosis (Fig. 16.19). These granulomatous lesions typically have a central zone of necrosis surrounded by a loose infiltrate of mononuclear leukocytes, neutrophils, and scattered multinucleated giant cells. At low magnification, the granulomatous inflammation often has an irregular, jagged outline, whereas the periglomerular inflammation usually has a more spherical configuration. Special stains for basement membranes, such as periodic acid–Schiff or methenamine silver stains, may be required to detect residual GBMs at the center of a periglomerular granuloma to distinguish it from interstitial granulomatous inflammation.

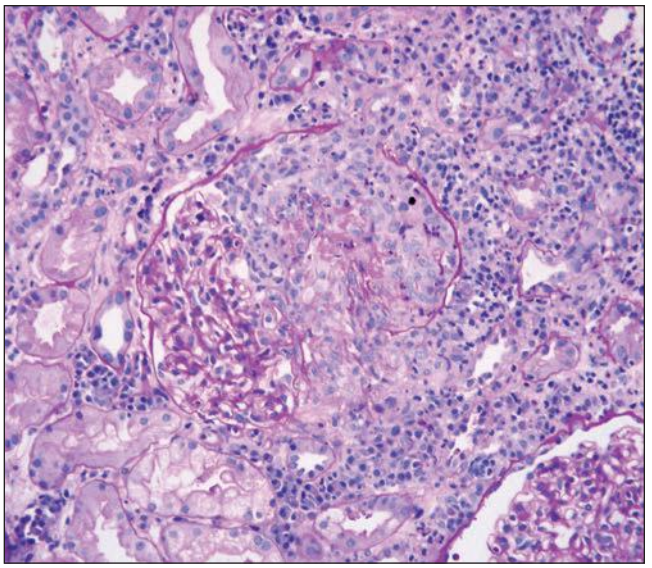


**FIGURE 16.18 Glomerulus affected by pauci-immune crescentic glomerulonephritis with a portion of the tuft on the left having no significant histologic changes, whereas the portion on the right has extensive necrosis with an associated cellular crescent and disruption of the Bowman capsule.** Also note that the tubulointerstitial tissue adjacent to the intact portion of the tuft on the left has only minimal inflammation, whereas the tubulointerstitial tissue adjacent to the inflamed portion of the tuft and the ruptured Bowman capsule is intensely inflamed. (PAS stain.)

Perivascular interstitial inflammation occurs adjacent to sites of segmental necrotizing inflammation in cortical arteries and arterioles and in medullary vasa recta (see Figs. 16.12, 16.15, and 16.16). The cortical perivascular infiltrates occasionally contain numerous neutrophils; however, mononuclear

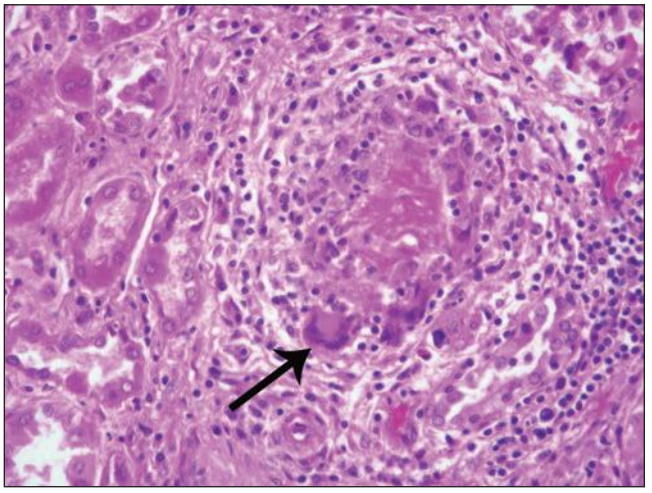


**FIGURE 16.19 Focus of granulomatous interstitial inflammation in a renal biopsy specimen with no apparent relationship to a glomerulus or vessel.** There is a central zone of necrosis surrounded by an admixture of neutrophils and mononuclear leukocytes and a few multinucleated giant cells (*arrow*). (H&E stain.)

leukocytes usually predominate. Eosinophils are often present and occasionally are numerous. Many eosinophils in the vasculitic and perivasculitic infiltrates can occur in MPA and GPA as well as in EGPA. Therefore, a diagnosis of EGPA cannot be made on the basis of numerous eosinophils in the vasculitic infiltrate, but rather, it requires a history of asthma and blood eosinophilia.

The medullary angiitis typically has extensive adjacent interstitial infiltration by neutrophils, marked leukocytoclasia, and hemorrhage (see Fig. 16.16). Medullary interstitial necrotizing granulomatous inflammation and papillary necrosis also occur in patients with pauci-immune small-vessel vasculitis (103) but are uncommon and are rarely identified in renal biopsy specimens.

As acute disease progresses, the interstitial edema is replaced by interstitial fibrosis. Focal tubular atrophy with flattening of epithelium and thickening of tubular basement membranes often develops. Areas of interstitial fibrosis and tubular atrophy have interstitial infiltration of chronic inflammatory cells with T lymphocytes predominating (see Fig. 16.10). Varying numbers of macrophages, plasma cells, and eosinophils are admixed. Atrophic tubules may have infiltrating lymphocytes on the epithelial side of the tubular basement membranes. Eisenberger et al. (23) compared the severity of tubulointerstitial disease in patients with ANCA-negative pauci-immune glomerulonephritis or ANCA-positive pauci-immune glomerulonephritis and found no significant difference.

## Extrarenal Pathology

The pathologic features of the necrotizing vasculitis lesions of MPA, GPA, and EGPA are the same in other organs as they are in the kidney. For example, leukocytoclastic angiitis affecting vasa recta resembles that in dermal venules, the necrotizing capillaritis in glomeruli is analogous to that in alveolar capillaries, and the necrotizing arteriolitis and arteritis in arteries within the kidney is histologically identical to the necrotizing arteriolitis and arteritis elsewhere in the body, such as epineural arteritis causing peripheral neuropathy, gastrointestinal arteritis causing focal ulceration and hemorrhage, and skeletal muscle arteritis causing myalgias. Histologic demonstration of necrotizing vasculitis alone, however, is not diagnostic for pauci-immune small-vessel vasculitis or for a specific category of ANCA small-vessel vasculitis without additional serologic, clinical, or pathologic data. For example, histologically indistinguishable necrotizing arteritis in a biopsy specimen from skeletal muscle, peripheral nerve, or skin can be caused by polyarteritis nodosa, MPA, GPA, and EGPA. Similarly, histologically indistinguishable leukocytoclastic angiitis in the dermis can be caused not only by the ANCA small-vessel vasculitides but also by IgA vasculitis (Henoch-Schönlein purpura [HSP]), cryoglobulinemic vasculitis, lupus vasculitis, serum sickness vasculitis, rheumatoid vasculitis, and other small-vessel vasculitides. Based on a biopsy specimen alone, a pathologist can provide only a descriptive diagnosis (such as necrotizing arteritis or leukocytoclastic angiitis) along with a differential diagnosis of categories of vasculitis that could be causing the lesions.

The necrotizing granulomatous inflammation of GPA most often affects the upper and lower respiratory tract (39), but it can occur elsewhere, for example, in the dermis and subcutaneous tissue, resulting in red, tender cutaneous nodules. The earliest lesions may contain predominantly neutrophils in essence forming a microabscess. The necrotizing lesions are characterized histologically by an irregular central zone of necrosis that may have an amphophilic or bluish hue because of finely dispersed nuclear debris. At low magnification, the multiple foci of irregular necrosis may impart an irregular geographic pattern. At the center of small lesions, and at the edge of the necrotic zone in larger lesions, one often sees numerous neutrophils, many undergoing leukocytoclasia. The periphery of the lesions has an admixture of mononuclear leukocytes, neutrophils, and occasionally eosinophils, with scattered multinucleated giant cells. Epithelioid macrophages may be numerous, but they do not have the compact arrangement seen in more prototypical granulomas, such as those of sarcoidosis. As the granulomatous inflammation matures, palisading elongated macrophages align at the interface between the inflammation and the central necrotic or sclerotic core. More chronic lesions have extensive fibroblastic proliferation and ultimately evolve into dense fibrotic scars. For a specimen that contains necrotizing granulomatous inflammation, the major nonvasculitic differential diagnostic considerations are mycobacterial and fungal infections.

The necrotizing granulomas of EGPA are similar to those of GPA, but they have predominantly eosinophils rather than neutrophils at the centers of early acute lesions, and the central zone of necrosis often is more acidophilic (37,56). The necrotic zone may contain numerous brightly acidophilic eosinophil granules and Charcot-Leyden crystals formed from annealed granule constituents. The granulomatous lesions of EGPA occur most often in the lungs and skin. In the latter location, nodular lesions are indistinguishable from those caused by GPA except for the tendency to contain more eosinophils. Nodular inflammatory skin lesions can be caused not only by vasculitis-associated granulomatous inflammation but also by arteritis in the dermis or subcutaneous tissue (dermal venulitis causes purpura rather than nodules). Therefore, cutaneous nodules can be a manifestation of not only GPA and EGPA but also any form of vasculitis that causes necrotizing arteritis. Cutaneous nodules caused by arteritis are more common in polyarteritis nodosa than in MPA.

Hemorrhagic necrotizing pulmonary alveolar capillaritis is a frequent, and often life-threatening, feature of GPA and MPA, but is uncommon in EGPA. As a consequence, the ANCA small-vessel vasculitides, primarily MPA and GPA, are the most common causes of pulmonary-renal vasculitic syndrome (24). The acute alveolar capillaritis is characterized by marked neutrophil infiltration in involved alveolar septa, often with leukocytoclasia. Special stains, such as methenamine silver, demonstrate extensive capillary basement membrane disruption. In general, the hemorrhagic capillaritis of anti-GBM disease (Goodpasture syndrome) has less conspicuous neutrophils and less destruction of alveolar capillary basement membranes than ANCA-associated alveolar capillaritis.

The focal nature of acute vasculitic and granulomatous lesions complicates diagnosis by biopsy because, even at sites of active acute injury, the biopsy specimen may contain tissue with only nonspecific acute and chronic inflammatory changes. For example, in a study of 126 head and neck biopsy specimens from patients with GPA, Devaney et al. (138) observed vasculitis with necrosis and granulomatous inflammation in 16%, vasculitis with granulomatous inflammation in 21%, and vasculitis with necrosis in 23%. The more distinctive acute

vasculitic and granulomatous lesions evolve into more nonspecific chronic sclerotic lesions, for example, pulmonary interstitial fibrosis and bronchiolitis obliterans (139). Therefore, acute necrotizing vasculitis and necrotizing granulomatous inflammation, although the most specific, are not the only pathologic findings in a lung biopsy specimen that support or raise the possibility of ANCA small-vessel vasculitis. Nonspecific acute and chronic inflammation and fibrosis, especially if associated with hemosiderin-laden macrophages, are consistent with but are not diagnostic of ANCA-associated pulmonary injury.

## Immunofluorescence Microscopy

By definition, pauci-immune crescentic glomerulonephritis does not have immunofluorescence microscopy findings that would be diagnostic for anti-GBM disease or immune complex disease. However, the term pauci-immune rather than nonimmune staining is used because many patients have a low level of staining for immunoglobulin (i.e., less than 2+ on a scale of 0 to 4+) as well as complement components (140). The paucity of staining for immunoglobulin is seen in renal-limited ANCA-associated necrotizing and crescentic glomerulonephritis as well as ANCA-associated systemic small-vessel vasculitis. Ronco et al. (141) were among the first investigators to report this observation. They detected glomerular staining for immunoglobulins in less than 20% of patients with GPA and in less than 10% of patients with "polyarteritis nodosa." Most of the latter group met the current definition for MPA because they had glomerulonephritis. Savage et al. (36) reported glomerular IgG in 15%, IgA in 10%, and IgM in 5% of 20 renal biopsies from patients with MPA. Jennette and Thomas (2) reported higher than 1+ staining for any immunoglobulin in only 14% of 71 patients with ANCA glomerulonephritis. D'Agati et al. (108) noted better than 1+ staining for immunoglobulin in 5% of 20 patients with MPA. Haas and Eustace (142) observed that many but not all patients with ANCA crescentic glomerulonephritis who have low-intensity staining for immunoglobulin by immunofluorescence microscopy also have scattered, usually small, electron-dense deposits by electron microscopy.

There is no standard criterion, however, for where to draw the line between pauci-immune crescentic glomerulonephritis and immune complex crescentic glomerulonephritis with respect to the amount or intensity of glomerular staining for immunoglobulin. There is an inverse relationship between the amount of staining for immunoglobulin in a specimen with crescentic glomerulonephritis and the frequency of ANCA positivity (Fig. 16.20). For example, in a study of 213 patients with more than 50% glomerular crescents (excluding patients with lupus glomerulonephritis or anti-GBM glomerulonephritis), 92% with no immunofluorescence staining for glomerular immunoglobulin were ANCA positive, 82% with trace to 1+ staining were ANCA positive, 23% with more than 2+ staining were ANCA positive, and 8% with higher than 3+ staining were ANCA positive (140). Thus, the more pauci-immune the specimen is, the more likely the patient is to have ANCA glomerulonephritis. However, ANCA-positivity does not influence the pathologic diagnosis or clinical management because ANCA-negative pauci-immune glomerulonephritis has the same pathologic and clinical features as ANCA-positive pauci-immune glomerulonephritis (23).

An important distinction to make when examining a specimen with pauci-immune crescentic glomerulonephritis is the staining in glomeruli or portions of glomeruli that do not have necrosis or sclerosis versus staining in glomeruli or portions of glomeruli that do have necrosis or sclerosis. As in other glomerular diseases, areas of glomerular sclerosis have irregular staining for C3, C1q, and IgM (11). Foci of glomerular necrosis also have variable staining for C3 and immunoglobulins and marked staining for fibrin (Fig. 16.21). Likewise, foci of fibrinoid necrosis in extrarenal vessels stain for fibrin. Crescents also stain for fibrin that is located in the interstices between cells (Fig. 16.22). The fibrin is the product of the

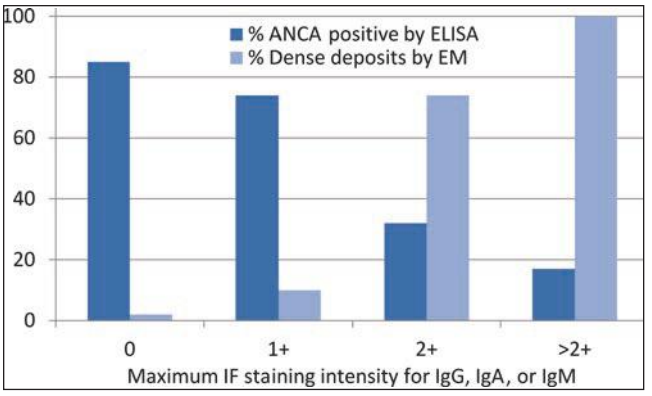



**FIGURE 16.20 Graph showing the relationship between the likelihood of a positive ANCA serology and identification of immune complex–type electron-dense deposits by electron microscopy on the *y*-axis as a function of the amount of staining for immunoglobulin on the *x*-axis.** The data are derived from 213 patients with crescentic glomerulonephritis (≥50% glomerular crescents) evaluated in the University of North Carolina Nephropathology Laboratory. Patients with anti-GBM disease and lupus nephritis were excluded from the study.

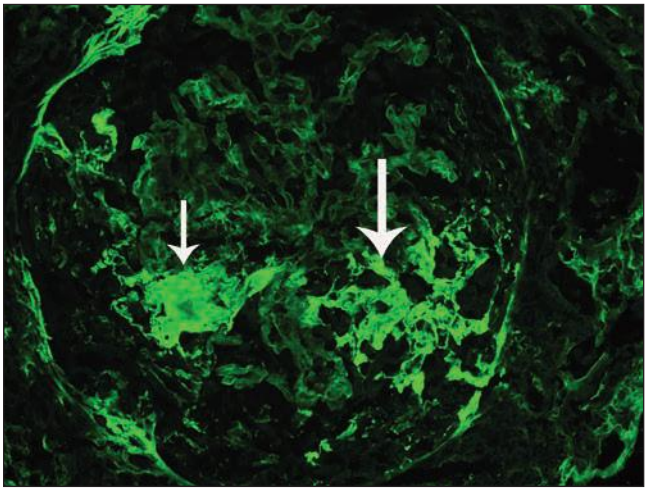


**FIGURE 16.21** Glomerulus from a patient with pauci-immune crescentic glomerulonephritis showing staining by immunofluorescence microscopy for fibrin at a site of segmental fibrinoid necrosis (*small arrow*) and a second focus of fibrin that appears to extend out from a site of fibrinoid necrosis into an adjacent crescent (*large arrow*). (Fluorescein isothiocyanate [FITC] antifibrin.)

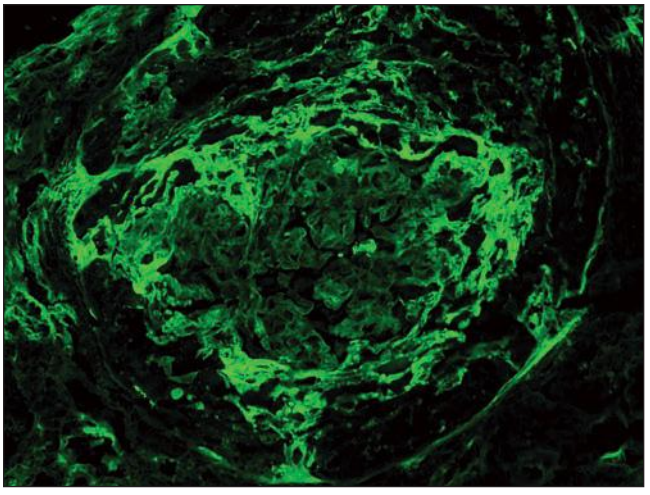


**FIGURE 16.22** Glomerulus from a patient with pauci-immune crescentic glomerulonephritis showing staining by immunofluorescence microscopy for fibrin in a large circumferential crescent. (FITC antifibrin.)

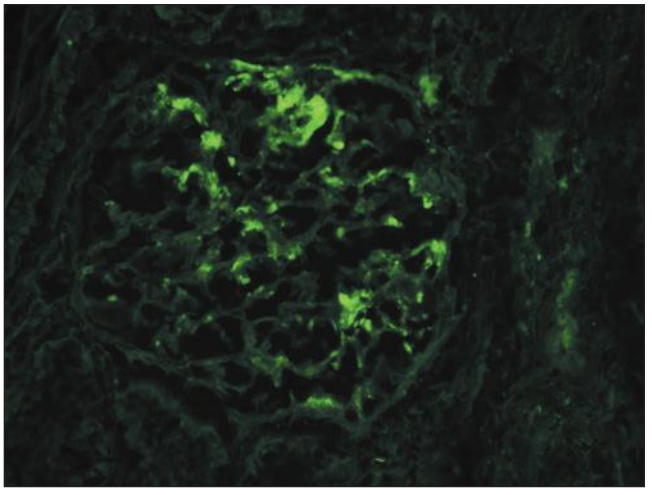


**FIGURE 16.23** Glomerulus from a patient with pauci-immune crescentic glomerulonephritis showing irregular predominantly mesangial staining by immunofluorescence microscopy for IgG. (FITC anti-IgG.)

activation of the coagulation system proteins in plasma that has spilled out of injured vessels into the perivascular tissue or into the Bowman space where they contact thrombogenic substances, such as tissue factor. Thus, fibrin staining is a marker for severe inflammatory injury to vessels. The staining for immunoglobulin and especially complement often is accentuated and may only be observed in areas of segmental necrosis or sclerosis. This greater correspondence with sites of injury differs from immune complex–mediated glomerulonephritis and anti-GBM glomerulonephritis in which the staining for immunoglobulin and complement is as strong if not stronger in glomeruli and glomerular segments that do not have injury. This may result from the difference in pathogenesis with immune deposits preceding the initiation of inflammation in immune complex and anti-GBM disease, versus initiation of immunoglobulin binding and complement activation only at the sites of injury in ANCA disease.

As noted earlier, the definition of "pauci-immune" is somewhat arbitrary (140). Most pathologists use a staining intensity of 2+ or less as the cutoff point. Using this definition for pauci-immune (i.e., 2+ or less staining), Vizjak et al. (11) determined that 96% of 135 patients with ANCA-associated glomerulonephritis were pauci-immune. In this study, there was no difference in the extent of staining for immunoglobulins or complement in patients with MPO-ANCA versus PR3-ANCA. When staining for immunoglobulins is present, it may be for any combination of IgG, IgM, or IgA. Staining for IgM is most frequent (11). The staining usually is confined to or predominantly in the mesangium (Fig. 16.23); however, the distribution is extremely variable. When the IgA staining is dominant or codominant, the possibility of concurrent ANCA disease and IgA nephropathy is a consideration. Some patients have very convincing features for concurrent ANCA glomerulonephritis and IgA nephropathy (124–128), but others have only very low-level staining that if seen in isolation in a renal biopsy specimen would not warrant a diagnosis of IgA nephropathy. Our arbitrary approach is to make a diagnosis of pauci-immune crescentic glomerulonephritis if there is IgA-dominant staining that is less than 2+ intensity in the setting of glomerular necrosis and crescents and to make a diagnosis of concurrent ANCA glomerulonephritis and IgA nephropathy if there is 2+ or greater IgA-dominant staining in a patient with necrosis, crescents, and ANCA. If the patient has IgA nephropathy with extensive necrosis and crescents but is ANCA negative, one can only conjecture about the possibility of concurrent ANCA-negative glomerulonephritis with the IgA nephropathy. Similarly, a cutoff must be established between pauci-immune crescentic glomerulonephritis with minor granular staining for IgG caused by subepithelial immune complex deposits versus ANCA glomerulonephritis concurrent with membranous glomerulonephritis (120–122,125,143). Our arbitrary approach is to make the latter diagnosis only if 50% of glomeruli have over 50% of the tufts involved with 2+ or greater granular staining for IgG in a patient with necrosis, crescents, and ANCA.

The presence of intense linear staining of glomeruli in an ANCA-positive patient with crescentic glomerulonephritis raises the possibility of concurrent anti-GBM disease (86–88). The presence of ANCA in these patients appears to alter the phenotype of disease; for example, patients with both ANCA and anti-GBM antibodies may have systemic small-vessel vasculitis that is not expected with anti-GBM disease alone. Thus, testing for ANCA may provide useful information not only in patients with pauci-immune glomerulonephritis and vasculitis but also in patients with anti-GBM glomerulonephritis or crescentic immune complex glomerulonephritis.

## Electron Microscopy

As would be expected from the results of immunofluorescence microscopy, most patients with pauci-immune crescentic glomerulonephritis, MPA, GPA, and EGPA have no or scanty glomerular or vascular immune complex–type electron-dense deposits (2,108,110,144). This absence or paucity of immune complex–type electron-dense deposits is a feature of pauci-immune crescentic glomerulonephritis that differentiates it from immune complex crescentic glomerulonephritis. As with immunofluorescence microscopy findings, however, a small

amount of scattered electron-dense material may be tolerated in the pauci-immune category (142). By electron microscopy, pauci-immune crescentic glomerulonephritis with no electron-dense deposits cannot be distinguished from anti-GBM glomerulonephritis, which also lacks electron-dense deposits. Extensive immune complex–type electron-dense deposits are not consistent with a diagnosis of pauci-immune glomerulonephritis; however, this does not rule out an ANCA-positive glomerulonephritis because ANCA-positivity, with associated glomerular necrosis and crescent formation, can be superimposed on immune complex glomerulonephritis that has conspicuous electron-dense deposits (119–128,142,143).

The ultrastructural features of pauci-immune crescentic glomerulonephritis are the same in patients with renal-limited disease as those in patients with systemic vasculitis (108). Breaks in GBMs and the Bowman capsule occur frequently. These can be identified in diagnostic transmission electron microscopy preparations (Fig. 16.24) and have been more dramatically demonstrated by scanning electron microscopy of basement membranes after removal of cellular elements (Fig. 16.25). Capillaries may have endothelial swelling or necrosis, thrombosis, and margination of neutrophils and monocytes. Crescents contain varying mixtures of fibrin tactoids, epithelial cells, and macrophages.

A thorough report of the ultrastructural features of pauci-immune crescentic glomerulonephritis and small-vessel vasculitis is provided in a study of MPA by D'Agati et al. (108). Early glomerular ultrastructural changes include focal endothelial swelling, focal expansion of the subendothelial zone, and focal endothelial necrosis and denudation of the GBM with thrombus formation containing fibrin tactoids and platelets (Figs. 16.26 and 16.27). Marginated neutrophils and monocytes may be present (Figs. 16.27 and 16.28). At sites of GBM rupture, the fibrin extends across the ruptured capillary walls into the Bowman space, usually in association with crescent formation (see Fig. 16.28). The fibrin typically is very electron dense and may have a fibrillary texture or fine periodicity. Elongated curvilinear aggregations of fibrin often are referred to as "fibrin tactoids." If extensive rupture of the Bowman capsule occurs, fibrin also is observed in the periglomerular interstitium, where it is accompanied by leukocyte infiltration. Electron-dense fibrin tactoids frequently occur in the interstices between the cells of cellular crescents (Fig. 16.29), which corresponds to the staining for fibrin in crescents that is seen by immunofluorescence microscopy (see Fig. 16.22).

The proportion of specimens with pauci-immune crescentic glomerulonephritis or ANCA glomerulonephritis that have glomerular electron-dense deposits will vary depending on the definitions and criteria used for case selection. For example, specimens with crescentic glomerulonephritis that have no staining for immunoglobulins by immunofluorescence microscopy will have a lower frequency (less than 5%) and smaller electron-dense deposits than patients who have 1+ to 2+ staining for immunoglobulin. In the cohort of patients with crescentic glomerulonephritis shown in Fig. 16.20, glomerular immune complex–type electron-dense deposits were identified in 2% (2 of 92) of specimens with no staining for

**FIGURE 16.25 Scanning electron micrograph of glomerular basement membranes from a patient with pauci-immune crescentic glomerulonephritis after digestion of all cellular elements.** A large gap and several small gaps can be seen. (From Bonsib SM. Glomerular basement membrane discontinuities: Scanning electron microscopic study of acellular glomeruli. *Am J Pathol* 1985;119:357.)

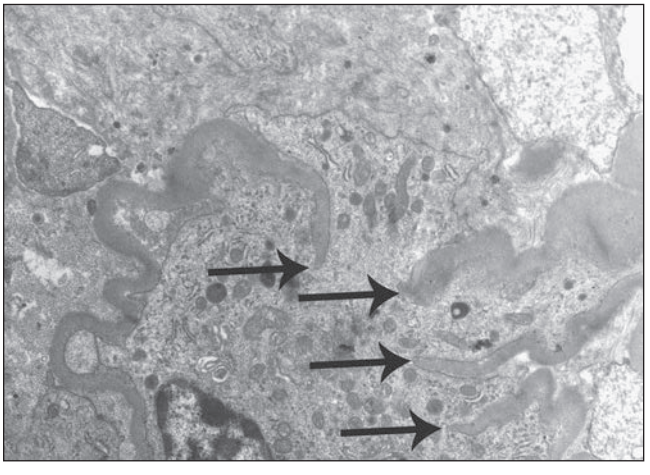


**FIGURE 16.24** Transmission electron micrograph showing extensive breaks (*arrows*) in the capillary wall glomerular basement membrane in a renal biopsy specimen from a patient with pauci-immune crescentic glomerulonephritis.

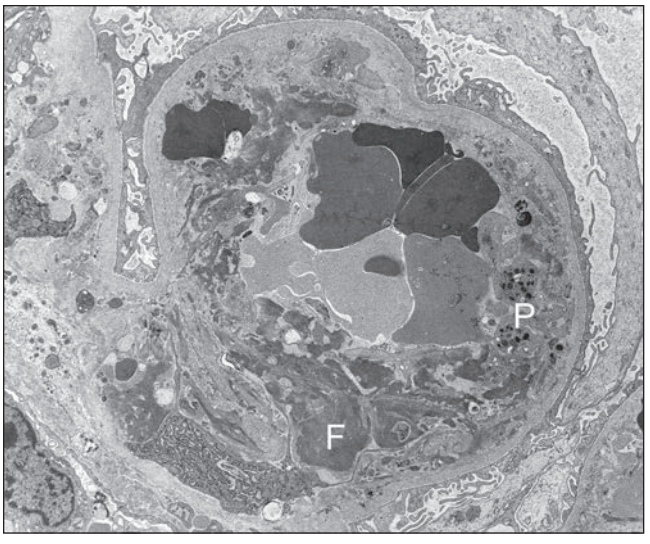



**FIGURE 16.26** Transmission electron micrograph of a glomerular capillary loop from a patient with pauci-immune crescentic glomerulonephritis showing acute endothelial disruption, adherence of platelets (*P*), and formation of fibrin (*F*) in the capillary wall.

immunoglobulins by immunofluorescence microscopy, 10% (7 of 72) of specimens with trace to 1+ staining, 74% (14/19) of specimens with 2+ staining, and 100% (30 of 30) of patients with greater than 2+ staining. Likewise, Haas and Eustace (142) retrospectively evaluated 126 renal biopsy specimens from ANCA-associated crescentic and/or necrotizing glomerulonephritis who were ANCA positive or had necrotizing arteritis in the biopsy specimen. When no dense deposits were seen by electron microscopy, none of the patients had more than 2+ staining for immunoglobulin or complement, and only 22% had 1+ staining. However, when electron-dense deposits were observed, which usually were scanty, 87% had some staining for immunoglobulin or complement, although only 12% had greater than 2+ staining. Thus, immune complex–type electron-dense deposits, especially if they are small and scattered, do not rule out pauci-immune crescentic glomerulonephritis or ANCA glomerulonephritis.

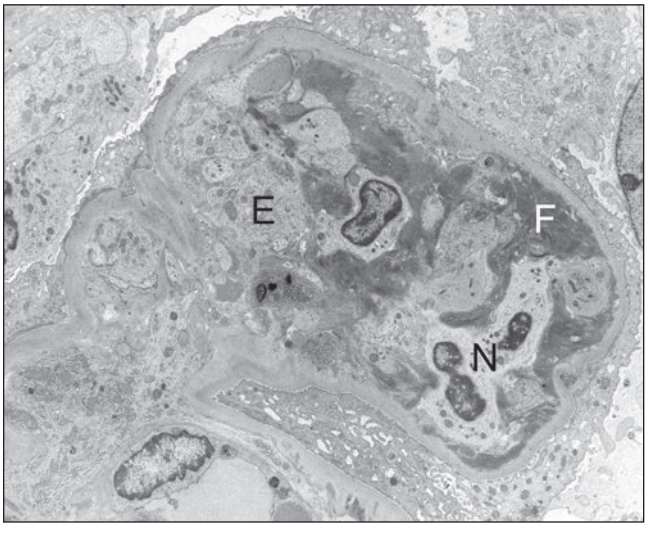



**FIGURE 16.27** Transmission electron micrograph of a glomerular capillary loop from a patient with pauci-immune crescentic glomerulonephritis showing a neutrophil (*N*), endothelial swelling (*E*) and focal denudation, and fibrin (*F*) thrombus formation.

Ultrastructural changes observed by D'Agati et al. (108) in inflamed arterioles and arteries parallel those seen in glomeruli. The earliest changes are endothelial swelling and necrosis with expansion of the subendothelial zone or accumulation of fibrin beneath endothelial cells. The basement membrane of arterioles and the internal elastica of arteries are disrupted as the inflammation progresses. Medial changes begin with intercellular edema and fibrin formation and progress to myocyte necrosis associated with mural infiltration by mononuclear leukocytes and neutrophils. Irregular electron-dense material consistent with fibrin is present in areas of medial degeneration, but no immune complex–type dense deposits are identified in most specimens. "Healed" vascular lesions have accumulations of granular dense material consistent with protein insudation that would correspond to hyalinosis by light microscopy and extensive deposition of elastic and collagenous material.

## ETIOLOGY AND PATHOGENESIS

As early as 1954, Godman and Churg (39) concluded that MPA, GPA, and EGPA were closely related pathogenetically as well as morphologically. The strong association of these three variants of small-vessel vasculitis as well as renal-limited pauci-immune crescentic glomerulonephritis with ANCA now supports this insightful conclusion. There are a number of lines of clinical and experimental evidence for the pathogenicity of ANCA IgG in the induction of pauci-immune crescentic glomerulonephritis and small-vessel vasculitis.

Clinical observations in patients with pauci-immune small-vessel vasculitis that support a pathogenic role for ANCA include the high frequency of ANCA in the blood; the general correlation of ANCA titers with disease activity (12,15,18) especially if sensitive capture assays are used (19,20); the increased risk for disease when there are higher levels of ANCA antigens on the surface of circulating neutrophils (145,146); the absence of evidence for other known pathogenic mechanisms (such as vascular wall immune complex localization) (2); the induction of ANCA as well as pauci-immune crescentic glomerulonephritis and small-vessel vasculitis by certain drugs, including propylthiouracil, penicillamine, minocycline, and hydralazine (61–68,147); and the efficacy of plasmapheresis and targeted B-cell therapy (discussed in detail later). There is one interesting report of a neonate who apparently developed glomerulonephritis and pulmonary hemorrhage as a result of transplacental passage of MPO-ANCA IgG from the mother to the fetus (148), but no additional examples have been reported, and there is one report of successful pregnancy and delivery of a healthy newborn despite transplacental transfer of MPO-ANCA from a mother with MPA (149).

The most compelling experimental evidence is the induction of pauci-immune crescentic glomerulonephritis and small-vessel vasculitis in experimental animals (150,151). Xiao et al. (152) induced a strong anti-MPO immune response

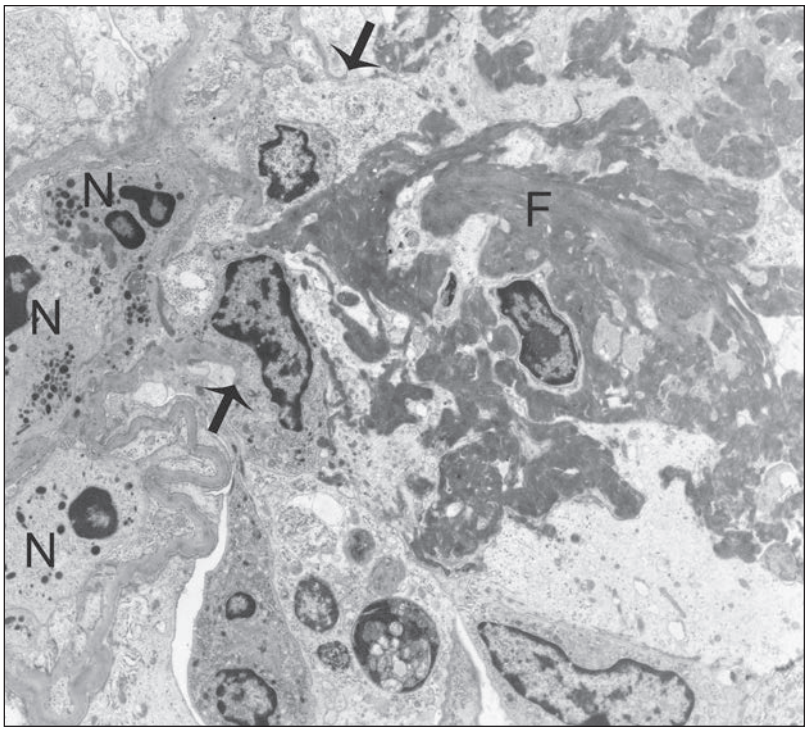



**FIGURE 16.28 Transmission electron micrograph of the edge of a glomerular segment (*left*) from a patient with pauci-immune crescentic glomerulonephritis.** The capillary lumens contain multiple neutrophils (*N*). The glomerular basement membrane is disrupted (between the two *arrows*). Electron-dense fibrin (*F*) appears to be spewing from the ruptured capillary into the Bowman space on the *right*.

in MPO knockout mice that were immunized with purified mouse MPO. Injection of this anti-MPO IgG (MPO-ANCA) into wild-type mice resulted in the development of a pauci-immune glomerulonephritis with segmental fibrinoid necrosis and crescent formation that closely resembled human ANCA glomerulonephritis. In addition, some but not all mice that received anti-MPO IgG developed small-vessel vasculitis, including alveolar capillaritis and necrotizing arteritis that was histologically indistinguishable from human ANCA disease. A few mice even developed necrotizing granulomatous inflammation mimicking GPA. The importance of neutrophils in this animal model was demonstrated by completely blocking the induction of glomerulonephritis and vasculitis by depletion of circulating neutrophils (116).

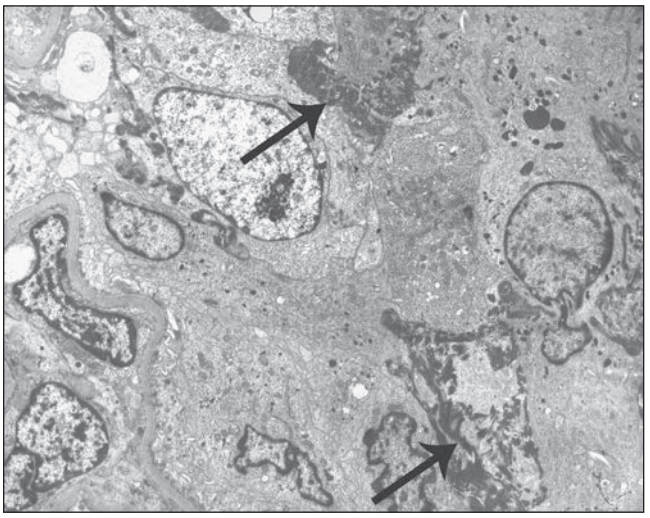


**FIGURE 16.29 Transmission electron micrograph of a cellular crescent from a patient with pauci-immune crescentic glomerulonephritis.** The edge of the glomerular tuft is on the left. There are electron-dense tactoids of fibrin (*arrows*) between the large epithelial/epithelioid cells of the crescent.

Little et al. (153) developed a model of ANCA disease in rats immunized with human MPO. The rats develop anti-MPO antibodies that cross-react with rat MPO, focal pauci-immune necrotizing and crescentic glomerulonephritis, and pulmonary capillaritis. The glomerulonephritis also can be induced by passive transfer of the antibodies by intravenous injection.

Numerous in vitro and in vivo experiments support the hypothetical pathogenic sequence of events depicted in Figure 16.30. Incubation of ANCA IgG with cytokine-primed neutrophils induces the release of toxic reactive oxygen species and the release of granule enzymes, some of which have lytic or toxic properties (154,155). Neutrophil priming by cytokines causes the expression of small amounts of ANCA antigens at the surface of neutrophils, where they can interact with ANCA (155). In ANCA disease patients, ANCA antigens are present on the surface of circulating neutrophils, and the amount correlates with disease activity (145,146). Conceptually, ANCA in the circulation can interact with ANCA antigens (MPO, PR3) on the surface of primed neutrophils in vivo and cause them to adhere to and injure vessel walls.

MPO and PR3 are not only in neutrophils but also in monocytes (81,156). As with neutrophils, incubation of monocytes with ANCA IgG causes activation with release of oxygen radicals (157) and inflammatory cytokines (158,159). Thus, activation of monocytes as well as neutrophils by ANCA could be involved in the pathogenesis of vascular injury in ANCA disease.

Data indicate that activation of primed neutrophils involves both Fc receptor engagement (160,161) and Fab′2 binding to cell surfaces (162,163). Thus, ANCA-induced leukocyte activation may use multiple pathways. For example, immune complexes containing ANCA IgG and ANCA antigens could form in the microenvironment around neutrophils and monocytes and could engage Fc receptors, or ANCA could bind directly to ANCA antigens on neutrophil and monocyte cell membranes and transduce an activation signal (164).

Neutrophils that have been activated by ANCA kill primed endothelial cells in vitro (165,166). This could explain why endothelial necrosis appears to be an early event in the development of the pathologic lesions of pauci-immune small-vessel vasculitis (108). Endothelial cell killing by ANCA-activated neutrophils requires adhesion of the neutrophils to endothelial cells by $\beta_2$-integrins (167). Cytokine-receptor interactions also are involved in the interaction of ANCA-activated neutrophils with endothelial cells (168–172).

ANCA also could react with antigens (e.g., MPO and PR3) passively adsorbed onto endothelial cells, thus, in essence, forming immune complexes in situ on the vessel wall. Vargunam et al. (171) showed that MPO binds to cultured endothelial cells in vitro by a charge-dependent mechanism and can subsequently react with ANCA to form immune complexes in situ. If immune complexes are forming on the surface of endothelial cells in ANCA vasculitis, this must be at low levels that are not demonstrable by standard immunofluorescence microscopy, because ANCA vasculitis typically has little or no vascular staining for immunoglobulins.

Observations in the murine model of anti-MPO–induced disease (66–69), immunohistologic studies of pauci-immune crescentic glomerulonephritis in patients with ANCA disease (75,78), and detection of complement activation fragments in the plasma of patients with ANCA disease indicate that activation of the alternative complement pathway is critically important for ANCA-induced vascular inflammation. Although typically there is a relative paucity of immunoglobulin and complement at sites of inflammatory injury in ANCA disease, there are localized deposits of immunoglobulin, especially components of the activated alternative pathway, at sites of inflammation, including glomerular lesions (173–175). One group has reported evidence for alternative pathway complement activation in the circulation of patients with ANCA disease (176). They observed that plasma levels of C3a, C5a, soluble C5b-9, and Bb were significantly higher in active disease than in remission, while plasma levels of properdin were significantly lower. There was no significant difference in the plasma levels of C4d between active stage and remission. The plasma level of Bb correlated with % cellular crescents and the Birmingham Vasculitis Activity Scores.

Studies using the anti-MPO–induced murine model of MPO-ANCA glomerulonephritis have convincingly demonstrated a critical pathogenic role for alternative complement pathway activation (177–180). Anti-MPO glomerulonephritis can be prevented by blockade of the alternative pathway but not the classic or lectin pathways (177). Activation of C5 is the critical step (178–180), which results in engagement of neutrophil C5a receptors by C5a. Two inflammatory amplification loops are established. Activation of neutrophils by ANCA releases factors that activate the alternative complement pathway, which in turn recruits and activates more neutrophils (see Fig. 16.30).

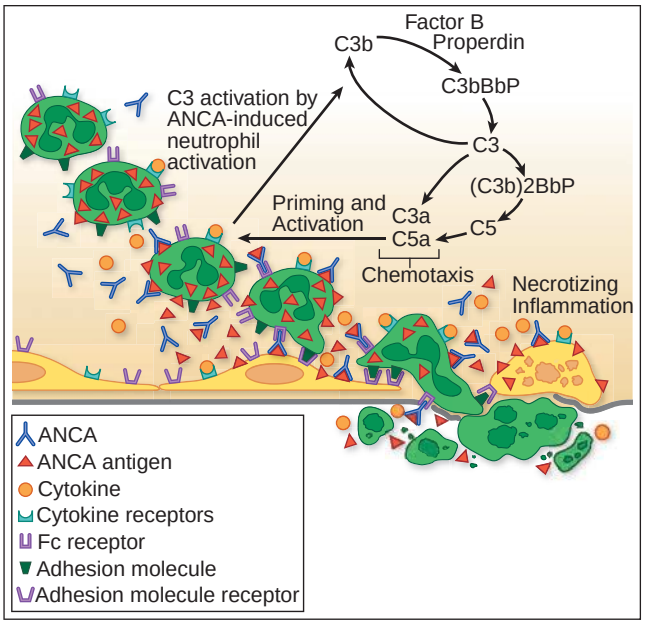



**FIGURE 16.30 Diagram depicting a hypothetical sequence of pathogenic events through which ANCA IgG induces neutrophils to cause vascular inflammation and necrosis.** Beginning in the upper left, engagement of cytokine receptors by cytokines stimulates the presentation of more ANCA antigens at the surface of neutrophils. These antigens then interact with ANCA in the microenvironment of neutrophils and activate neutrophils both by Fc receptor engagement and by Fab′2 binding to antigen on the cell surface. Fc receptors can engage ANCA bound to antigen at the cell surface or can engage Fc receptors on ANCA bound to antigens adsorbed onto the tissue (i.e., localized immune complexes formed in situ). Once activated by ANCA, neutrophils release factors that activate the alternative complement pathway, which generates C5a that attracts more neutrophils and primes neutrophils to be activated by ANCA. Activated neutrophils adhere to endothelial cells via adhesion molecules and release toxic factors that cause vessel wall necrosis (*lower right*).

Thus, the clinical and experimental evidence support the following pathogenic mechanism. ANCA small-vessel vasculitis occurs in patients with circulating ANCA who develop a synergistic inflammatory process, such as a viral respiratory tract infection, that primes neutrophils and monocytes so they can be activated by ANCA. Engagement of receptors on neutrophils by cytokines enhances the availability of ANCA antigens at the surface of neutrophils for interaction with ANCA. Neutrophils are activated by both Fc receptor engagement and Fab′2 binding to antigens on the neutrophil surface. Fc receptors can engage ANCA bound to antigen at the cell surface or can engage Fc receptors on ANCA bound to antigens in immune complexes that form in the microenvironment adjacent to the activated neutrophils, including small amounts of immune complexes formed in situ at sites of inflammation. Activated neutrophils release factors that activate the alternative complement pathway, which amplifies the inflammation and attracts and activates more neutrophils. Activated neutrophils adhere to endothelial cells via adhesion molecules and release toxic factors that cause vessel wall necrosis.

Approximately 90% of patients report a “flu-like illness” shortly before the onset of the signs and symptoms of ANCA

small-vessel vasculitis (44). This finding could be an indication that high levels of circulating cytokines, for example, secondary to a viral infection, are causing the fever, aches, and pains of "flu." High levels of circulating cytokines would prime neutrophils and monocytes to express ANCA antigens on their surfaces that would interact with circulating ANCA. This concept is supported by the observation that the glomerulonephritis in mice induced by the injection of anti-MPO IgG is worsened by injection of bacterial lipopolysaccharide, which results in increased levels of circulating tumor necrosis factor–α (172).

The review of the pathogenesis thus far provides convincing evidence that ANCA can cause glomerulonephritis and vasculitis and suggests some of the mechanisms. But why do patients develop an autoimmune response against neutrophil proteins? Pendergraft et al. (181) have made intriguing observations that suggest that an immune response to microbial pathogens might be the immunogenic trigger. They serendipitously discovered that patients with PR3-ANCA disease not only have circulating antibodies that react with PR3 but also circulating antibodies that react with peptides that are complementary to PR3. A complementary peptide is the product of the antisense strand of DNA that is complementary to the sense strand. In other words, patients with PR3-ANCA have antibodies not only to peptides made by the sense strand of the PR3 gene but also have a separate set of antibodies against peptides made by the antisense strand that is complementary to the sense strand. In addition, antibodies against complementary PR3 peptides (anti-cPR3) bind to anti-PR3 antibodies. In fact, anti-PR3 and anti-cPR3 comprise an anti-idiotypic pair. Further, certain infectious pathogens that are known to be associated with PR3-ANCA disease, such as *Staphylococcus aureus*, Ross River virus, and *Entamoeba histolytica*, contain proteins that have peptides that closely mimic the complementary peptides of PR3. These observations were the basis for a theory for the immunogenesis of the ANCA autoimmune response that proposes that the initiating event, in a predisposed individual, is an immune response to an infectious pathogen (such as Ross River virus) that carries a peptide that is a close mimic of the complementary (antisense) peptide of cPR3. The individual then makes anti-idiotypic antibodies in response to the anti-cPR3. Unfortunately, these anti-idiotypic antibodies recognize the sense peptides that are complementary to the antisense peptides (cPR3), and these sense peptides are part of the PR3 molecule. Pendergraft et al. (181) obtained additional support for this theory by immunizing mice with human cPR3 and observing that the mice developed antibodies not only against cPR3 (anti-cPR3) but also against PR3 (anti-PR3) and demonstrated that these antibodies were an idiotypic pair. A historical note of interest is that the very first report of ANCA in 1982 by Davies et al. (79) was titled "Segmental necrotising glomerulonephritis with antineutrophil antibody: possible arbovirus aetiology?" Davies suggested this etiology because all of his patients with c-ANCA also had positive serology for Ross River virus. Ross River virus contains a peptide that is a close mimic of a complementary peptide of PR3 (181).

## DIFFERENTIAL DIAGNOSIS

Pauci-immune crescentic glomerulonephritis and pauci-immune small-vessel vasculitis must be distinguished from immune complex–mediated glomerulonephritis and small-vessel vasculitis and anti-GBM disease. This may be difficult or impossible by light microscopy alone; however, immunohistology and serology usually result in an actionable diagnosis.

Immune complex glomerulonephritis, whether limited to the kidney or a component of a systemic small-vessel vasculitis, typically has more glomerular hypercellularity, often with thickened capillary walls, in contrast to pauci-immune crescentic glomerulonephritis, which often has little or no hypercellularity and no thickening of capillary walls in nonnecrotic segments and glomeruli. By light microscopy, anti-GBM glomerulonephritis is indistinguishable from pauci-immune crescentic glomerulonephritis because it, too, typically has conspicuous fibrinoid necrosis and crescent formation in the absence of substantial endocapillary hypercellularity or thickening of capillary walls. The accumulation of immune complexes in the glomeruli of patients with immune complex glomerulonephritis stimulates more endocapillary proliferation and less necrosis than the activation of neutrophils within glomeruli by ANCA or anti-GBM antibodies.

Immune complex–mediated vasculitides that affect the kidneys and must be distinguished from ANCA small-vessel vasculitis include IgA vasculitis (HSP), cryoglobulinemic vasculitis, and lupus vasculitis (22). Immune complex vasculitis can be induced by autoimmune responses, as in lupus vasculitis, or by immune responses to exogenous agents, such as viral antigens (182,183). The immune complex vasculitides that most often affect the kidneys are discussed in detail in other chapters of this book, so they are only briefly reviewed here, primarily to compare and contrast them with the pauci-immune ANCA-associated small-vessel vasculitides. The reader is referred to the appropriate chapters for detailed descriptions of the immune complex small-vessel vasculitides. IgA vasculitis (HSP) is discussed in Chapter 12, renal vasculitic lesions of lupus nephritis are discussed in Chapter 14, and cryoglobulinemic vasculitis is discussed in Chapters 22 and 23.

The clinical and light microscopic manifestations of the various types of immune complex–mediated small-vessel vasculitis overlap with each other as well as with the manifestations of pauci-immune small-vessel vasculitides (see Table 16.2). For example, a patient with nephritis, arthralgias, abdominal pain, purpura, and leukocytoclastic angiitis observed in a skin biopsy specimen could have IgA vasculitis (HSP), cryoglobulinemic vasculitis, lupus vasculitis, or an ANCA small-vessel vasculitis such as MPA. Although this patient meets the historical clinical criteria for a diagnosis of IgA vasculitis, the modern definition of this syndrome requires that the vasculitis be caused by IgA-dominant immune complexes (26,27). This additional criterion is important for separating IgA vasculitis from other small-vessel vasculitides that have different prognoses and appropriate treatments. For example, if the patient described in this paragraph, in fact, has IgA vasculitis, the prognosis is good, and supportive care without immunosuppressive treatment is appropriate in most patients; however, if the patient has ANCA-positive MPA, the prognosis is grave unless aggressive immunosuppressive treatment is instituted. Thus, the diagnostic differentiation among different types of small-vessel vasculitis is important to patient care.

Immune complex vasculitis most often affects venules, especially dermal venules, and capillaries, especially glomerular capillaries. Therefore, purpura and nephritis are frequent clinical manifestations of immune complex vasculitis, along with

nonspecific constitutional symptoms of systemic inflammation, such as fever, arthralgias, and myalgias.

Immune complex localization in arterial walls can cause necrotizing lesions that are identical to those of arterial involvement by the pauci-immune ANCA small-vessel vasculitides. The vascular inflammation results from activation of many humoral and cellular inflammatory effector systems, with complement and neutrophils playing major roles (184). Immune complex arteritis tends to affect smaller arteries, for example, in abdominal viscera and peripheral nerves, causing abdominal pain and peripheral neuropathy, respectively. Leukocytoclastic angiitis, for example, in dermal vessels, has the same basic light microscopic histologic features whether caused by immune complexes or associated with ANCA. A distinguishing pathologic feature is the presence of immune complex–type deposits by immunofluorescence microscopy and electron microscopy in the former and their absence in the latter. For example, IgA-dominant immune deposits indicate IgA vasculitis, and IgM-dominant deposits raise the possibility of cryoglobulinemic vasculitis.

The glomerulonephritis that is a component of systemic immune complex small-vessel vasculitis typically has much more hypercellularity and much less necrosis than the glomerulonephritis seen with pauci-immune small-vessel vasculitis. For example, the glomerulonephritis of IgA vasculitis is pathologically indistinguishable from IgA nephropathy (185) and thus has a spectrum of proliferative expressions including ranging from focal to diffuse. The most common phenotype of glomerulonephritis that occurs as a component of cryoglobulinemic vasculitis is type I immunoglobulin-positive membranoproliferative glomerulonephritis, sometimes with a distinctive microtubular ultrastructure in the dense deposits and often associated with hepatitis C infection (183,186).

Exceptions to the proposition that proliferative glomerulonephritis and thick capillary walls suggest immune complex disease rather than pauci-immune disease are the instances of apparent concurrent immune complex and ANCA disease. This has been reported most often for membranous glomerulonephritis (119–121,143) and IgA nephropathy (125–128), diabetic glomerulosclerosis (129), and lupus glomerulonephritis (122,130). The concurrent ANCA disease apparently is contributing to disease induction because the clinical course and the severity of crescent formation and necrosis is more severe in patients with combined immune complex glomerulonephritis and ANCA compared with the usual presentation of the disease in the absence of ANCA.

A particularly problematic differential diagnosis arises in patients who are ANCA positive and have severe glomerulonephritis, sometimes with systemic small-vessel vasculitis, but are found to have immune complex glomerulonephritis rather than pauci-immune glomerulonephritis. For example, well-documented examples of concurrent infectious endocarditis, immune complex glomerulonephritis, and ANCA (typically PR3-ANCA) have been reported (187–191). Most of these have responded well to antibiotic therapy and have not required immunosuppression. Given the circumstances, immunosuppressive therapy may have been detrimental to patient outcome.

However, the differential diagnostic considerations in patients with infectious endocarditis are even more complicated because some patients, who usually are ANCA negative, have a pauci-immune crescentic glomerulonephritis (190,191). For example, in a series of nine renal biopsy specimens and seven autopsy specimens with infectious endocarditis glomerulonephritis, Majumdar et al. (191) observed focal pauci-immune necrotizing ("vasculitic") glomerulonephritis in 11, acute diffuse proliferative glomerulonephritis in 3, and type I membranoproliferative glomerulonephritis in 2. Thus, in their series, pauci-immune glomerulonephritis was more common than immune complex glomerulonephritis and had an appearance that was indistinguishable from ANCA glomerulonephritis. Some patients with ANCA-negative, endocarditis-associated, pauci-immune crescentic glomerulonephritis seem to do well with antibiotic therapy alone; however, at least some may require immunomodulatory therapy (190).

Segmental necrotizing lupus glomerulonephritis also is in the differential diagnosis for ANCA glomerulonephritis and pauci-immune crescentic glomerulonephritis. This pattern of glomerular injury in lupus nephritis was first reported by Schwartz et al. (192). Some lupus patients have substantial segmental necrosis and crescent formation with only mild hypercellularity and scanty immune deposits, thus resembling ANCA pauci-immune crescentic glomerulonephritis. These patients usually are ANCA negative; however, Masani et al. (123) reported a patient with pauci-immune crescentic glomerulonephritis who was MPO-ANCA–positive and apparently represented concurrent lupus and ANCA disease. Approximately 20% of patients with systemic lupus erythematosus have a positive ANCA, almost always p-ANCA rather than c-ANCA and usually specific for myeloperoxidase (MPO-ANCA) or lactoferrin (LF-ANCA) (123,193). Although there are a few intriguing anecdotes (123), multiple studies have provided conflicting findings but have not proven a frequent role for ANCA in the pathogenesis of glomerulonephritis in patients with systemic lupus erythematosus. The differential diagnosis for pauci-immune crescentic glomerulonephritis, especially if there is a positive antinuclear antibody test without overt systemic lupus erythematosus, includes drug-induced ANCA disease. The renal pathologic features of drug-associated ANCA disease are indistinguishable from those of idiopathic ANCA disease. An unusually high titer of MPO-ANCA should raise suspicion of drug-induced disease (70,71,147). Propylthiouracil, hydralazine, methimazole, minocycline, and a number of other drugs apparently induce circulating MPO-ANCA and pauci-immune crescentic glomerulonephritis and systemic small-vessel vasculitis (61–71,147,194). Cocaine-induced ANCA disease is most likely caused by admixed levamisole (70).

## CLINICAL COURSE, PROGNOSIS, THERAPY, AND CLINICOPATHOLOGIC CORRELATIONS

Pauci-immune crescentic glomerulonephritis, with or without accompanying systemic vasculitis, is an aggressive renal disease that warrants aggressive immunosuppression; however, remission can be induced in most patients with expeditious treatment (35,44,195–203). Without treatment, pauci-immune crescentic glomerulonephritis and small-vessel vasculitis have a 1-year mortality of 80%, whereas with treatment, there is a 75% 5-year patient and renal survival (200). Treatment has three phases: remission induction, remission maintenance, and

relapse treatment (198). Cyclophosphamide combined with corticosteroids is the most commonly used induction therapy (196,200). Corticosteroids often are begun as intravenous pulses of methylprednisolone followed by tapering with oral prednisone. Cyclophosphamide is given either orally or intravenously. The intravenous route is as effective as the oral route at inducing remission and has less leukopenia and fewer infectious complications; however, there are more relapses (200). Combination therapy with cyclophosphamide and corticosteroids results in improvement in more than 90% of patients and complete remission in approximately 75% (33,35,44). After remission is achieved, which usually occurs within 3 months, corticosteroids and cyclophosphamide often are discontinued. Many patients have relapses that require additional immunosuppressive treatment (35,196). A regimen similar to that used for induction often is used for relapses. Approximately 70% of patients with relapses enter remission with retreatment (35). The 10% or so of patients who are resistant to conventional therapy are problematic. Various alternative induction therapies have been tried including administration of intravenous gamma globulin, anti–B-cell antibodies, and plasma exchange (196,200–204).

In a study of 70 patients with ANCA-positive pauci-immune crescentic glomerulonephritis, Falk et al. (44) obtained 75% overall renal survival at 24 months using high-dose corticosteroids or corticosteroids combined with cyclophosphamide. There was no difference in renal survival between patients with renal-limited disease and those with systemic vasculitis. Oral cyclophosphamide and intravenous cyclophosphamide were equally effective for inducing remission. In a follow-up study by the same group that evaluated remission and relapse in 107 patients with ANCA pauci-immune crescentic glomerulonephritis, patients treated with corticosteroids alone had a lower remission rate and three times greater risk for relapse compared with patients treated with corticosteroids combined with cyclophosphamide (35). In a parallel study of the same cohort, serum creatinine levels at the time of diagnosis and institution of treatment were the best predictor of outcome (53), which highlights the importance of rapid pathologic diagnosis of ANCA crescentic glomerulonephritis so that appropriate treatment can begin promptly. Bomback et al. (54) have shown that immunosuppressive therapy is beneficial to renal and patient survival even in patients greater than 80 years old.

The EUVAS has provided extremely important data on the treatment of ANCA glomerulonephritis and vasculitis. Studies by this collaborative group have concluded that cyclophosphamide is an effective drug for induction of remission but that cumulative toxicity should be reduced by using alternative potentially less toxic drugs for maintenance of remission, such as mycophenolate mofetil and azathioprine (196,197,200).

Plasmapheresis appears to improve outcomes in patients with pulmonary hemorrhage and severe renal failure at the time of diagnosis (205–207). The EUVAS investigated the efficacy of plasma exchange versus intravenous methylprednisolone as induction therapy in patients with substantial renal failure (207). Plasmapheresis improved the rate of recovery from renal failure. Patient survival and adverse events were similar in both groups. Plasmapheresis was associated with a 24% reduced risk of progression to ESRD (43% to 19% at 1 year).

The effectiveness of induction therapy with an anti–B-cell antibody has been tested in patients with ANCA disease using rituximab (Rituxan), which is a chimeric monoclonal antibody against the CD20 (201–203). Two randomized control trials demonstrated noninferiority of rituximab versus cyclophosphamide (201–203). There was no reduction in adverse events with Rituxan in either study. An extension of one of these studies demonstrated that rituximab was as effective as conventional immunosuppressive therapy for the induction and maintenance of remissions for 18 months (208).

Patients with MPO-ANCA may have a better prognosis than those with PR3-ANCA (10,53,204) even though they have more extensive and more chronic renal disease at presentation (11,200). Patients with PR3-ANCA have more extrarenal organ manifestations and respiratory tract granulomatous inflammation (204) and a greater relapse rate after induction of remission (209). Even in the absence of granulomatous disease, patients with PR3-ANCA have a worse prognosis with more rapid deterioration of renal function and higher mortality (53,204). This is in spite of the observation that patients with MPO-ANCA have more extensive chronic renal disease at the time of initial renal biopsy (11,12). More chronicity of the renal disease in the initial biopsy may be related to delayed diagnosis and treatment as a result of fewer extrarenal manifestations in MPO-ANCA disease patients compared with PR3-ANCA patients. Vizjak et al. (11) estimated that duration of disease prior to renal biopsy was 14.8 ± 26.6 months in patients with PR3-ANCA compared with 8.5 ± 12.3 months in patients with MPO-ANCA.

Patients with ANCA crescentic glomerulonephritis have a better response to treatment than patients with anti-GBM glomerulonephritis, even when they have similar initial degrees of renal dysfunction and glomerular histologic changes (10,89,195). Patients with both ANCA and anti-GBM disease have a prognosis that is more like that of anti-GBM disease and thus is worse than ANCA disease (89,205).

Factors that independently correlate with an overall poor outcome include older age, higher serum creatinine at presentation, and pulmonary hemorrhage (53,200). Respiratory tract disease and PR3-ANCA (vs. MPO-ANCA) are predictors of greater relapse rate (47). In a study by Bajema et al. (47), the pathologic finding that correlated best with renal function at the time of biopsy and during follow-up was the percentage of histologically normal glomeruli. Other features that correlated with renal function included glomerular sclerosis, interstitial leukocyte infiltration, tubular necrosis, and tubular atrophy (47). A study by the same group (46) concluded that the glomerular filtration rate (GFR) at 18 months after diagnosis correlated best with interstitial fibrosis and tubular atrophy, whereas glomerular crescents and necrosis correlated with the extent of recovery of renal function (i.e., the GFR at 18 month minus the GFR at diagnosis). As discussed earlier, pathologic classification based on the proportions of normal, crescentic, and sclerotic glomeruli helps predict renal outcome (49,134–136), with patients with ≥ 50% normal glomeruli having the best outcome and patients with ≥ 50% globally sclerotic glomeruli having the worst outcome. Berden et al. (107) also evaluated the importance of tubulointerstitial inflammation in predicting outcome among patients treated with a rituximab-based regimen in the Randomized Trial of Rituximab versus Cyclophosphamide in ANCA-Associated Vasculitis trial. Both CD3(+) T-cell tubulitis and tubular atrophy correlated with estimated GFR at 12 months; tubular atrophy remained an

independent predictor of renal outcome at 24 months ($P < 0.01$).

Several studies conclude that chronic injury at the time that treatment is begun is irreversible and is likely to result in a poor renal outcome if it is severe enough, whereas active inflammatory lesions may be suppressed or reversed by treatment and thus are predictors that there will be a response to anti-inflammatory and immunosuppressive treatment.

## REFERENCES


1. Stilmant MM, Bolton WK, Sturgill BC, et al. Crescentic glomerulonephritis without immune deposits: clinicopathologic features. *Kidney Int* 1979;15:184.
2. Jennette JC, Thomas DB. Crescentic glomerulonephritis. *Nephrol Dial Transplant* 2001;16(Suppl 6):80.
3. Falk RJ, Jennette JC. ANCA disease: where is this field going? *J Am Soc Nephrol* 2010;21:745.
4. Croker BP, Lee T, Gunnells JC. Clinical and pathologic features of polyarteritis nodosa and its renal-limited variant: primary crescentic and necrotizing glomerulonephritis. *Hum Pathol* 1987;18:38.
5. Angangco R, Thiru S, Esnault VL, et al. Does truly "idiopathic" crescentic glomerulonephritis exist? *Nephrol Dial Transplant* 1994;9:630.
6. Falk RJ, Jennette JC. Anti-neutrophil cytoplasmic autoantibodies with specificity for myeloperoxidase in patients with systemic vasculitis and idiopathic necrotizing and crescentic glomerulonephritis. *N Engl J Med* 1988;318:1651.
7. Andrassy K. Rapidly progressive glomerulonephritis and Wegener's granulomatosis. *Nephrologie* 1992;13:279.
8. Bindi P, Mougenot B, Mentre F, et al. Necrotizing crescentic glomerulonephritis without significant immune deposits: a clinical and serological study. *Q J Med* 1993;86:55.
9. Tervaert JW, Goldschmeding R, Elema JD, et al. Autoantibodies against myeloid lysosomal enzymes in crescentic glomerulonephritis. *Kidney Int* 1990;37:799.
10. Saxena R, Bygren P, Rasmussen N, et al. Circulating autoantibodies in patients with extracapillary glomerulonephritis. *Nephrol Dial Transplant* 1991;6:389.
11. Vizjak A, Rott T, Koselj-Kajtna M, et al. Histologic and immunohistologic study and clinical presentation of ANCA-associated glomerulonephritis with correlation to ANCA antigen specificity. *Am J Kidney Dis* 2003;41:539.
12. Hauer HA, Bajema IM, van Houwelingen HC, et al. Renal histology in ANCA-associated vasculitis: differences between diagnostic and serologic subgroups. *Kidney Int* 2002;61:80.
13. Hagen EC, Daha MR, Hermans J, et al. Diagnostic value of standardized assays for anti-neutrophil cytoplasmic antibodies in idiopathic systemic vasculitis. EC/BCR Project for ANCA Assay Standardization. *Kidney Int* 1998;53:743.
14. Jennette JC, Falk RJ. Antineutrophil cytoplasmic autoantibodies in inflammatory bowel disease. *Am J Clin Pathol* 1993;99:221.
15. Kallenberg CG, Brouwer E, Weening JJ, et al. Anti-neutrophil cytoplasmic antibodies: current diagnostic and pathophysiological potential. *Kidney Int* 1994;46:1.
16. Lim LC, Taylor JG III, Schmitz JL, et al. Diagnostic usefulness of antineutrophil cytoplasmic autoantibody serology: comparative evaluation of commercial indirect fluorescent antibody kits and enzyme immunoassay kits. *Am J Clin Pathol* 1999;111:363.
17. Savage CO. ANCA-associated renal vasculitis. *Kidney Int* 2001;60:1614.
18. Savige J, Davies D, Falk RJ, et al. Antineutrophil cytoplasmic antibodies and associated diseases: a review of the clinical and laboratory features. *Kidney Int* 2000;57:846.
19. Tervaert JW, Damoiseaux J. Antineutrophil cytoplasmic autoantibodies: How are they detected and what is their use for diagnosis, classification and follow-up? *Clin Rev Allergy Immunol* 2012;43:211.
20. Holle JU, Herrmann K, Gross WL, et al. Comparative analysis of different commercial ELISA systems for the detection of anti-neutrophil cytoplasm antibodies in ANCA-associated vasculitides. *Clin Exp Rheumatol* 2012;30(1 Suppl 70):S66.
21. Jennette JC. Rapidly progressive crescentic glomerulonephritis. *Kidney Int* 2003;63:1164.
22. Jennette JC, Falk RJ. Small-vessel vasculitis. *N Engl J Med* 1997;337:1512.
23. Eisenberger U, Fakhouri F, Vanhille P, et al. ANCA-negative pauci-immune renal vasculitis: histology and outcome. *Nephrol Dial Transplant* 2005;20:1392.
24. Niles JL, Bottinger EP, Saurina GR, et al. The syndrome of lung hemorrhage and nephritis is usually an ANCA-associated condition. *Arch Intern Med* 1996;156:440.
25. Bonsib SM, Walker WP. Pulmonary-renal syndrome: clinical similarity amidst etiologic diversity. *Mod Pathol* 1989;2:129.
26. Jennette JC, Falk RJ, Andrassy K, et al. Nomenclature of systemic vasculitides. Proposal of an international consensus conference. *Arthritis Rheum* 1994;37:187.
27. Jennette JC, Falk RJ, Bacon PA, et al. 2012 revised International Chapel Hill Consensus Conference Nomenclature of Vasculitides. *Arthritis Rheum* 2013;65:1.
28. Guillevin L, Lhote F, Gherardi R. Polyarteritis nodosa, microscopic polyangiitis, and Churg-Strauss syndrome: clinical aspects, neurologic manifestations, and treatment. *Neurol Clin* 1997;15:865.
29. Guillevin L, Lhote F, Amouroux J, et al. Antineutrophil cytoplasmic antibodies, abnormal angiograms and pathological findings in polyarteritis nodosa and Churg-Strauss syndrome: indications for the classification of vasculitides of the polyarteritis Nodosa Group. *Br J Rheumatol* 1996;35:958.
30. Lhote F, Guillevin L. Polyarteritis nodosa, microscopic polyangiitis, and Churg-Strauss syndrome: clinical aspects and treatment. *Rheum Dis Clin North Am* 1995;21:911.
31. Duna GF, Galperin C, Hoffman GS. Wegener's granulomatosis. *Rheum Dis Clin North Am* 1995;21:949.
32. Fauci AS, Haynes BF, Katz P, et al. Wegener's granulomatosis: prospective clinical and therapeutic experience with 85 patients for 21 years. *Ann Intern Med* 1983;98:76.
33. Hoffman GS, Kerr GS, Leavitt RY, et al. Wegener granulomatosis: an analysis of 158 patients. *Ann Intern Med* 1992;116:488.
34. Leavitt RY, Fauci AS, Bloch DA, et al. The American College of Rheumatology 1990 criteria for the classification of Wegener's granulomatosis. *Arthritis Rheum* 1990;33:1101.
35. Nachman PH, Hogan SL, Jennette JC, et al. Treatment response and relapse in antineutrophil cytoplasmic autoantibody-associated microscopic polyangiitis and glomerulonephritis. *J Am Soc Nephrol* 1996;7:33.
36. Savage CO, Winearls CG, Evans DJ, et al. Microscopic polyarteritis: presentation, pathology and prognosis. *Q J Med* 1985;56:467.
37. Churg J, Strauss L. Allergic granulomatosis, allergic angiitis and periarteritis nodosa. *Am J Pathol* 1951;27:277.
38. Kikuchi Y, Ikehata N, Tajima O, et al. Glomerular lesions in patients with Churg-Strauss syndrome and the anti-myeloperoxidase antibody. *Clin Nephrol* 2001;55:429.
39. Godman GC, Churg J. Wegener's granulomatosis: pathology and review of the literature. *Arch Pathol Lab Med* 1954;58:533.
40. Clutterbuck EJ, Evans DJ, Pusey CD. Renal involvement in Churg-Strauss syndrome. *Nephrol Dial Transplant* 1990;5:161.
41. Keogh KA, Specks U. Churg-Strauss syndrome. *Semin Respir Crit Care Med* 2006;27:148–157.
42. Whyte AF, Smith WB, Sinkar SN, et al. Clinical and laboratory characteristics of 19 patients with Churg-Strauss syndrome from a single South Australian centre. *Intern Med J* 2013;43:784.
43. Jennette JC. Antineutrophil cytoplasmic autoantibody-associated diseases: a pathologist's perspective. *Am J Kidney Dis* 1991;18:164.
44. Falk RJ, Hogan S, Carey TS, et al. Clinical course of anti-neutrophil cytoplasmic autoantibody associated glomerulonephritis and systemic vasculitis. The Glomerular Disease Collaborative Network. *Ann Intern Med* 1990;113:656.
45. Gayraud M, Guillevin L, le Toumelin P, et al. Long-term followup of polyarteritis nodosa, microscopic polyangiitis, and Churg-Strauss syndrome: analysis of four prospective trials including 278 patients. *Arthritis Rheum* 2001;44:666.
46. Hauer HA, Bajema IM, van Houwelingen HC, et al. Determinants of outcome in ANCA-associated glomerulonephritis: a prospective clinico-histopathological analysis of 96 patients. *Kidney Int* 2002;62:1732.

47. Bajema IM, Hagen EC, Hermans J, et al. Kidney biopsy as a predictor for renal outcome in ANCA-associated necrotizing glomerulonephritis. *Kidney Int* 1999;56:1751.
48. de Lind van Wijngaarden RA, Hauer HA, Wolterbeek R, et al. Clinical and histologic determinants of renal outcome in ANCA-associated vasculitis: a prospective analysis of 100 patients with severe renal involvement. *J Am Soc Nephrol* 2006;17:2264.
49. Berden AE, Ferrario F, Hagen EH, et al. Histopathological classification of ANCA-associated glomerulonephritis. *Kidney Int* 2010;21:1628–1636.
50. Lionaki S, Blyth ER, Hogan SL, et al. Classification of antineutrophil cytoplasmic autoantibody vasculitides: the role of antineutrophil cytoplasmic autoantibody specificity for myeloperoxidase or proteinase 3 in disease recognition and prognosis. *Arthritis Rheum* 2012;64:3452.
51. Woodworth TG, Abuelo JG, Esparza A. Severe glomerulonephritis with late emergence of classic Wegener's granulomatosis. Report of 4 cases and review of the literature. *Medicine* 1987;66:181.
52. Guillevin L, Durand-Gasselin B, Cevallos R, et al. Microscopic polyangiitis: clinical and laboratory findings in eighty-five patients. *Arthritis Rheum* 1999;42:421.
53. Hogan SL, Nachman PH, Wilkman AS, et al. Prognostic markers in patients with antineutrophil cytoplasmic autoantibody-associated microscopic polyangiitis and glomerulonephritis. *J Am Soc Nephrol* 1996;7:23.
54. Bomback AS, Appel GB, Radhakrishnan J, et al. ANCA-associated glomerulonephritis in the very elderly. *Kidney Int* 2011;79:757–764.
55. Masi AT, Hunder GG, Lie JT, et al. The American College of Rheumatology 1990 criteria for the classification of Churg-Strauss syndrome (allergic granulomatosis and angiitis). *Arthritis Rheum* 1990;33:1094.
56. Lanham JG, Churg J. Churg-Strauss syndrome. In: Churg J, ed. *Systemic Vasculitides*. New York: Igaku-Shoin, 1991:101.
57. Lyons PA, Rayner TF, Trivedi S, et al. Genetically distinct subsets within ANCA-associated vasculitis. *N Engl J Med* 2012;367:214.
58. Neild GH, Cameron JS, Lessof MH, et al. Relapsing polychondritis with crescentic glomerulonephritis. *Br Med J* 1978;1:743.
59. Geffriaud-Ricouard C, Noel LH, Chauveau D, et al. Clinical spectrum associated with ANCA of defined antigen specificities in 98 selected patients. *Clin Nephrol* 1993;39:125.
60. Espinoza LR, Richman A, Bocanegra T, et al. Immune complex-mediated renal involvement in relapsing polychondritis. *Am J Med* 1981;71:181.
61. Morita S, Ueda Y, Eguchi K. Anti-thyroid drug-induced ANCA-associated vasculitis: a case report and review of the literature. *Endocr J* 2000;47:467.
62. D'Cruz D, Chesser AM, Lightowler C, et al. Antineutrophil cytoplasmic antibody-positive crescentic glomerulonephritis associated with antithyroid drug treatment. *Br J Rheumatol* 1995;34:1090.
63. Dolman KM, Gans RO, Vervaat TJ, et al. Vasculitis and antineutrophil cytoplasmic autoantibodies associated with propylthiouracil therapy. *Lancet* 1993;342:651.
64. Vogt BA, Kim Y, Jennette JC, et al. Antineutrophil cytoplasmic autoantibody-positive crescentic glomerulonephritis as a complication of treatment with propylthiouracil in children. *J Pediatr* 1994;124:986.
65. Devogelaer JP, Pirson Y, Vandenbroucke JM, et al. D-penicillamine induced crescentic glomerulonephritis: report and review of the literature. *J Rheumatol* 1987;14:1036.
66. Ntoso KA, Tomaszewski JE, Jimenez SA, et al. Penicillamine-induced rapidly progressive glomerulonephritis in patients with progressive systemic sclerosis: successful treatment of two patients and a review of the literature. *Am J Kidney Dis* 1986;8:159.
67. Nassberger L, Johansson AC, Bjorck S, et al. Antibodies to neutrophil granulocyte myeloperoxidase and elastase: autoimmune responses in glomerulonephritis due to hydralazine treatment. *J Intern Med* 1991;229:261.
68. Lenert P, Icardi M, Dahmoush L. ANA (+) ANCA (+) systemic vasculitis associated with the use of minocycline: case-based review. *Clin Rheumatol* 2013;32:1099.
69. Mathieson PW, Peat DS, Short A, et al. Coexistent membranous nephropathy and ANCA-positive crescentic glomerulonephritis in association with penicillamine. *Nephrol Dial Transplant* 1996;11:863.
70. Pearson T, Bremmer M, Cohen J, et al. Vasculopathy related to cocaine adulterated with levamisole: a review of the literature. *Dermatol Online J* 2012;18:1.
71. McGrath MM, Isakova T, Rennke HG, et al. Contaminated cocaine and antineutrophil cytoplasmic antibody-associated disease. *Clin J Am Soc Nephrol* 2011;6:2799.
72. Marco H, Mirapeix E, Arcos E, et al. Long-term outcome of antineutrophil cytoplasmic antibody-associated small vessel vasculitis after renal transplantation. *Clin Transplant* 2013;27:338–347.
73. Rostaing L, Modesto A, Oksman F, et al. Outcome of patients with antineutrophil cytoplasmic antibody–associated vasculitis following cadaveric kidney transplantation. *Am J Kidney Dis* 1997;9:96.
74. Nachman PH, Segelmark M, Westman K, et al. Recurrent ANCA-associated small vessel vasculitis after transplantation: a pooled analysis. *Kidney Int* 1999;56:1544.
75. Ferrario F, Tadros MT, Napodano P, et al. Critical re-evaluation of 41 cases of "idiopathic" crescentic glomerulonephritis. *Clin Nephrol* 1994;41:1.
76. Ferrario F, Rastaldi MP. Necrotizing-crescentic glomerulonephritis in ANCA-associated vasculitis: the role of monocytes. *Nephrol Dial Transplant* 1999;14:1627–1631.
77. Sinico RA, Di Toma L, Maggiore U, et al. Renal involvement in Churg-Strauss syndrome. *Am J Kidney Dis* 2006;47:770.
78. Jennette JC, Hipp CG. The epithelial antigen phenotype of glomerular crescent cells. *Am J Clin Pathol* 1986;86:274.
79. Davies DJ, Moran JE, Niall JF, et al. Segmental necrotising glomerulonephritis with antineutrophil antibody: possible arbovirus aetiology? *Br Med J (Clin Res Ed)* 1982;285:606.
80. Hall JB, Wadham BM, Wood CJ, et al. Vasculitis and glomerulonephritis: A subgroup with an antineutrophil cytoplasmic antibody. *Aust N Z J Med* 1984;14:277.
81. van der Woude FJ, Rasmussen N, Lobatto S, et al. Autoantibodies against neutrophils and monocytes: tool for diagnosis and marker of disease activity in Wegener's granulomatosis. *Lancet* 1985;1:425.
82. Savage CO, Winearls CG, Jones S, et al. Prospective study of radioimmunoassay for antibodies against neutrophil cytoplasm in diagnosis of systemic vasculitis. *Lancet* 1987;1:1389.
83. Venning MC, Arfeen S, Bird AG. Antibodies to neutrophil cytoplasmic antigen in systemic vasculitis. *Lancet* 1987;2:850.
84. Wathen CW, Harrison DJ. Circulating anti-neutrophil antibodies in systemic vasculitis. *Lancet* 1987;1:1037.
85. Bosch X, Mirapeix E, Font J, et al. Prognostic implication of anti-neutrophil cytoplasmic autoantibodies with myeloperoxidase specificity in antiglomerular basement membrane disease. *Clin Nephrol* 1991;36:107.
86. Bosch X, Mirapeix E, Font J, et al. Anti-myeloperoxidase autoantibodies in patients with necrotizing glomerular and alveolar capillaritis. *Am J Kidney Dis* 1992;20:231.
87. Jayne DR, Marshall PD, Jones SJ, et al. Autoantibodies to GBM and neutrophil cytoplasm in rapidly progressive glomerulonephritis. *Kidney Int* 1990;37:965.
88. Short AK, Esnault VL, Lockwood CM. Anti-neutrophil cytoplasm antibodies and anti-glomerular basement membrane antibodies: two coexisting distinct autoreactivities detectable in patients with rapidly progressive glomerulonephritis. *Am J Kidney Dis* 1995;26:439.
89. Rutgers A, Slot M, van Paassen P, et al. Coexistence of anti-glomerular basement membrane antibodies and myeloperoxidase-ANCAs in crescentic glomerulonephritis. *Am J Kidney Dis* 2005;46:253.
90. Bonsib SM, Goeken JA, Kemp JD, et al. Coexistent anti-neutrophil cytoplasmic antibody and antiglomerular basement membrane antibody associated disease: report of six cases. *Mod Pathol* 1993;6:526.
91. Hellmark T, Niles JL, Collins AB, et al. Comparison of anti-GBM antibodies in sera with or without ANCA. *J Am Soc Nephrol* 1997;8:376.
92. Charles LA, Falk RJ, Jennette JC. Reactivity of anti-neutrophil cytoplasmic autoantibodies with HL-60 cells. *Clin Immunol Immunopathol* 1989;53(Pt 1):243.
93. Goldschmeding R, van der Schoot CE, ten Bokkel Huinink D, et al. Wegener's granulomatosis autoantibodies identify a novel diisopropylfluorophosphate-binding protein in the lysosomes of normal human neutrophils. *J Clin Invest* 1989;84:1577.
94. Jennette JC, Hoidal JH, Falk RJ. Specificity of anti-neutrophil cytoplasmic autoantibodies for proteinase 3. *Blood* 1990;75:2263.
95. Niles JL, McCluskey RT, Ahmad MF, et al. Wegener's granulomatosis autoantigen is a novel neutrophil serine proteinase. *Blood* 1989;74:1888.

96. Savige J, Gillis D, Benson E, et al. International Consensus Statement on testing and reporting of Antineutrophil Cytoplasmic Antibodies (ANCA). *Am J Clin Pathol* 1999;111:507.
97. Roth AJ, Ooi JD, Hess JJ, et al. Epitope specificity determines pathogenicity and detectability in ANCA-associated vasculitis.*J Clin Invest* 2013;123:1773.
98. Kain R, Exner M, Brandes R, et al. Molecular mimicry in pauci-immune focal necrotizing GN. *Nat Med* 2008;14:1088.
99. Kain R, Tadema H, McKinney EF, et al. High prevalence of autoantibodies to hLAMP-2 in anti-neutrophil cytoplasmic antibody-associated vasculitis. *J Am Soc Nephrol* 2012;23:556.
100. Roth AJ, Brown MC, Smith RN, et al. Anti-LAMP-2 antibodies are not prevalent in patients with antineutrophil cytoplasmic autoantibody glomerulonephritis. *J Am Soc Nephrol* 2012;23:545.
101. Heptinstall RH. Polyarteritis (periarteritis) nodosa, Wegener's syndrome, and other forms of vasculitis. In: *Pathology of the Kidney*, 4th ed. Boston: Little, Brown and Company, 1992:1100.
102. Watanabe T, Nagafuchi Y, Yoshikawa Y, et al. Renal papillary necrosis associated with Wegener's granulomatosis. *Hum Pathol* 1983;14:551.
103. Bonsib SM, Goeken JA, Fandel T, et al. Necrotizing medullary lesions in patients with ANCA associated renal disease. *Mod Pathol* 1994;7:181.
104. Ronco P, Mignon F, Laneo Y, et al. Ureteral stenosis in Wegener's granulomatosis: Report of a case. *Nephron* 1982;30:201.
105. Metselaar HJ, ten Kate FJ, Weimar W. Ureter obstruction as a complication of Wegener's granulomatosis. *Eur Urol* 1985;11:63.
106. Rich LM, Piering WF. Ureteral stenosis due to recurrent Wegener's granulomatosis after kidney transplantation. *J Am Soc Nephrol* 1994;4:1516.
107. Berden AE, Jones RB, Erasmus DD, et al. Tubular lesions predict renal outcome in antineutrophil cytoplasmic antibody-associated glomerulonephritis after rituximab therapy. *J Am Soc Nephrol* 2012;23:313–321.
108. D'Agati V, Chander P, Nash M, et al. Idiopathic microscopic polyarteritis nodosa: ultrastructural observations on the renal vascular and glomerular lesions. *Am J Kidney Dis* 1986;7:95.
109. Hauer HA, Bajema IM, de Heer E, et al. Distribution of renal lesions in idiopathic systemic vasculitis: a three-dimensional analysis of 87 glomeruli. *Am J Kidney Dis* 2000;36:257.
110. Weiss MA, Crissman JD. Renal biopsy findings in Wegener's granulomatosis: segmental necrotizing glomerulonephritis with glomerular thrombosis. *Hum Pathol* 1984;15:943.
111. Bajema IM, Hagen EC, de Heer E, et al. Colocalization of ANCA-antigens and fibrinoid necrosis in ANCA-associated vasculitis. *Kidney Int* 2001;60:2025.
112. Jennette JC. Implications for pathogenesis of patterns of injury in small- and medium-sized vessel vasculitis. *Cleve Clin J Med* 2002;69:SII33.
113. Jennette JC, Wilkman AS, Falk RJ. Anti-neutrophil cytoplasmic autoantibody-associated glomerulonephritis and vasculitis. *Am J Pathol* 1989;135:921.
114. Bajema IM, Hagen EC, Ferrario F, et al. Renal granulomas in systemic vasculitis. EC/BCR Project for ANCA-Assay Standardization. *Clin Nephrol* 1997;48:16.
115. Ohashi N, Sugiura T, Isozaki T, et al. Anti-glomerular basement membrane antibody-induced glomerulonephritis with periglomerular granulomatous reaction and massive renal eosinophilic infiltration. *Am J Kidney Dis* 2003;42:E28.
116. Xiao H, Heeringa P, Liu Z, et al. The role of neutrophils in the induction of glomerulonephritis by anti-myeloperoxidase antibodies. *Am J Pathol* 2005;167:39.
117. Cunningham MA, Huang XR, Dowling JP, et al. Prominence of cell-mediated immunity effectors in "pauci-immune" glomerulonephritis. *J Am Soc Nephrol* 1999;10:499.
118. Weidner S, Carl M, Riess R, et al. Histologic analysis of renal leukocyte infiltration in antineutrophil cytoplasmic antibody-associated vasculitis: importance of monocyte and neutrophil infiltration in tissue damage. *Arthritis Rheum* 2004;50:3651.
119. Dwyer KM, Agar JW, Hill PA, et al. Membranous nephropathy and antineutrophil cytoplasmic antibody-associated glomerulonephritis: a report of 2 cases. *Clin Nephrol* 2001;56:394.
120. Taniguchi Y, Yorioka N, Kumagai J, et al. Myeloperoxidase antineutrophil cytoplasmic antibody-positive necrotizing crescentic glomerulonephritis and membranous glomerulonephropathy. *Clin Nephrol* 1999;52:253.
121. Tse WY, Howie AJ, Adu D, et al. Association of vasculitic glomerulonephritis with membranous nephropathy: a report of 10 cases. *Nephrol Dial Transplant* 1997;12:1017.
122. Marshall S, Dressler R, D'Agati V. Membranous lupus nephritis with antineutrophil cytoplasmic antibody-associated segmental necrotizing and crescentic glomerulonephritis. *Am J Kidney Dis* 1997;29:119.
123. Masani NN, Imbriano LJ, D'Agati VD, et al. SLE and rapidly progressive glomerulonephritis. *Am J Kidney Dis* 2005;45:950.
124. Haas M, Jafri J, Bartosh SM, et al. ANCA-associated crescentic glomerulonephritis with mesangial IgA deposits. *Am J Kidney Dis* 2000;36:709.
125. Richer C, Mouthon L, Cohen P, et al. IgA glomerulonephritis associated with microscopic polyangiitis or Churg-Strauss syndrome. *Clin Nephrol* 1999;52:47.
126. Vrtovsnik F, Queffeulou G, Skhiri H, et al. Simultaneous IgA nephropathy and Wegener's granulomatosis—overlap or coincidence (the role of renal biopsy). *Nephrol Dial Transplant* 1999;14:1266.
127. Rollino C, Mazzucco G, Basolo B, et al. cANCA positivity in a case of IgA glomerulonephritis (IgAGN) with necrotizing lesions. *Nephrol Dial Transplant* 1999;14:797.
128. Allmaras E, Nowack R, Andrassy K, et al. Rapidly progressive IgA nephropathy with anti-myeloperoxidase antibodies benefits from immunosuppression. *Clin Nephrol* 1997;48:269.
129. Nasr SH, D'Agati VD, Said SM, et al. Pauci-immune crescentic glomerulonephritis superimposed on diabetic glomerulosclerosis. *Clin J Am Soc Nephrol* 2008;3:1282–1288.
130. Nasr SH, D'Agati VD, Park HR, et al. Necrotizing and crescentic lupus nephritis with antineutrophil cytoplasmic antibody seropositivity. *Clin J Am Soc Nephrol* 2008;3:682–690.
131. Hauer HA, Bajema IM, Hagen EC, et al. Long-term renal injury in ANCA-associated vasculitis: an analysis of 31 patients with follow-up biopsies. *Nephrol Dial Transplant* 2002;17:587.
132. D'Agati V, Jennette JC, Silva FG. *Non-Neoplastic Renal Disease*. Washington: American Registry of Pathology, 2005:385.
133. Lockwood CM. Antineutrophil cytoplasmic autoantibodies: the nephrologist's perspective. *Am J Kidney Dis* 1991;18:171.
134. Chang DY, Wu LH, Liu G, et al. Re-evaluation of the histopathologic classification of ANCA-associated glomerulonephritis: a study of 121 patients in a single center. *Nephrol Dial Transplant* 2012;27:2343.
135. Ellis CL, Manno RL, Havill JP, Racusen LC, Geetha D. Validation of the new classification of pauci-immune glomerulonephritis in a United States cohort and its correlation with renal outcome. *BMC Nephrol* 2013;14:210.
136. Iwakiri T, Fujimoto S, Kitagawa K, et al. Validation of a newly proposed histopathological classification in Japanese patients with anti-neutrophil cytoplasmic antibody-associated glomerulonephritis. *BMC Nephrol* 2013;14:125.
137. Jennette JC, Falk RJ. The pathology of vasculitis involving the kidney. *Am J Kidney Dis* 1994;24:130.
138. Devaney KO, Travis WD, Hoffman G, et al. Interpretation of head and neck biopsies in Wegener's granulomatosis: a pathologic study of 126 biopsies in 70 patients. *Am J Surg Pathol* 1990;14:555.
139. Gaudin PB, Askin FB, Falk RJ, et al. The pathologic spectrum of pulmonary lesions in patients with anti-neutrophil cytoplasmic autoantibodies specific for anti-proteinase 3 and anti-myeloperoxidase. *Am J Clin Pathol* 1995;104:7.
140. Harris AA, Falk RJ, Jennette JC. Crescentic glomerulonephritis with a paucity of glomerular immunoglobulin localization. *Am J Kidney Dis* 1998;32:179.
141. Ronco P, Verroust P, Mignon F, et al. Immunopathological studies of polyarteritis nodosa and Wegener's granulomatosis: a report of 43 patients with 51 renal biopsies. *Q J Med* 1983;52:212.
142. Haas M, Eustace JA. Immune complex deposits in ANCA-associated crescentic glomerulonephritis: a study of 126 cases. *Kidney Int* 2004;65:2145.
143. Gaber LW, Wall BM, Cooke CR. Coexistence of anti-neutrophil cytoplasmic antibody-associated glomerulonephritis and membranous glomerulopathy. *Am J Clin Pathol* 1993;99:211.
144. Serra A, Cameron JS, Turner DR, et al. Vasculitis affecting the kidney: presentation, histopathology and long-term outcome. *Q J Med* 1984;53:181.

145. Schreiber A, Busjahn A, Luft FC, et al. Membrane expression of proteinase 3 is genetically determined. *J Am Soc Nephrol* 2003;14:68.
146. Schreiber A, Luft FC, Kettritz R. Membrane proteinase 3 expression and ANCA-induced neutrophil activation. *Kidney Int* 2004;65:2172.
147. Choi HK, Merkel PA, Walker AM, et al. Drug-associated antineutrophil cytoplasmic antibody-positive vasculitis: prevalence among patients with high titers of anti-myeloperoxidase antibodies. *Arthritis Rheum* 2000;43:405.
148. Schlieben DJ, Korbet SM, Kimura RE, et al. Pulmonary-renal syndrome in a newborn with placental transmission of ANCAs. *Am J Kidney Dis* 2005;45:758.
149. Silva F, Specks U, Sethi S, et al. Successful pregnancy and delivery of a healthy newborn despite transplacental transfer of antimyeloperoxidase antibodies from a mother with microscopic polyangiitis. *Am J Kidney Dis* 2009;54:542.
150. Jennette JC, Falk RJ, Hu P, et al. Pathogenesis of anti-neutrophil cytoplasmic autoantibody associated small vessel vasculitis. *Annu Rev Pathol* 2013;8:139–160.
151. Jennette JC, Xiao H, Falk R, et al. Experimental models of vasculitis and glomerulonephritis induced by antineutrophil cytoplasmic autoantibodies. *Contrib Nephrol* 2011;169:211–220.
152. Xiao H, Heeringa P, Hu P, et al. Antineutrophil cytoplasmic autoantibodies specific for myeloperoxidase cause glomerulonephritis and vasculitis in mice. *J Clin Invest* 2002;110:955.
153. Little MA, Smyth CL, Yadav R, et al. Antineutrophil cytoplasm antibodies directed against myeloperoxidase augment leukocyte-microvascular interactions in vivo. *Blood* 2005;106:2050.
154. Falk RJ, Terrell RS, Charles LA, et al. Anti-neutrophil cytoplasmic autoantibodies induce neutrophils to degranulate and produce oxygen radicals in vitro. *Proc Natl Acad Sci U S A* 1990;87:4115.
155. Charles LA, Caldas ML, Falk RJ, et al. Antibodies against granule proteins activate neutrophils in vitro. *J Leukoc Biol* 1991;50:539.
156. Charles LA, Falk RJ, Jennette JC. Reactivity of antineutrophil cytoplasmic autoantibodies with mononuclear phagocytes. *J Leukoc Biol* 1992;51:65.
157. Weidner S, Neupert W, Goppelt-Struebe M, et al. Antineutrophil cytoplasmic antibodies induce human monocytes to produce oxygen radicals in vitro. *Arthritis Rheum* 2001;44:1698.
158. Ralston DR, Marsh CB, Lowe MP, et al. Antineutrophil cytoplasmic antibodies induce monocyte IL-8 release: role of surface proteinase-3, alpha1-antitrypsin, and Fcgamma receptors. *J Clin Invest* 1997;100:1416.
159. Casselman BL, Kilgore KS, Miller BF, et al. Antibodies to neutrophil cytoplasmic antigens induce monocyte chemoattractant protein-1 secretion from human monocytes. *J Lab Clin Med* 1995;126:495.
160. Mulder AH, Stegeman CA, Kallenberg CG, et al. Activation of granulocytes by anti-neutrophil cytoplasmic antibodies (ANCA) in Wegener's granulomatosis: a predominant role for the IgG3 subclass of ANCA autoantibodies developing to myeloperoxidase and proteinase 3 in systemic vasculitis stimulate neutrophil cytotoxicity toward cultured endothelial cells. *Clin Exp Immunol* 1992;141:335.
161. Porges AJ, Redecha PB, Kimberly WT, et al. Anti-neutrophil cytoplasmic antibodies engage and activate human neutrophils via Fc gamma RIIa. *J Immunol* 1994;153:1271.
162. Kettritz R, Jennette JC, Falk RJ. Cross-linking of ANCA-antigens stimulates superoxide release by human neutrophils. *J Am Soc Nephrol* 1997;8:386.
163. Williams JM, Ben Smith A, Hewins P, et al. Activation of the G(i) heterotrimeric G protein by ANCA IgG F(ab')2 fragments is necessary but not sufficient to stimulate the recruitment of those downstream mediators used by intact ANCA IgG. *J Am Soc Nephrol* 2003;14:661.
164. Savage CO, Gaskin G, Pusey CD, et al. Myeloperoxidase binds to vascular endothelial cells, is recognized by ANCA and can enhance complement dependent cytotoxicity. *Adv Exp Med Biol* 1993;336:121.
165. Ewert BH, Jennette JC, Falk RJ. Anti-myeloperoxidase antibodies stimulate neutrophils to damage human endothelial cells. *Kidney Int* 1992;41:375.
166. Savage CO, Pottinger BE, Gaskin G, et al. Autoantibodies developing to myeloperoxidase and proteinase 3 in systemic vasculitis stimulate neutrophil cytotoxicity toward cultured endothelial cells. *Am J Pathol* 1992;141:335.
167. Ewert BH, Becker ME, Jennette JC, et al. Antimyeloperoxidase antibodies induce neutrophil adherence to cultured human endothelial cells. *Ren Fail* 1995;17:125.
168. Radford DJ, Luu NT, Hewins P, et al. Antineutrophil cytoplasmic antibodies stabilize adhesion and promote migration of flowing neutrophils on endothelial cells. *Arthritis Rheum* 2001;44:2851.
169. Radford DJ, Savage CO, Nash GB. Treatment of rolling neutrophils with antineutrophil cytoplasmic antibodies causes conversion to firm integrin-mediated adhesion. *Arthritis Rheum* 2000;43:1337.
170. Calderwood JW, Williams JM, Morgan MD, et al. ANCA induces beta2 integrin and CXC chemokine-dependent neutrophil-endothelial cell interactions that mimic those of highly cytokine-activated endothelium. *J Leukoc Biol* 2005;77:33.
171. Vargunam M, Adu D, Taylor CM, et al. Endothelium myeloperoxidase-antimyeloperoxidase interaction in vasculitis. *Nephrol Dial Transplant* 1992;7:1077.
172. Huugen D, Xiao H, van Esch A, et al. Aggravation of anti-myeloperoxidase antibody-induced glomerulonephritis by bacterial lipopolysaccharide: role of tumor necrosis factor-alpha. *Am J Pathol* 2005;167:47.
173. Chen M, Xing GQ, Yu F, et al. Complement deposition in renal histopathology of patients with ANCA-associated pauci-immune glomerulonephritis. *Nephrol Dial Transplant* 2009;24:1247–1252.
174. Kawashima S, Arimura Y, Sano K, et al. Immunopathologic co-localization of MPO, IgG, and C3 in glomeruli in human MPO-ANCA-associated glomerulonephritis. *Clin Nephrol* 2013;79:292.
175. Xing GQ, Chen M, Liu G, et al. Complement activation is involved in renal damage in human antineutrophil cytoplasmic autoantibody associated pauci-immune vasculitis. *J Clin Immunol* 2009;29:282–291.
176. Gou SJ, Yuan J, Chen M, et al. Circulating complement activation in patients with anti-neutrophil cytoplasmic antibody-associated vasculitis. *Kidney Int* 2013;83:129–137.
177. Xiao H, Schreiber A, Heeringa P, et al. Alternative complement pathway in the pathogenesis of disease mediated by anti-neutrophil cytoplasmic autoantibodies. *Am J Pathol* 2007;170:52–64.
178. Huugen D, van Esch A, Xiao H, et al. Inhibition of complement factor C5 protects against anti-myeloperoxidase antibody-mediated glomerulonephritis in mice. *Kidney Int* 2007;71:646–654.
179. Schreiber A, Xiao H, Jennette JC, et al. C5a receptor mediates neutrophil activation and ANCA-induced glomerulonephritis. *J Am Soc Nephrol*, 2009;20:289–298.
180. Xiao H, Dairaghi DJ, Powers JP, et al. C5a receptor (CD88) blockade protects against MPO-ANCA glomerulonephritis. *J Am Soc Nephrol* 2013;25:225–231.
181. Pendergraft WF III, Preston GA, Shah RR, et al. Autoimmunity is triggered by cPR-3(105–201), a protein complementary to human autoantigen proteinase-3. *Nat Med* 2004;10:72.
182. Gower RG, Sausker WF, Kohler PF, et al. Small vessel vasculitis caused by hepatitis B virus immune complexes: small vessel vasculitis and HBsAG. *J Allergy Clin Immunol* 1978;62:222.
183. Agnello V. The aetiology of mixed cryoglobulinaemia associated with hepatitis C virus infection. *Scand J Immunol* 1995;42:179.
184. Cochrane CG, Koffler D. Immune complex disease in experimental animals and man. *Adv Immunol* 1973;16:185.
185. Robson WL, Leung AK. Henoch-Schönlein purpura. *Adv Pediatr* 1994;41:163.
186. D'Amico G, Fornasieri A. Cryoglobulinemic glomerulonephritis: a membranoproliferative glomerulonephritis induced by hepatitis C virus. *Am J Kidney Dis* 1995;25:361.
187. Bauer A, Jabs WJ, Sufke S, et al. Vasculitic purpura with antineutrophil cytoplasmic antibody-positive acute renal failure in a patient with *Streptococcus bovis* case and *Neisseria subflava* bacteremia and subacute endocarditis. *Clin Nephrol* 2004;62:144.
188. Choi HK, Lamprecht P, Niles JL, et al. Subacute bacterial endocarditis with positive cytoplasmic antineutrophil cytoplasmic antibodies and anti-proteinase 3 antibodies. *Arthritis Rheum* 2000;43:226.
189. Subra JF, Michelet C, Laporte J, et al. The presence of cytoplasmic antineutrophil cytoplasmic antibodies (C-ANCA) in the course of subacute bacterial endocarditis with glomerular involvement, coincidence or association? *Clin Nephrol* 1998;49:15.

190. Couzi L, Morel D, Deminiere C, et al. An unusual endocarditis-induced crescentic glomerulonephritis treated by plasmapheresis. *Clin Nephrol* 2004;62:461.
191. Majumdar A, Chowdhary S, Ferreira MA, et al. Renal pathological findings in infective endocarditis. *Nephrol Dial Transplant* 2000;15:1782.
192. Schwartz MM, Roberts JL, Lewis EJ. Necrotizing glomerulitis of systemic lupus erythematosus. *Hum Pathol* 1983;14:158.
193. Sen D, Isenberg DA. Antineutrophil cytoplasmic autoantibodies in systemic lupus erythematosus. *Lupus* 2003;12:651.
194. Bonaci-Nikolic B, Nikolic MM, Andrejevic S, et al. Antineutrophil cytoplasmic antibody (ANCA)-associated autoimmune diseases induced by antithyroid drugs: comparison with idiopathic ANCA vasculitides. *Arthritis Res Ther* 2005;7:R1072.
195. Saxena R, Bygren P, Arvastson B, et al. Circulating autoantibodies as serological markers in the differential diagnosis of pulmonary renal syndrome. *J Intern Med* 1995;238:143.
196. Mukhtyar C, Guillevin L, Cid MC, et al. EULAR recommendations for the management of primary small and medium vessel vasculitis. *Ann Rheum Dis* 2009;68:310–317.
197. Jayne D, Rasmussen N, Andrassy K, et al. A randomized trial of maintenance therapy for vasculitis associated with antineutrophil cytoplasmic autoantibodies. *N Engl J Med* 2003;349:36.
198. Bacon PA. Therapy of vasculitis. *J Rheumatol* 1994;21:788.
199. Guillevin L, Cevallos R, Durand-Gasselin B, et al. Treatment of glomerulonephritis in microscopic polyangiitis and Churg-Strauss syndrome: Indications of plasma exchanges, meta-analysis of 2 randomized studies on 140 patients, 32 with glomerulonephritis. *Ann Med Interne* 1997;148:198.
200. Little MA, Pusey CD. Glomerulonephritis due to antineutrophil cytoplasm antibody-associated vasculitis: An update on approaches to management. *Nephrology (Carlton)* 2005;10:368.
201. Jones RB, Tervaert JW, Hauser T, et al. Rituximab versus cyclophosphamide in ANCA-associated renal vasculitis. *N Engl J Med* 2010;363:211–220.
202. Stone JH, Merkel PA, Spiera R, et al. Rituximab versus cyclophosphamide for ANCA-associated vasculitis. *N Engl J Med* 2010;363:221–232.
203. Falk RJ, Jennette JC. Rituximab in ANCA-associated disease. *N Engl J Med* 2010;363:285–286.
204. Franssen CF, Stegeman CA, Kallenberg CG, et al. Antiproteinase 3- and antimyeloperoxidase-associated vasculitis. *Kidney Int* 2000;57:2195.
205. Levy JB, Hammad T, Coulthart A, et al. Clinical features and outcome of patients with both ANCA and anti-GBM antibodies. *Kidney Int* 2004;66:1535.
206. Klemmer PJ, Chalermskulrat W, Reif MS, et al. Plasmapheresis therapy for diffuse alveolar hemorrhage in patients with small-vessel vasculitis. *Am J Kidney Dis* 2003;42:1149–1153.
207. Jayne DR, Gaskin G, Rasmussen N, et al. Randomized trial of plasma exchange or high-dosage methylprednisolone as adjunctive therapy for severe renal vasculitis. *J Am Soc Nephrol* 2007;18:2180–2188.
208. Specks U, Merkel PA, Seo P, et al. Efficacy of remission-induction regimens for ANCA-associated vasculitis. *N Engl J Med* 2013;369:417.
209. Hogan SL, Falk RJ, Chin H, et al. Predictors of relapse and treatment resistance in antineutrophil cytoplasmic antibody-associated small-vessel vasculitis. *Ann Intern Med* 2005;143:621.

CHAPTER 17

J. Charles Jennette • Harsharan K. Singh

# Renal Involvement in Polyarteritis Nodosa, Kawasaki Disease, Takayasu Arteritis, and Giant Cell Arteritis



## BACKGROUND

### Nomenclature of Systemic Vasculitis

Vasculitis is vessel wall inflammation. Perivascular leukocyte infiltration alone is not vasculitis. Diapedesis of leukocytes through the walls of vessels, usually postcapillary venules, that does not cause pathologic vessel wall injury is not vasculitis. The chronic phase of vasculitis may be characterized by sclerosis without inflammation; thus, in this instance, a diagnosis of vasculitis is made based on evidence indicating that the sclerosis was preceded by inflammation. Nonspecific chronic inflammation with infiltration by predominantly lymphocytes in the setting of arteriosclerosis should not be considered vasculitis.

Vasculitis can affect any type of vessel in any organ and thus results in a broad range of clinical signs and symptoms. Because the kidneys have numerous and diverse vessels, they are a frequent target for many types of vasculitis, especially those that affect predominantly small vessels such as capillaries, venules, arterioles, and small arteries. The medullary vasa recta can be affected by small-vessel vasculitides. Renal veins are rarely if ever involved directly by noninfectious vasculitides.

No approach to classifying vasculitides is universally accepted. Different names have been used for the same type of vasculitis, and specific types of vasculitis have been given multiple names. The approach used in this chapter is the 2012 Chapel Hill Consensus Conference Nomenclature System (Table 17.1; Fig. 17.1) (1), which is a modification of the 1994 Chapel Hill Consensus Conference Nomenclature System (2) that was used in the previous edition of this book. Three major categories of vasculitis are large-vessel vasculitis, medium-vessel vasculitis, and small-vessel vasculitis. Although these names imply that the size of the vessels affected by inflammation is the major basis for categorizing vasculitides, this is a gross oversimplification because many factors must be taken into consideration to classify a vasculitis precisely, including the type of vessel involved, the pattern of inflammatory injury, distribution of organ involvement, immunopathologic findings in the tissue and blood, and clinical features. As demonstrated in Figure 17.1, substantial overlap exists among categories with respect to the size of involved vessels. Therefore, diagnostic categorization requires a relatively complex integration of not only pathologic data but also immunologic and clinical data to reach the most appropriate diagnosis.

In the kidney, small-vessel vasculitides, such as IgA vasculitis (Henoch-Schönlein purpura), cryoglobulinemic vasculitis, anti–glomerular basement membrane (GBM) disease,

**TABLE 17.1 Names and definitions adopted by the 2012 Chapel Hill Consensus Conference on the Nomenclature of Vasculitis**

| CHCC 2012 names[a] | CHCC 2012 definitions |
|---|---|
| **Large-vessel vasculitis (LVV)** | Vasculitis affecting large arteries more often than other vasculitides. Large arteries are the aorta and its major branches. Any size artery may be affected. |
| Takayasu arteritis (TA) | Arteritis, often granulomatous, predominantly affecting the aorta and/or its major branches. Onset usually in patients younger than 50 |
| Giant cell arteritis (GCA) | Arteritis, often granulomatous, usually affecting the aorta and/or its major branches, with a predilection for the branches of the carotid and vertebral arteries. Often involves the temporal artery. Onset usually in patients older than 50 and often associated with polymyalgia rheumatica |
| **Medium-vessel vasculitis (MVV)** | Vasculitis predominantly affecting medium arteries defined as the main visceral arteries and their branches. Any size of artery may be affected. Inflammatory aneurysms and stenoses are common. |
| Polyarteritis nodosa (PAN) | Necrotizing arteritis of medium or small arteries without glomerulonephritis or vasculitis in arterioles, capillaries, or venules; not associated with ANCA |
| Kawasaki disease (TA) | Arteritis associated with the mucocutaneous lymph node syndrome and predominantly affecting medium and small arteries. Coronary arteries are often involved. Aorta and large arteries may be involved. Usually occurs in infants and young children |
| **Small-vessel vasculitis (SVV)** | Vasculitis predominantly affecting small vessels, defined as small intraparenchymal arteries, arterioles, capillaries, and venules. Medium arteries and veins may be affected. |
| ANCA-associated vasculitis (AAV) | Necrotizing vasculitis, with few or no immune deposits, predominantly affecting small vessels (i.e., capillaries, venules, arterioles, and small arteries), associated with MPO-ANCA or PR3-ANCA. Not all patients have ANCA. Add a prefix indicating ANCA reactivity, for example, PR3-ANCA, MPO-ANCA, ANCA-negative. |
| *Microscopic polyangiitis (MPA)* | Necrotizing vasculitis, with few or no immune deposits, predominantly affecting small vessels (i.e., capillaries, venules, or arterioles). Necrotizing arteritis involving small and medium arteries may be present. Necrotizing glomerulonephritis is very common. Pulmonary capillaritis often occurs. Granulomatous inflammation is absent. |
| *Granulomatosis with polyangiitis (Wegener) (GPA)* | Necrotizing granulomatous inflammation usually involving the upper and lower respiratory tract and necrotizing vasculitis affecting predominantly small vessels (e.g., capillaries, venules, arterioles, arteries, and veins). Necrotizing glomerulonephritis is common. |
| *Eosinophilic granulomatosis with polyangiitis (Churg-Strauss) (EGPA)* | Eosinophil-rich and necrotizing granulomatous inflammation often involving the respiratory tract, and necrotizing vasculitis predominantly affecting small to medium vessels and associated with asthma and eosinophilia. ANCA is more frequent when glomerulonephritis is present. |
| Immune complex vasculitis | Vasculitis with moderate to marked vessel wall deposits of immunoglobulin and/or complement components predominantly affecting small vessels (i.e., capillaries, venules, arterioles, and small arteries). Glomerulonephritis is frequent. |
| *Anti-GBM disease* | Vasculitis affecting glomerular capillaries, pulmonary capillaries, or both, with basement membrane deposition of anti–basement membrane autoantibodies. Lung involvement causes pulmonary hemorrhage, and renal involvement causes glomerulonephritis with necrosis and crescents. |
| *Cryoglobulinemic vasculitis* | Vasculitis with cryoglobulin immune deposits affecting small vessels (predominantly capillaries, venules, or arterioles) and associated with cryoglobulins in serum. Skin, glomeruli, and peripheral nerves are often involved. |
| *IgA vasculitis (Henoch-Schönlein)* | Vasculitis, with IgA1-dominant immune deposits, affecting small vessels (predominantly capillaries, venules, or arterioles). Often involves skin and gut and frequently causes arthritis. Glomerulonephritis indistinguishable from IgA nephropathy may occur. |
| *Hypocomplementemic urticarial vasculitis (Anti-C1q vasculitis)* | Vasculitis accompanied by urticaria and hypocomplementemia affecting small vessels (i.e., capillaries, venules, or arterioles) and associated with anti-C1q antibodies. Glomerulonephritis, arthritis, obstructive pulmonary disease, and ocular inflammation are common. |

**TABLE 17.1 Names and definitions adopted by the 2012 Chapel Hill Consensus Conference on the Nomenclature of Vasculitis (*Continued*)**

| CHCC 2012 names[a] | CHCC 2012 definitions |
|---|---|
| **Single organ vasculitis (SOV)** | Vasculitis in arteries or veins of any size in a single organ that has no features that indicate that it is a limited expression of a systemic vasculitis. The involved organ and vessel type should be included in the name (e.g., cutaneous small-vessel vasculitis, testicular vasculitis, central nervous system vasculitis). Vasculitis distribution may be unifocal or multifocal (diffuse) within an organ. Some patients originally diagnosed with SOV will develop additional disease manifestations that warrant redefining the case as one of the systemic vasculitides (e.g., cutaneous arteritis later becoming systemic polyarteritis nodosa). |
| **Vasculitis associated with systemic disease** | Vasculitis that is associated with and may be secondary to (caused by) a systemic disease. The name (diagnosis) should have a prefix term specifying the systemic disease (e.g., rheumatoid vasculitis, lupus vasculitis). |
| **Vasculitis associated with probable etiology** | Vasculitis that is associated with a probable specific etiology. The name (diagnosis) should have a prefix term specifying the association (e.g., hydralazine-associated microscopic polyangiitis, hepatitis B virus–associated vasculitis, hepatitis C virus–associated cryoglobulinemic vasculitis). |

[a]The Variable Vessel Vasculitis category, which includes Behcet disease and Cogan syndrome, is not included in this table because renal disease is not a significant component of this category.

From Jennette JC, Falk RJ, Bacon PA, et al. 2012 revised International Chapel Hill Consensus Conference Nomenclature of Vasculitides. *Arthritis Rheum* 2013;65:1–11.

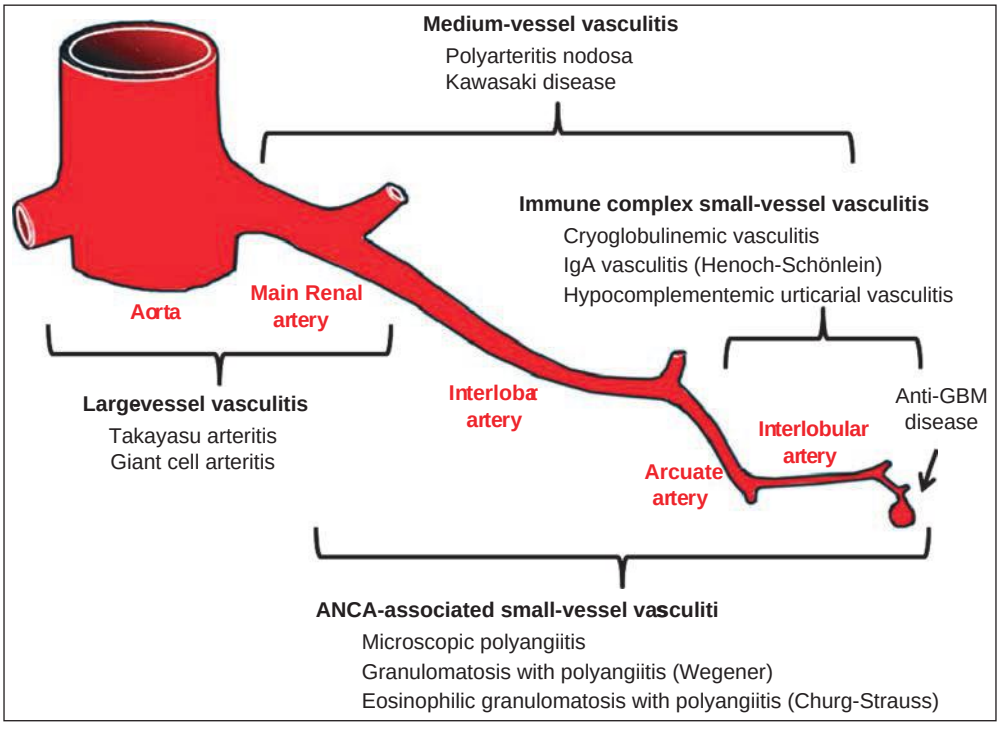



**FIGURE 17.1 Diagram of the renal vasculature with brackets indicating the predominant distribution of vessel involvement by different forms of vasculitis (and an *arrow* indicating that anti-GBM disease only affects glomeruli).** Notice that arteries can be affected by large-, medium-, and small-vessel vasculitis. ANCA-associated vasculitis more often affects arteries than does immune complex small-vessel vasculitis. If glomerulonephritis is a component of a systemic vasculitis, by definition, the vasculitis is a small-vessel vasculitis.

microscopic polyangiitis (MPA), and granulomatosis with polyangiitis (GPA) (Wegener granulomatosis), most often affect glomerular capillaries and thus cause glomerulonephritis (1,3,4). Renal involvement by small-vessel vasculitis usually manifests as nephritis with hematuria, proteinuria, and renal insufficiency. Different small-vessel vasculitides have different frequencies of arterial involvement; for example, IgA vasculitis and cryoglobulinemic vasculitis rarely affect intrarenal arteries, whereas MPA and GPA more often affect intrarenal arteries although this may not be sampled in a renal biopsy specimen. The interlobular arteries are the arteries most often affected by small-vessel vasculitis. The small-vessel vasculitides are discussed in detail in several other chapters in this book, including Chapter 16 (antineutrophil cytoplasmic autoantibodies [ANCA] small-vessel vasculitis), Chapter 12 (IgA vasculitis), and Chapter 22 (cryoglobulinemic vasculitis), and thus are mentioned in this chapter only in the context of the nomenclature and differential diagnosis of medium- and large-vessel vasculitis.

Medium-vessel vasculitis, such as polyarteritis nodosa (PAN) and Kawasaki disease (KD), may affect any of the renal arteries but most often involves the interlobar and arcuate arteries (2). Clinically significant renal involvement is more common with PAN than with KD; however, postmortem examinations reveal that both PAN (5) and KD (6) often affect the kidneys. PAN and KD are forms of necrotizing arteritis characterized by segmental transmural necrosis, often with aneurysm formation. This necrosis may result in rupture and hemorrhage, although partial or complete occlusion with resultant ischemia is more common and may cause infarction. The narrowing or obliteration of lumina is caused by various combinations of inflammation, thrombosis, and sclerosis. Renal involvement by medium-vessel vasculitides often causes abdominal pain from visceral inflammation and ischemia. Renal rupture with hemorrhage into the retroperitoneum or peritoneal space is an uncommon but potentially life-threatening complication. Hematuria (resulting from renal infarction and hemorrhage from inflamed arteries rather than glomerulonephritis) is a frequent consequence of renal involvement by medium-vessel vasculitis, but renal insufficiency and substantial proteinuria are less frequent than in the small-vessel vasculitides.

In literature prior to the 1990s, polyarteritis nodosa (PAN) was not uniformly separated from microscopic polyangiitis (MPA) (formerly called microscopic polyarteritis or microscopic periarteritis) (2). In the Chapel Hill Nomenclature System approach to classification used in this book, the presence or absence of glomerulonephritis is a major distinguishing feature between PAN and MPA (1,2). According to this classification system, PAN affects only arteries and thus does not cause glomerulonephritis, whereas MPA has a predilection for vessels other than arteries (especially venules and capillaries) and frequently causes necrotizing glomerulonephritis. This approach of separating PAN from MPA is supported by the strong association of ANCA with MPA but not PAN (7).

Large-vessel vasculitides, such as giant cell arteritis (GCA) and Takayasu arteritis, affect predominantly the main renal artery or its aortic ostium, although they also may involve intraparenchymal renal arteries (8,9). These chronic inflammatory and sclerosing vascular diseases often cause narrowing of arterial lumens with distal ischemia, but alternating segments with stenosis and dilation may occur. Pathologic involvement of renal vessels may be clinically silent or cause renovascular hypertension, especially in patients with Takayasu arteritis (10).

Before discussing in detail the pathologic features of vasculitides in the kidneys, some of the historical background that underlies our understanding of the pathology and classification of vasculitides is reviewed.

## Historical Background of Necrotizing Arteritis

Most medium-vessel vasculitides and small-vessel vasculitides have extensive vascular necrosis during the acute phase, a finding that differs from the more indolent and granulomatous inflammation of the large-vessel vasculitides. In other words, the initial arterial involvement by small- and medium-vessel vasculitides is a necrotizing arteritis and that of the large-vessel vasculitides is a chronic granulomatous inflammation (11). Unlike the large-vessel vasculitides, which rarely affect vessels within the kidney parenchyma that are sampled in renal biopsy specimens, the medium-vessel vasculitides and especially the small-vessel vasculitides often involve the kidney parenchyma, with the latter frequently causing significant renal dysfunction. The necrotizing vascular inflammation is characterized by segmental fibrinoid necrosis with neutrophilic infiltration (11,12). The neutrophils rapidly undergo apoptosis and necrosis with nuclear fragmentation (leukocytoclasia). This light microscopic lesion is similar in arteries, arterioles, capillaries, venules, and veins (12). By definition, a major distinction between the medium-vessel vasculitides and the small-vessel vasculitides is that the former involve only arteries, whereas the latter may involve arteries, arterioles, capillaries, and venules (1,2). Thus, in a patient with systemic vasculitis, the presence of glomerulonephritis is diagnostic for some form of small-vessel vasculitis.

Both infectious and noninfectious vasculitides cause acute necrotizing inflammation of vessels. Infectious vasculitis may affect any type of vessel, although particular infections have a predilection for certain types of vessels. Direct infection of vessel walls, for example, by *Rickettsia* or *Neisseria*, incites inflammation. Infections also can cause vasculitis by generating pathogenic immune complexes, for example, mixed cryoglobulin immune complexes induced by hepatitis C virus (HCV) infection and resulting in cryoglobulinemic vasculitis (discussed in detail in Chapter 22). Infectious vasculitis caused by direct invasion of vessel walls is not discussed in this chapter.

Noninfectious necrotizing inflammation of arteries was first recognized in the context of PAN (13), whereas noninfectious necrotizing inflammation of vessels smaller than arteries (i.e., arterioles, capillaries, and venules) was first appreciated in patients with purpura (14).

The initial recognition of necrotizing arteritis began with the gross observance of nodular aneurysmal lesions in arteries. Rokitansky (15) gave one of the first gross descriptions of systemic necrotizing arteritis in a report on arterial aneurysms in 1852; however, Kussmaul, who was a clinician who studied pathology under Rokitansky and Virchow, and the pathologist Maier published the first definitive report in 1866 (13). Their patient had fever, anorexia, muscle weakness, myalgias, paresthesias, abdominal pain, and oliguria. He died, and an autopsy revealed nodular inflammatory lesions in medium-sized and small arteries throughout the body. These authors observed white-yellow nodular arterial lesions in the kidneys that were most conspicuous at the corticomedullary junction. They also noted the presence of infarcts. Thus, their patient had features very similar to those in Figure 17.2. Kussmaul and Maier (13) named the disease *periarteritis nodosa* to emphasize what they

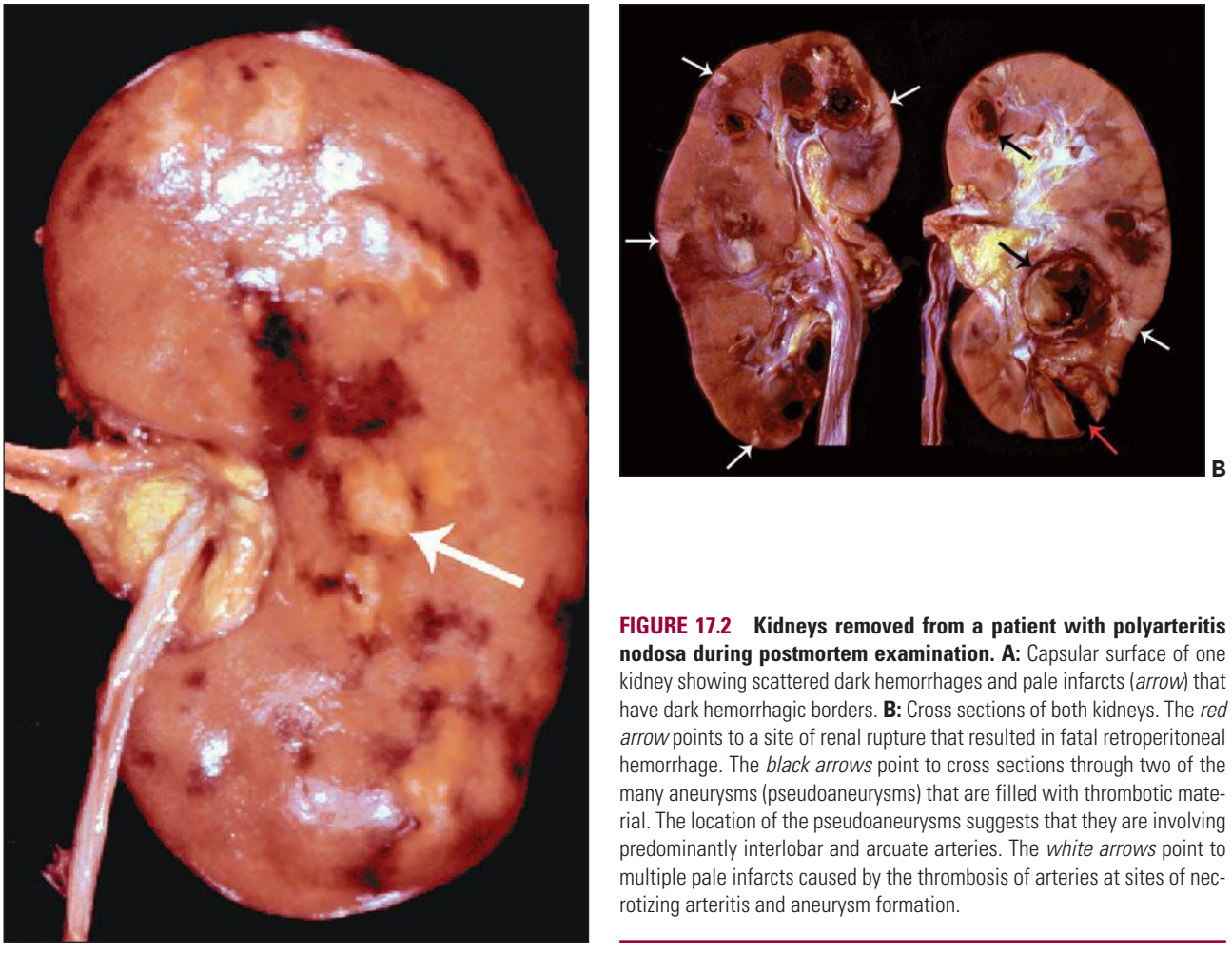



**FIGURE 17.2 Kidneys removed from a patient with polyarteritis nodosa during postmortem examination. A:** Capsular surface of one kidney showing scattered dark hemorrhages and pale infarcts (*arrow*) that have dark hemorrhagic borders. **B:** Cross sections of both kidneys. The *red arrow* points to a site of renal rupture that resulted in fatal retroperitoneal hemorrhage. The *black arrows* point to cross sections through two of the many aneurysms (pseudoaneurysms) that are filled with thrombotic material. The location of the pseudoaneurysms suggests that they are involving predominantly interlobar and arcuate arteries. The *white arrows* point to multiple pale infarcts caused by the thrombosis of arteries at sites of necrotizing arteritis and aneurysm formation.

perceived as predominantly perivascular inflammation that produced focal nodular vascular lesions.

In 1903, Ferrari (16) introduced the term *polyarteritis* to emphasize the involvement of multiple arteries and to do away with the misleading term *periarteritis* because he recognized the transmural character of the disease and illustrated this beautifully with color drawings in his publication. This term was advocated further by Dickson in 1908 (17), who also noted the frequency of renal involvement by arteritis resulting in thrombosis and infarction. Currently, the name *polyarteritis nodosa* is generally used, rather than *periarteritis nodosa* (1).

Until distinctive subcategories of necrotizing arteritis were recognized, PAN became a wastebasket diagnostic category. In essence, any patient with necrotizing arteritis was said to have PAN, although many of these patients had different clinical and pathologic features (18–21). Unfortunately, for many years after the recognition of pathologically and clinically distinct subcategories of necrotizing arteritis, some pathologists continued to lump different variants of necrotizing arteritis under the term PAN. Many studies, however, have clearly demonstrated that histologically identical necrotizing arteritis occurs as a component of many clinically, pathologically, and pathogenetically distinct types of vasculitis, some of which have concurrent vasculitis in vessels smaller than arteries (i.e., the small-vessel vasculitides) (1). The natural history, prognosis, and most appropriate treatment differ among these categories of necrotizing vasculitis. Thus, their recognition is not merely an academic exercise, but rather has major implications for prognosis and treatment.

By the 1920s, a variant of systemic necrotizing vasculitis with arteritis as well as inflammation of small vessels including small arteries, arterioles, venules, and capillaries was recognized (20,21). One of the earliest pathologic features found to distinguish among clinically distinctive categories of necrotizing vasculitis was glomerulonephritis. In 1930, Arkin (5) published one of the best early pathologic descriptions of systemic necrotizing arteritis and observed two basic forms, one with a predominance of grossly visible vascular nodules and a second "microscopic form" in which the vasculitis could be identified only with the aid of microscopy. Arkin pointed out that the kidneys were a major target organ for both the gross and microscopic forms of arteritis. In a 1948 publication, Davson et al. (22) focused on renal involvement in patients with necrotizing arteritis and concluded that an important feature that divided the microscopic form of arteritis from the form with conspicuous grossly identifiable arterial nodules was the presence in the former but not the latter of extensive necrotizing glomerulonephritis. Also in 1948, Zeek et al. (23) published similar observations. These investigators noted that one group of patients had extensive necrotizing arteritis with grossly discernible nodules and no glomerulonephritis, whereas another group had

microscopic arteritis that was accompanied by necrotizing glomerulonephritis. They considered the former to be true PAN, and they called the latter *hypersensitivity angiitis* because they thought it could be caused by an allergic response possibly to a drug (23,24). The term *hypersensitivity angiitis* has subsequently been used for many different patterns of vasculitis, including relatively nonspecific cutaneous manifestations of drug hypersensitivity. As a consequence, this name has little diagnostic utility and probably should be abandoned as a diagnostic term.

Thus, by the 1940s, strong evidence indicated that patients with necrotizing arteritis could be divided into two major categories: those with systemic necrotizing arteritis with conspicuous gross nodular inflammatory arterial lesions but no glomerulonephritis and those predominant involvement of small arteries as well as involvement of vessels smaller than arteries, such as glomerular capillaries. This latter category was variable called *microscopic polyarteritis*, *microscopic periarteritis*, or *hypersensitivity angiitis*. In an analysis of 38 autopsy cases with necrotizing arteritis, Heptinstall (25) confirmed the usefulness of the presence or absence of glomerulonephritis as a criterion for separating PAN from microscopic polyarteritis. Using clinical, renal biopsy, serologic, and angiographic data, Guillevin et al. (7,26) have demonstrated that this distinction between PAN and MPA not only is possible and appropriate but also is of major value in guiding patient management.

While microscopic polyarteritis was emerging as a discrete diagnosis, two other variants of vasculitis were separated from PAN: Wegener granulomatosis and Churg-Strauss syndrome. Although these diseases frequently have vasculitis in vessels other than arteries, both had initially been included in the PAN category because patients often had necrotizing arterial lesions that were histologically identical to those in patients with PAN. Klinger (27) initially reported granulomatosis with polyangiitis (GPA) in 1931 as a "borderline type of periarteritis nodosa." This disease subsequently was described in more detail by his former schoolmate Wegener (28), which prompted widespread adoption of the term Wegener granulomatosis until this term was replaced by granulomatosis with polyangiitis (29). In a landmark publication, Godman and Churg (19) published the definitive description of GPA in 1954. They recognized the following triad of features: necrotizing "angiitis," necrotizing inflammation of the respiratory tract, and necrotizing glomerulonephritis. Subsequently, patients with limited expressions of GPA were recognized who did not manifest all the features, such as patients with no glomerulonephritis (30).

Another variant of necrotizing vasculitis was reported by Churg and Strauss in 1951 (18). In this same article, the authors also pointed out that what was called *periarteritis nodosa* was a heterogeneous group of vasculitides. They described 13 patients with asthma, eosinophilia, granulomatous inflammation, necrotizing vasculitis (including necrotizing arteritis), and focal necrotizing glomerulonephritis. They noted that similar patients had previously been reported as having PAN occurring in patients with asthma. This distinct variant of necrotizing vasculitis has been called Churg-Strauss syndrome, but the name proposed by the Chapel Hill Consensus Conference is eosinophilic granulomatosis with polyangiitis (EGPA) (1).

In their landmark 1954 article that elucidated the features of GPA, Godman and Churg (19) concluded that MPA, GPA, and EGPA are closely related to each other and are distinct from PAN. More recent data support this conclusion, for example, the strong association of MPA, GPA, and EGPA (when glomerulonephritis is present), but not PAN, with ANCA and the strong association of a subset of PAN with hepatitis B infection (7,26,31–34), but not MPA, GPA, or EGPA.

The 1994 Chapel Hill Consensus Conference proposed that the term *microscopic polyarteritis* be replaced with the term *microscopic polyangiitis* because many patients with this category of vasculitis have inflammation in vessels other than arteries, such as arterioles, capillaries, and venules (1). When this approach is used, MPA is a more frequent diagnosis than is PAN, especially in patients who are seen by nephrologists and nephropathologists.

The major necrotizing arteritis in the medium-vessel vasculitis category other than PAN is Kawasaki disease (KD). The mucocutaneous lymph node syndrome (MCLNS), which is the hallmark of KD, was first described by Tomisaku Kawasaki in 1967 (33) and consists of polymorphous erythematous rash, erythema of the palms and soles, indurative edema of the extremities followed by desquamation, erythema of the oropharyngeal mucosa, conjunctivitis, and nonsuppurative lymphadenopathy (34,35). In a study of autopsy cases, Tanaka, Naoe, and Kawasaki determined that necrotizing arteritis involving medium and small arteries is an important component of KD and may affect the kidneys, especially the interlobar arteries (6,36). Because KD usually occurs in children younger than 5 years of age and is associated with necrotizing arteritis, some examples have been reported under the designation *infantile PAN* (34,37). However, KD is easily separated from PAN by the characteristic MCLNS. Necrotizing arteritis caused by PAN, as well as arteritis as a component of a small-vessel vasculitis, such as MPA, occasionally occurs in children and should not be misdiagnosed as KD.

In summary, evaluation of patients with necrotizing arteritis has revealed multiple clinically and pathologically distinctive entities, some that affect predominantly, if not exclusively, arteries (i.e., medium-vessel vasculitides) and others that affect not only arteries but also vessels smaller than arteries (i.e., small-vessel vasculitides). In fact, many patients with small-vessel vasculitis have involvement exclusively of vessels other than arteries, for example, glomerulonephritis, pulmonary alveolar capillaritis, and cutaneous dermal venulitis.

## Historical Background of Large-Vessel Vasculitis

Large-vessel vasculitides affect the aorta and its major branches more often than does small-vessel vasculitis or medium-vessel vasculitis (see Fig. 17.1 and Table 17.1) (1,38). Renal parenchymal involvement is less frequent with large-vessel vasculitis compared to medium- and small-vessel vasculitis. Nevertheless, large-vessel vasculitides can affect any type of artery in the kidney although the main renal arteries and their first- and second-order branches are affected most often. Takayasu arteritis and giant cell arteritis are the two major categories of large-vessel vasculitis. Both cause chronic inflammation in arteries that is characterized by mononuclear leukocyte infiltration with a predominance of monocytes, macrophages, and T lymphocytes (11,38). Often, but not always, the inflammation has a granulomatous character with numerous macrophages, sometimes with multinucleated giant cells. Advanced disease is characterized predominantly by sclerosis rather than by inflammation, a finding that may complicate pathologic diagnosis because it can be confused clinically and pathologically with

arteriosclerosis and atherosclerosis. The arterial inflammation and resultant scarring cause narrowing of lumina that, in turn, causes ischemic symptoms, for example, pulselessness, claudication, and renovascular hypertension.

A comprehensive clinical description of Takayasu arteritis was made by Savory in 1856 (39), although patients with pulseless disease had been reported in the medical literature since the mid-eighteenth century (40). The disease is named after Mikito Takayasu, a Japanese ophthalmologist who reported the ocular complications of this disease in 1908, even though he did not recognize the underlying vasculitic features (41). The reduced vascular perfusion caused by the narrowing of arteries initially led to the realization that this disease affects arteries and also is the basis for another designation for this disease, "pulseless disease."

The temporal artery involvement of GCA brought this disease to the attention of Hutchinson in 1890 (42). Over the next 50 years, the widespread aortic and arterial distribution and the granulomatous nature of this disease became apparent (43), and shortly thereafter, the association with polymyalgia rheumatica was recognized (44,45). The term *giant cell arteritis* is more appropriate than *temporal arteritis* for this category of vasculitis because not all patients with GCA have temporal artery involvement, and vasculitides other than GCA (e.g., PAN, MPA, GPA) can cause temporal artery inflammation (2,46). Glomerulonephritis is not a feature of GCA; thus, if a patient has clinical evidence of glomerulonephritis and temporal artery inflammation, the diagnosis is probably not GCA, but rather some form of small-vessel vasculitis with temporal artery involvement.

The subsequent sections of this chapter review the clinical and pathologic features of PAN, KD, Takayasu arteritis, and GCA, in this order, with an emphasis on the renal involvement. Various forms of immune complex small-vessel vasculitis are reviewed in multiple chapters, and ANCA small-vessel vasculitis is reviewed in Chapter 16.

## POLYARTERITIS NODOSA

### Clinical Presentation

The literature prior to 1990 that describes the clinical and pathologic features of PAN is problematic because, as pointed out in the previous section, some investigators studied cohorts that included patients with both PAN and MPA. In this chapter, the Chapel Hill Consensus Conference Nomenclature, which defines PAN as necrotizing arteritis of medium and small arteries without glomerulonephritis, will be followed (see Table 17.1 and Fig. 17.1) (1). Not only do PAN and MPA differ by vessel involvement and serology but also in natural history, prognosis, and response to treatment (7,31,32,47).

Arkin (5) concluded that the major clinical manifestations of PAN are fever, peripheral neuropathy, myalgias, abdominal pain, and signs and symptoms of renal disease. These continue to be major manifestations today (31,47). As with the literature on pathologic features, one must determine whether data on clinical manifestations are derived from patients with PAN alone (as defined in this chapter) or from patients with PAN and MPA in this chapter. The former has a much more restricted spectrum of clinical manifestations than the latter. Guillevin et al. (26,31) and others (47,48) have elucidated the differences between the clinical manifestations of these two variants of necrotizing vasculitis (Table 17.2 ). The data in this table show that the presence of ANCA is a strong marker for MPA versus PAN.

When defined by the Chapel Hill Nomenclature System, the prevalence in Europe of PAN is approximately 31 per million compared with 25 per million for MPA, 24 per million for GPA, and 11 per million for EGPA (49). Most patients with PAN present with nonspecific constitutional symptoms, such as fever, malaise, arthralgias, myalgias, and weight loss (47). Peripheral neuropathy, typically in the form of mononeuritis multiplex, is a common clinical manifestation of arteritis that occurs with both medium- and small-vessel vasculitis (26). Peripheral neuropathy occurs in approximately 75% of patients with PAN (47), which is caused primarily by inflammation of small epineural arteries. Signs and symptoms of gastrointestinal involvement occur in approximately half of PAN patients, for example, abdominal pain and blood in the stool. Bowel infarction is uncommon, and perforation is rare. The major cutaneous manifestations of PAN are inflammatory nodules, infarction, and livedo reticularis, whereas purpura is the most common cutaneous manifestation of MPA and other small-vessel vasculitides (50). Overall, skin involvement is less common in PAN than in MPA (50). Renal involvement by PAN manifests as flank pain and hematuria and rarely as retroperitoneal hemorrhage from rupture of an aneurysm. Pain caused by rupture of a renal aneurysm in PAN can be severe enough to present as an acute abdomen (51). Renovascular hypertension occurs in up to one third of patients, but it is only rarely malignant. Arterial aneurysms may be detected by imaging in patients with PAN, but this is not specific because any necrotizing arteritis that affects arteries large enough to be resolved by imaging can produce detectable aneurysms.

Table 17.2 shows some of the clinical distinctions between PAN and MPA (47). A major distinction between PAN and MPA is the presence of glomerulonephritis in MPA but not PAN (22,25,31,48). Other distinguishing clinical features are the typical absence of clinical manifestations of vasculitic pulmonary disease in PAN and frequent involvement of the lungs in MPA and other ANCA-associated small-vessel vasculitides as a consequence of alveolar capillaritis or necrotizing granulomatosis. As early as 1957, Rose and Spencer (52) realized that the presence or absence of pulmonary disease distinguishes among distinctive forms of necrotizing arteritis, just as Dickson (17), Davson et al. (22), and Heptinstall (25) determined that the presence or absence of glomerulonephritis is a distinguishing feature. In both instances, clinical evidence of necrotizing capillaritis (alveolar or glomerular) is used as a marker for MPA that is not shared by PAN.

### Pathologic Findings

#### Gross Pathology

The kidneys, along with the gastrointestinal tract and the heart, are frequently involved in PAN. The gross abnormalities in the kidneys are the result of arterial aneurysms, thrombosis, infarction, and hemorrhage (Fig. 17.2). The aneurysms often are pseudoaneurysms in that they are not the result of vessel wall dilation but rather result from erosion of necrotizing inflammation through the vessel wall into the perivascular tissue. In Heptinstall's series (25) of 20 patients with PAN, 5 had aneurysms, 12 had infarcts, and 1 had a perirenal hematoma.

**TABLE 17.2 Comparison between polyarteritis nodosa and microscopic polyangiitis with respect to clinical features and outcomes**

| | Polyarteritis nodosa ($n = 36$) | Microscopic polyangiitis ($n = 36$) | $P$ |
|---|---|---|---|
| **Clinical feature** | | | |
| Male/female | 24/12 | 21/15 | 0.47 |
| Age | 54.8 ± 15.1 yr | 60.3 ± 16.4 yr | 0.18 |
| Fever | 53% | 56% | 0.81 |
| Weight loss | 69% | 64% | 0.74 |
| Myalgias | 56% | 56% | 0.89 |
| Arthralgias | 44% | 56% | 0.28 |
| Positive ANCA[a] | 4% | 58% | <0.0001 |
| Peripheral neuropathy | 75% | 56% | 0.08 |
| GI involvement | 53% | 22% | 0.007 |
| Skin involvement[a] | 28% | 53% | 0.03 |
| Testicular disease | 21% | 5% | 0.19 |
| Renal involvement | 22% | 19% | 0.77 |
| Renal insufficiency | 19% | 25% | 0.57 |
| Cardiac involvement | 17% | 17% | 1.0 |
| Pulmonary disease[a] | 6% | 22% | 0.04 |
| **Outcome** | | | |
| Death | 36% | 33% | 0.73 |
| Relapse | 17% | 36% | 0.06 |
| Number of relapses[a] | 0.19 ± 0.47 | 0.58 ± 1.09 | 0.03 |

[a]Features with a statistically significant difference ($P < 0.05$) between polyarteritis nodosa and microscopic polyangiitis and thus are of greatest value in differential diagnosis. Note that ANCA is by far the most significant difference.

Data from Agard C, Mouthon L, Mahr A, et al. Microscopic polyangiitis and polyarteritis nodosa: How and when do they start? *Arthritis Rheum* 2003;49:709.

Viewed through the capsular surface, infarcts are pale with hemorrhagic borders (see Fig. 17.2A). Viewed on the cut surface of the kidney, renal infarcts usually are roughly wedge shaped, with their bases touching or almost touching the capsular surface of the cortex (see Fig. 17.2B). The lesions are pale yellow after the reddish pigments have leached out of the necrotic cells. There often is a peripheral zone that is red to white, depending on the relative content of hemorrhage versus neutrophils, respectively. This is subsequently replaced by a thin rim of granulation tissue that is red and somewhat glassy. Acute infarcts may bulge slightly above the renal surface, but as the lesions age, they become depressed, especially once scarring has developed.

Any type of artery in the kidney can be affected by PAN, from the main renal artery to the smallest interlobular arteries, although the main renal artery is rarely involved. The interlobar that course in the renal sinus and enter the parenchyma through the columns of Bertin and the arcuate arteries that arise from the interlobar arteries are involved most often. When larger arteries are involved, nodular inflammatory lesions and pseudoaneurysms can be observed grossly (see Fig. 17.2B), but if only small arteries are affected (i.e., interlobular arteries), the arteritis will be observed only microscopically. The vascular origin of the nodular lesions is seen best when vessels are cut longitudinally or obliquely because they appear as focal swellings. The inflammation has a predilection for arterial branch points (25,26). Arterial lesions are observed most often at the corticomedullary junction and in the renal sinus tissue adjacent to the columns of Bertin because of the predilection for involvement of arcuate and interlobar arteries, respectively. Lesions that are composed predominantly of fibrinoid necrosis and leukocyte infiltration are pale, whereas aneurysms, especially when they contain thrombi, are dark red (see Fig. 17.2B). Inflamed arteries with pseudoaneurysms also may be thrombosed.

An uncommon but potentially lethal renal complication of PAN is rupture of an aneurysm with retroperitoneal or peritoneal cavity hemorrhage (51,53–55). This complication may be confined to a small perirenal hematoma, or it may result in exsanguination into the peritoneal cavity or retroperitoneum as was the case with the patient whose kidney is shown in Figure 17.2. The presence of perirenal hemorrhage should prompt a search for a ruptured aneurysm.

## Light Microscopy

The light microscopic features of PAN vary over time. Lesions of different ages may be observed simultaneously (Fig. 17.3). They begin with fibrinoid necrosis and acute inflammation, evolve through chronic inflammation and fibroplasia, and culminate in varying degrees of vascular sclerosis. Although published in 1930, the description of the pathologic changes in PAN made by Arkin (5) has stood the test of time. He described an acute phase, a healing phase, and a healed phase. The acute phase has fibrinoid necrosis and infiltration of predominantly neutrophils with varying numbers of admixed eosinophils (Fig. 17.4). The necrosis and inflammation are initially confined to the inner portion of the arterial wall but eventually may become transmural, with extension of the necrosis and inflammation

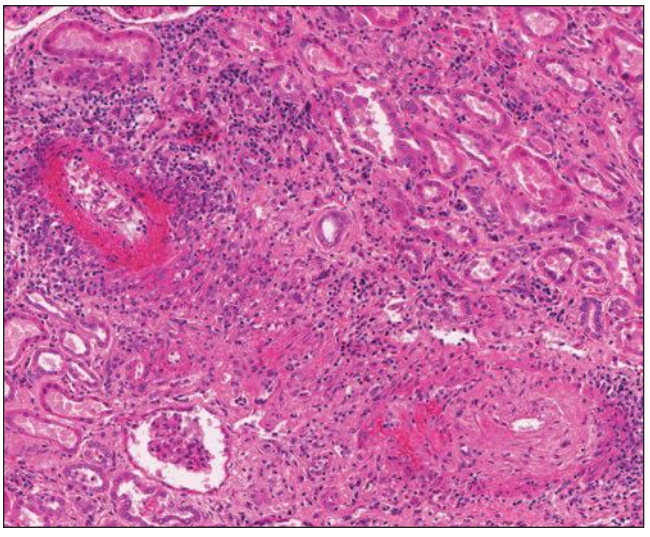


**FIGURE 17.3 Postmortem kidney from a patient with polyarteritis nodosa showing involvement of two arteries with two different phases of necrotizing arteritis.** The artery in the upper left (which is shown at higher magnification in Fig. 17.4) has circumferential fibrinoid necrosis with marked perivascular inflammation, whereas the artery in the lower right has marked fibrosis of the intima and media surrounded by a lesser degree of residual fibrinoid necrosis and inflammation. Note also the simplification (flattening) of the proximal tubular epithelium in the lower left corner of the photomicrograph indicative of acute tubular epithelial injury. (H&E.)

into the perivascular tissue. The inflammatory and necrotizing arterial lesions are segmental. Thus, they may be present in a specimen but missed in some planes of section. As illustrated in the diagram in Figure 17.5, the level of artery wall that appears to be involved by the necrotizing inflammation is affected by the plane of section. The necrosis and inflammation appear to spread out laterally along the artery wall once they penetrate the muscularis and extend into the adventitia. Thus, at some planes of section (e.g., level C in Fig. 17.5), the inflammation and necrosis appear to be perivascular rather than vascular. This may have been why Kussmaul and Maier (13) named the disease "periarteritis nodosa."

This erosion of vascular and perivascular tissue by the necrotizing process is the basis for the "aneurysm" formation (see Fig. 17.5). These areas of luminal dilation thus are not true aneurysms with dilation of the vessel wall, but rather, they are pseudoaneurysms caused by destruction of the vessel wall. The lesions are segmental with intervening segments of normal artery walls; therefore, multiple levels of section may be required to identify the vasculitis when it is sparse. The fibrinoid necrosis is deeply acidophilic on hematoxylin and eosin–stained sections and fuchsinophilic (red) on trichrome-stained sections. The latter distinguishes fibrinoid material from the collagen that will replace it in the healed phase and will stain green or blue depending on the counterstain used in the Masson trichrome stain. Fibrinoid necrosis looks like fibrin because it has a high content of fibrin, as can be documented by immunohistologic examination using antifibrin antibodies

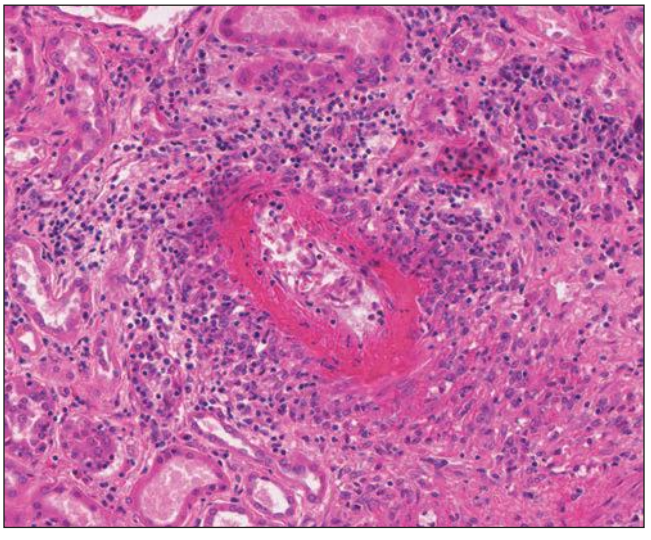


**FIGURE 17.4 Higher magnification of the specimen illustrated in Figure 17.3 showing bright red ragged fibrinoid necrosis that has virtually replaced the destroyed muscularis.** The surrounding inflammatory infiltrate includes neutrophils, eosinophils, lymphocytes, monocytes, and macrophages. (H&E.)

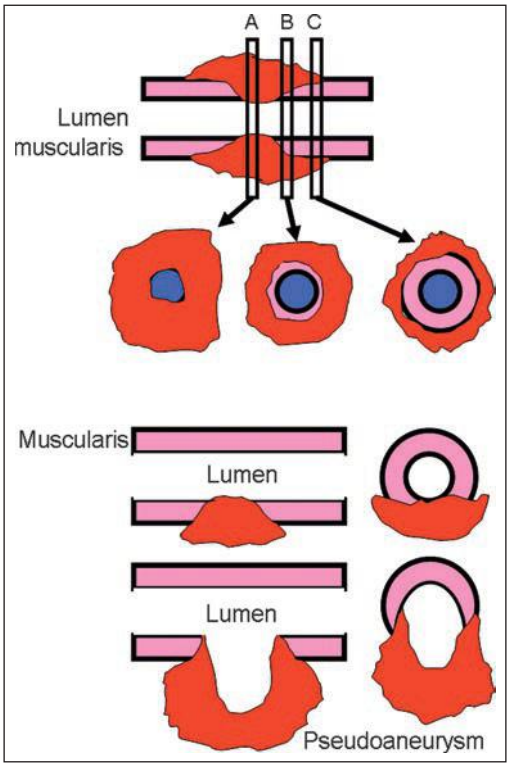



**FIGURE 17.5 Diagram depicting the histologic distribution of fibrinoid necrosis (*dark red material*) in the walls of arteries (*pink*) and perivascular tissue.** Because the necrotizing lesions are segmental, the plane of section will influence the appearance of the lesion **(top)**. For example, at level *C* in the diagram, the necrosis appears to be perivascular. The bottom view illustrates that the dilation of an artery lumen that is caused by necrotizing arteritis is primarily by erosion of the necrosis through the vessel wall and into the perivascular tissue. Thus, this is a pseudoaneurysm rather than a true aneurysm even though it is an aneurysm by convention.

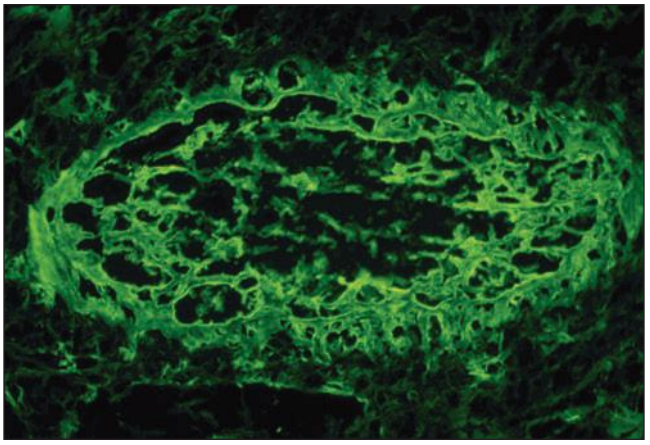


**FIGURE 17.6** Immunofluorescence microscopy of an arcuate artery from a postmortem kidney from a patient with polyarteritis nodosa showing irregular circumferential transmural staining for fibrin that would correspond to a histologic picture similar to that in Figure 17.4. (Fluorescein isothiocyanate [FITC] antifibrin.)

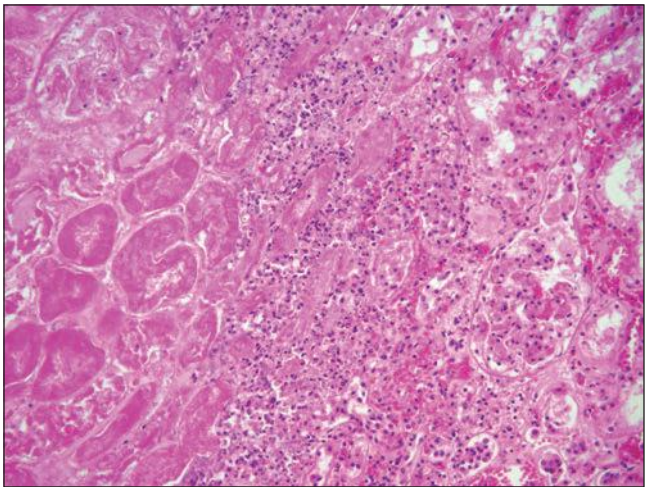


**FIGURE 17.8** Postmortem kidney from a patient with polyarteritis nodosa showing a zone of infarction with coagulative necrosis on the left and a zone of acute inflammation in the middle at the interface between the infarct and viable but congested cortical parenchyma on the right. (H&E.)

(Fig. 17.6). The fibrinoid material accumulates as a result of plasma constituents, including fibrinogen and the other coagulation proteins, spilling into the zones of necrosis, where the coagulation factors contact thrombogenic material resulting in fibrin formation (11). When this happens in the arterial lumen, it is designated thrombosis, and when it occurs within the zones of tissue necrosis, it is fibrinoid necrosis.

In addition to the perivascular inflammation at sites of arteritis (see Fig. 17.4), the major renal tubulointerstitial histologic finding is coagulative necrosis secondary to infarction (Figs. 17.7 and 17.8). This has the same histologic features as any renal infarct. In the acute phase, coagulative necrosis is evident, which may have a peripheral zone of acute inflammation with neutrophilic infiltration (see Fig. 17.8). In a needle biopsy specimen, this can be mistaken for acute tubulointerstitial nephritis. Glomeruli immediately adjacent to the infarcts may have reactive changes, including hypercellularity, necrosis, and rarely even reactive epithelial changes that mimic true crescent formation. This picture should not be confused with the glomerulonephritis that is a component of small-vessel vasculitis such as MPA. The coagulative necrosis of an infarct is ultimately replaced by fibrous tissue. The narrowing of arteries by the lesions of PAN may cause sublethal ischemia that results in tubular epithelial simplification in the acute phase (see Figs. 17.3 and 17.4) and tubular atrophy and interstitial fibrosis in the chronic phase. Such ischemia may be the basis for the

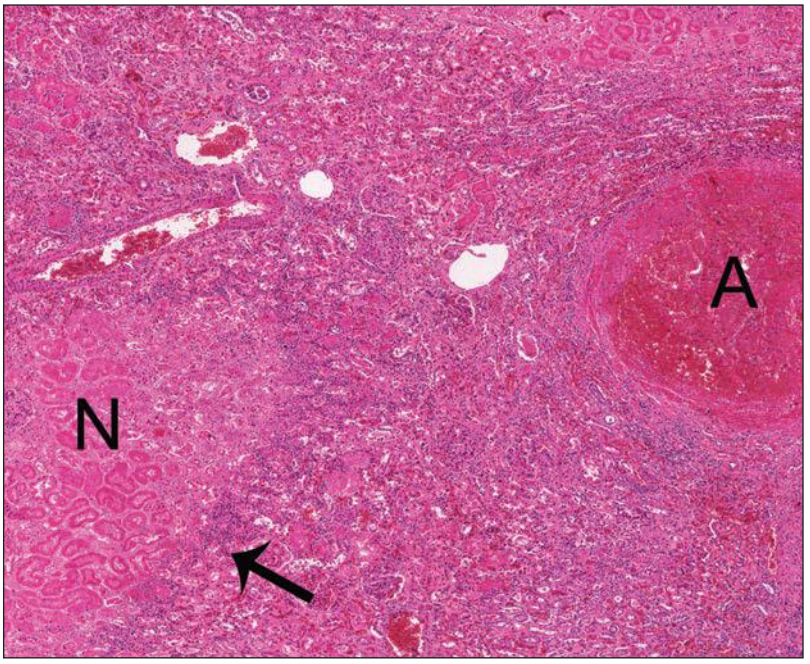



**FIGURE 17.7** Postmortem kidney from a patient with polyarteritis nodosa showing an aneurysm (pseudoaneurysm) filled with a thrombus (*A*), a zone of infarction with coagulative necrosis (*N*), and zone of acute inflammation at the interface between the infarct and viable cortical parenchyma (*arrow*). (H&E.)

hypertension that often accompanies PAN. The hypertension may reach the malignant range and may produce superimposed microangiopathic changes caused by the malignant hypertension (22). These effects of malignant hypertension may include fibrinoid necrosis of arterioles and segmental necrosis of glomeruli that should not be misinterpreted as a component of the vasculitis. In a patient with malignant hypertension, the absence of leukocytes or leukocytoclasia at sites of microvascular necrosis suggests a hypertensive origin for the necrotizing small-vessel injury.

The acute phase evolves into a healing phase with fibrous tissue formation replacing the fibrinoid necrosis and thrombosis and mononuclear leukocytes replacing the neutrophils. Marked intimal thickening often is a component of the mesenchymal remodeling in the arterial wall (Figs. 17.3 and 17.9). The healed stage has progressively denser collagen in the fibrotic arterial wall and perivascular tissue. Thrombosed arteries may be recanalized. The arterial sclerosis of healed arteritis is distinguished from that of advanced benign hypertensive arteriosclerosis by disruption of the internal elastic lamina and the replacement of medial smooth muscle cells by fibrous tissue (22). In the early phase of arterial fibrosis, there may be residual foci of fibrinoid necrosis that reveal the initial cause of injury (see Fig. 17.7). Arteries with advanced sclerosis caused by necrotizing arteritis may be difficult to distinguish from severe arteriosclerosis caused by hypertension unless a stain is used to demarcate the elastica and identify fragmentation caused by the necrotizing inflammation (Fig. 17.10).

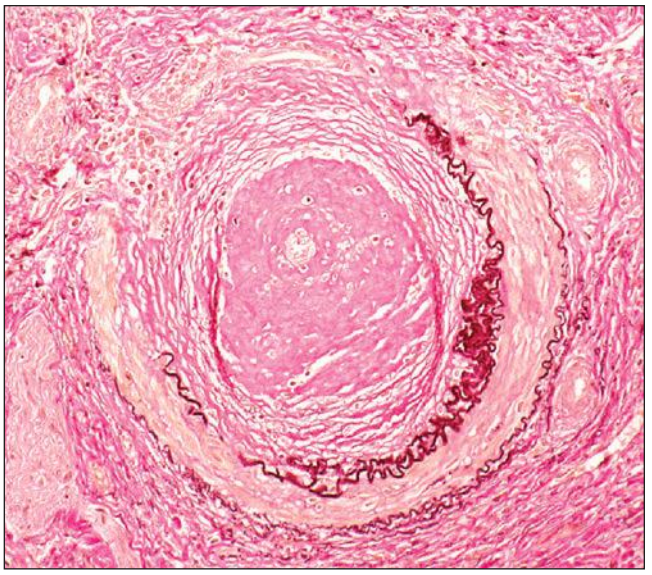


**FIGURE 17.10 Severely sclerotic artery with occlusion of the lumen and disruption of the internal and external elastica caused by earlier PAN-induced necrotizing inflammation.** At this late stage, the histopathology does not allow differentiation between a medium- or small-vessel vasculitis versus a large-vessel vasculitis. (Jones silver stain.)

## Immunofluorescence and Electron Microscopy

Most published immunofluorescence microscopy and electron microscopy data pertain to MPA, rather than to PAN. Although immune complexes have been suggested as a possible cause for PAN, immunofluorescence microscopy and electron microscopy typically do not reveal evidence of vascular immune complex deposition. Even in PAN that is associated with hepatitis, glomeruli typically have no significant immune complex–type staining for immunoglobulin by immunofluorescence microscopy and no immune complex–type electron-dense deposits by electron microscopy (56,57). In acute lesions, immunofluorescence microscopy demonstrates staining of fibrinoid material and thrombi with antisera specific for fibrin (see Fig. 17.6). The necrotic lesions also often have irregular staining for complement components and immunoglobulins, especially IgM, as is true for all fibrinoid necrosis irrespective of cause. Histologically unremarkable arterial walls adjacent to the inflamed segments have no staining for immunoglobulins or complement.

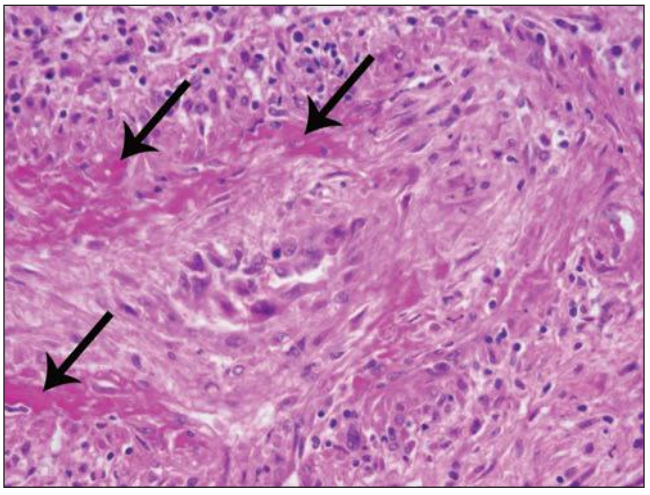


**FIGURE 17.9 Arcuate artery from a postmortem kidney from a patient with polyarteritis nodosa showing extensive narrowing of the lumen by marked fibrotic intimal thickening.** The muscularis is poorly defined and has focal fibrosis as well as focal residual red ragged fibrinoid necrosis (*arrows*). There is a relatively scanty associated infiltration of predominantly mononuclear leukocytes. (H&E.)

## Etiology and Pathogenesis

The etiology and pathogenesis of PAN are incompletely understood. There appear to be multiple causes and pathogenic mechanisms that can result in a pattern of arteritis consistent with PAN. Some patients with PAN have hepatitis B virus infection, a finding that has raised the possibility of an infection-induced immune complex pathogenesis (31,58,59). However, overall, less than 10% of patients with PAN have serologic evidence for hepatitis B infection (59). Hepatitis B infection also is associated with some forms of secondary membranous glomerulonephritis and proliferative glomerulonephritis and with small-vessel vasculitis that often causes dermal leukocytoclastic angiitis (58). The evidence that hepatitis B infection induces vascular immune complex deposition is much stronger in the hepatitis-associated glomerulonephritis and small-vessel vasculitis than in PAN. In fact, patients with hepatitis B infection who have PAN but no glomerulonephritis have no immune complex–type deposits in glomeruli (57). Other infections that have been rarely associated

with PAN include hepatitis C, HIV, and parvovirus B19 (60). In one study of 161 patients with HCV-associated vasculitis, HCV-PAN accounted for approximately 20% and cryoglobulinemic vasculitis for 80% (61).

ANCA are frequent in patients with MPA and appear to play a pathogenic role; however, ANCA are not frequent in patients who have arteritis that is not accompanied by inflammatory involvement of capillaries or venules (7,26,31,32). Thus, ANCA are not incriminated in the pathogenesis of PAN, and the prevalence of ANCA in MPA, but not in PAN, indicates that these diseases are etiologically different.

## Differential Diagnosis

PAN must be distinguished not only from MPA but also any of the many other forms of vasculitis that cause necrotizing arteritis. The clinical manifestations at the time of presentation often do not allow differentiation between PAN and MPA unless there is overt clinical evidence for glomerulonephritis or pulmonary capillaritis, or a positive ANCA, either of which essentially rules out PAN (7,47,48,56). Extensive pulmonary hemorrhage caused by capillaritis rules out PAN in favor of MPA, and pulmonary hemorrhage caused by necrotizing granulomatous inflammation rules out PAN in favor of GPA or EGPA (1). Asthma and blood eosinophilia in a patient with arteritis supports a diagnosis of EGPA; however, eosinophilia alone can be seen with PAN. In a young child with necrotizing arteritis with renal involvement, evidence for the MCLNS should be sought to distinguish between PAN and KD (37). In addition, the histologic pattern of arteritis can help distinguish between PAN and KD. The acute lesions of PAN typically have extensive fibrinoid material, whereas the acute lesions of KD have lesser amounts of fibrinoid material and more edema (11).

In a renal biopsy specimen, or in a specimen from other tissue for that matter, the necrotizing arteritis of PAN cannot be distinguished from necrotizing arteritis caused by many other forms of vasculitis, for example, MPA, GPA, and EGPA (3,11). This usually is not a problem because histologic evidence for glomerulonephritis is identified in more than 90% of renal biopsy specimens from patients with MPA and GPA who have clinical evidence for renal disease. However, rare renal biopsy specimens from patients with MPA and GPA, especially if they are small, contain only arteritis. A definitive diagnosis of PAN should never be made on the sole basis of identifying necrotizing arteritis in a renal biopsy specimen. The same holds true for any other type of biopsy, such as nerve, muscle, skin, or gut.

The differential diagnosis for necrotizing arteritis in the kidney and elsewhere also includes arteritis secondary to a systemic autoimmune disease, for example, systemic lupus erythematosus and rheumatoid arthritis (1,62). The association between necrotizing arteritis in a renal biopsy specimen and lupus usually is apparent because of the concurrent pathologic features of lupus glomerulonephritis (Fig. 17.11). Unlike idiopathic PAN, immunohistology reveals that lupus-associated arteritis has immune complex immunoglobulin and complement in vessel walls.

During gross inspection of a postmortem or nephrectomy kidney that has been sectioned, large aneurysms (pseudoaneurysms) from which blood or thrombus has been lost during sectioning can be mistaken for infectious cavitary lesions (see Fig. 17.2B). Histologic examination readily resolves this misinterpretation.

## Clinical Course, Prognosis, Therapy, and Clinicopathologic Correlations

PAN is treated with corticosteroids and cytotoxic drugs, usually cyclophosphamide (31,59,60). The 10-year survival rate with treatment is approximately 70% to 80%. Relapses are rare in patients with PAN, unlike the more frequent relapses in MPA (31,60). Table 17.2 indicates that only 17% of the 36 PAN patients in the cohort developed a relapse. Relapse is more likely if diagnosis and treatment are delayed (47).

PAN that is associated with hepatitis B requires specialized treatment aimed at controlling both the arteritis and the hepatitis (59,60). One approach (60) uses initial administration of corticosteroids to control the most life-threatening manifestations of the PAN, which are most severe during the first weeks of the disease. Corticosteroids are stopped to

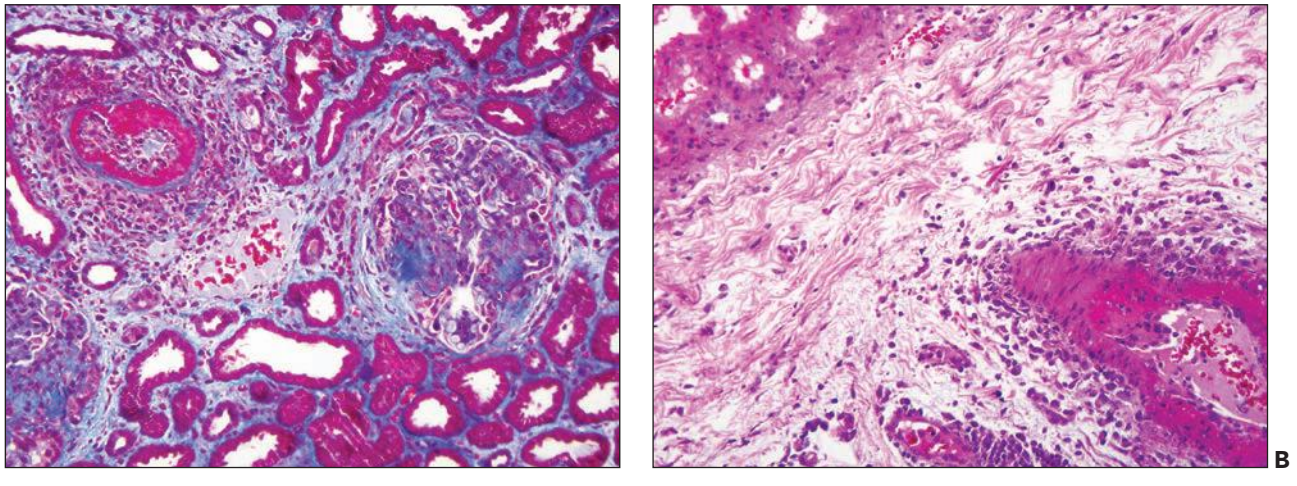



**FIGURE 17.11** Renal biopsy specimen from a patient with systemic lupus erythematosus that demonstrated a necrotizing arteritis in the cortex along with diffuse proliferative and sclerosing class IV-G A/C lupus glomerulonephritis **(A)** (Masson trichrome stain) as well as necrotizing arteritis in the perirenal adipose tissue **(B)** (H&E stain).

facilitate immune defense against the hepatitis, which is monitored by seroconversion from hepatitis B e antigen positivity to hepatitis B e antibody positivity. Recovery from the hepatitis also is enhanced with antiviral agents (59,60).

Although not a component of standard care, surgical intervention may be required in rare circumstances such as bowel infarction, life-threatening rupture of an aneurysm, or uncontrollable hypertension caused by renal artery involvement (60,63). In the latter instance, renal artery repair and autotransplantation have been successful (63).

# KAWASAKI DISEASE

## Clinical Presentation

Kawasaki disease is an acute febrile illness that almost always occurs in children, with a peak incidence in the first year of life (64–66). The incidence of KD is highest in children of Japanese ancestry. In North America, the incidence is approximately 32/100,000 in children of Asian ancestry; 17/100,000 African Americans; 11/100,000 Hispanics; and 9/100,000 Caucasians (65). Approximately 80% to 85% of children are younger than 5 years of age at the time of onset (64).

The hallmark of KD is the mucocutaneous lymph node syndrome (MCLNS), which includes polymorphous erythematous rash, erythema of the palms and soles, indurative edema of the extremities followed by desquamation, erythema of the oropharyngeal mucosa, conjunctivitis, and nonsuppurative lymphadenopathy. KD often has a component of necrotizing arteritis that can be mistaken for PAN (36,37,66–69). This feature has resulted in some confusion over the relationship of the so-called infantile polyarteritis nodosa with KD. Most cases reported as infantile PAN are, in fact, KD (34,37). Differentiation of KD from PAN is important because KD usually is effectively controlled with aspirin and intravenous γ-globulin therapy (64,65), whereas PAN requires corticosteroids and possible immunosuppressive drugs (59,60).

Clinically significant renal disease in patients with KD is rare, although postmortem examination reveals inflammatory lesions in renal arteries in one fourth (67) to three fourths (6) of patients. The difference in frequency of renal involvement pathologically may be a result of different sampling times because the frequency of the visceral vasculitic lesions of KD peaks during the first week of the illness and is markedly reduced after 1 month (6). Anecdotal reports exist of glomerulonephritis and hemolytic-uremic syndrome in patients with KD (70), but these reports may reflect chance associations. Renovascular hypertension caused by postvasculitic stenosis of the renal artery is a rare renal complication (71). There is one report of hydronephrosis resulting from obstruction of the pelviureteric junction caused by KD arteritis (72).

## Pathologic Findings

### Gross Pathology

The arteritis of KD affects small- and medium-sized arteries, and it has a predilection for the coronary arteries (6,67–69, 73–75). Aneurysms (pseudoaneurysms) and thrombosis may occur. Thrombosis of inflamed coronary arteries in patients with KD is the most common cause of childhood myocardial infarction.

The kidneys are second to the heart in frequency of vasculitic lesions, affecting the kidneys of 50% to 75% of patients who die from KD (6,75). In the kidneys, vasculitic lesions usually affect interlobar arteries, occasionally affect main renal and arcuate arteries, and only rarely involve interlobular arteries (6). Therefore, the nodular inflammatory vascular lesions are observed most often in the renal sinus tissue adjacent to the calyces and columns of Bertin and within the columns of Bertin. The lesions appear as pale yellow or dark red nodules depending on the degree of inflammation versus thrombosis, respectively.

### Light Microscopy

The acute arteritis of KD is a necrotizing process, but it typically has less conspicuous fibrinoid change and more edema than does PAN (Figs. 17.12 to 17.14) (6,65,68,75). Early inflammatory changes are most pronounced in the intima and media. The intima is thickened by infiltrating leukocytes. The media develops edema, vacuolization of myocytes, disassociation of myocytes, and focal rupture of the internal elastic lamina. During the first few days, neutrophils are most conspicuous (6), but even in the acute phase, monocytes and macrophages may predominate (76). The arteritis eventually becomes transmural in some segments with necrosis and extensive disruption of elastic laminae in the most severely inflamed areas. Weakening or total destruction of the muscularis may result in aneurysm formation (see Figs. 17.13 and 17.14). Thrombosis may occur at sites of arteritis, resulting in distal ischemia. Myocardial infarction is a well-recognized complication of KD coronary arteritis, but renal infarction is rare (75).

An analysis of seven kidney specimens from children who died from KD revealed that inflammatory infiltrates at sites of arteritis in interlobar arteries contained an extremely high proportion of monocytes/macrophages (CD68-positive cells), most with the morphology of macrophages (Fig. 17.15A) (73). Early intimal lesions had almost exclusively CD68-positive cells immediately beneath the endothelium (Fig. 17.15B). Most infiltrates in the intima, muscularis, and adventitia had approximately 90% CD68-positive cells. The few remaining

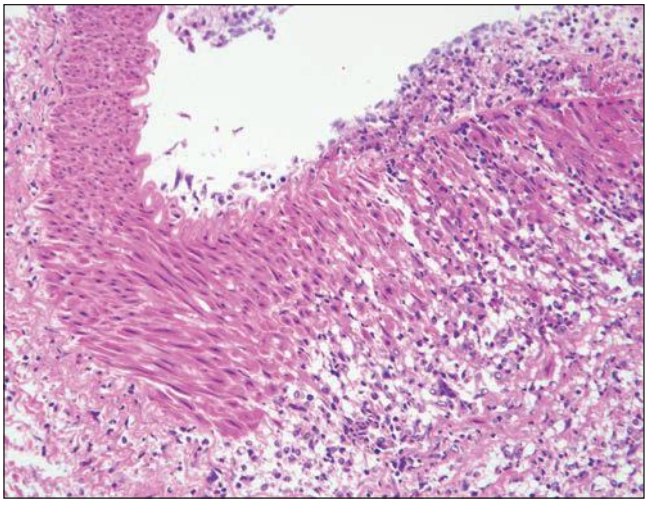
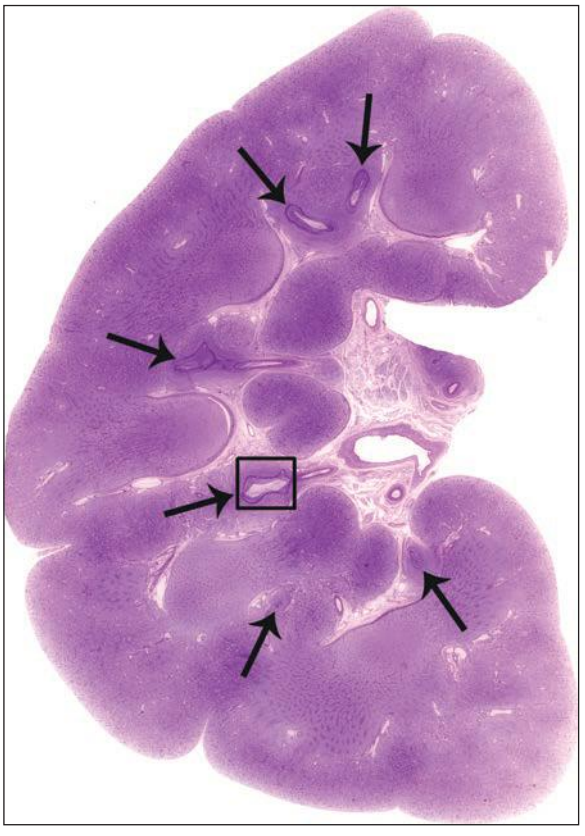


**FIGURE 17.12 Interlobar artery from the kidney of an infant who died from Kawasaki disease showing segmental infiltration of the intima, muscularis, and adventitia by predominantly mononuclear leukocytes.** Note the marked edema at the site of necrosis of the muscularis and the absence of significant fibrinoid material. (H&E.)



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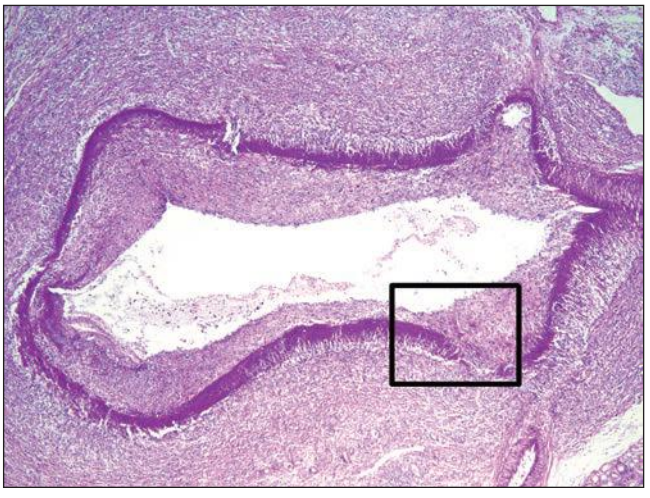

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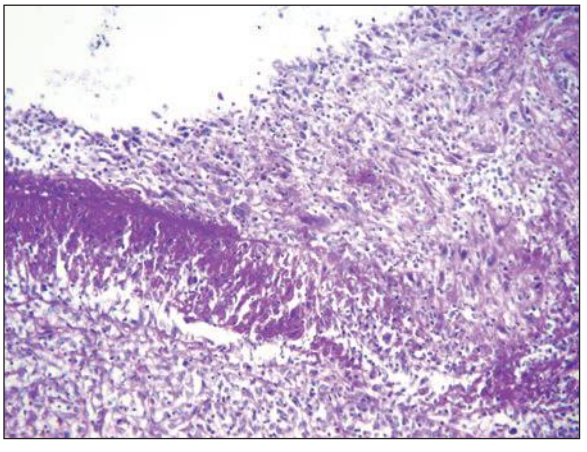

C

**FIGURE 17.13** **A:** Whole mount of a PAS-stained cross section of a postmortem kidney from an infant with Kawasaki disease showing multiple sites of necrotizing arteritis (*arrows*) that are all involving interlobar arteries. Note that there is a dilation of the artery in the *box* (aneurysm) relative to the caliber of the uninvolved segment of the artery just outside the box. **B:** Higher magnification of the arteritis demarcated by the *box* in showing a darkly staining, partially disrupted muscularis; irregularly thickened intima; and prominent perivascular infiltrate. **C:** Higher magnification of the arteritis demarcated by the *box* showing transmural inflammation that has completely destroyed the muscularis on the right. The muscularis on the left is partially destroyed and has focal edema and slight focal accumulation of fibrinoid material. The infiltrate contains predominantly mononuclear leukocytes that are primarily monocytes and macrophages. (PAS stain.) (Courtesy of Shiro Naoe and Kei Takahashi of Toho University, Japan.)

cells were mostly CD3-positive T lymphocytes, and more often were CD8-positive rather than CD4-positive. CD20-positive B lymphocytes were rare. An unexpected finding was increased numbers of CD68-positive monocytes/macrophages in glomeruli (Fig. 17.15C). Specimens with severe arteritis had 1.4 CD68-positive cells/glomerulus, moderate arteritis specimens had 2.3 CD68-positive cells/glomerulus, and mild arteritis specimens had 0.04 CD68-positive cells/glomerulus. Even though immunohistology demonstrated increased macrophages in glomeruli, no glomerular hypercellularity could be discerned by light microscopy in routinely stained sections. Theoretically, the glomerular macrophages may have broken away from the inflamed surface of larger arteries and embolized to the glomerular capillaries.

Renal tubulointerstitial lesions in KD are absent or nonspecific. Focal intestinal mononuclear cell infiltrates with prominent IgA-positive plasma cells have been described in kidneys of patients undergoing autopsy; however, IgA-positive plasma cells are more conspicuous in the coronary arteries and respiratory tract (77).

As with other forms of arteritis, acute inflammatory lesions evolve into sclerotic lesions that often are accompanied by intimal thickening and gaps in the elastic laminae (68).

## Etiology and Pathogenesis

The etiology and pathogenesis of KD are unknown (64–66). Although most cases are sporadic, a tendency for the disease to occasionally manifest endemic and epidemic characteristics

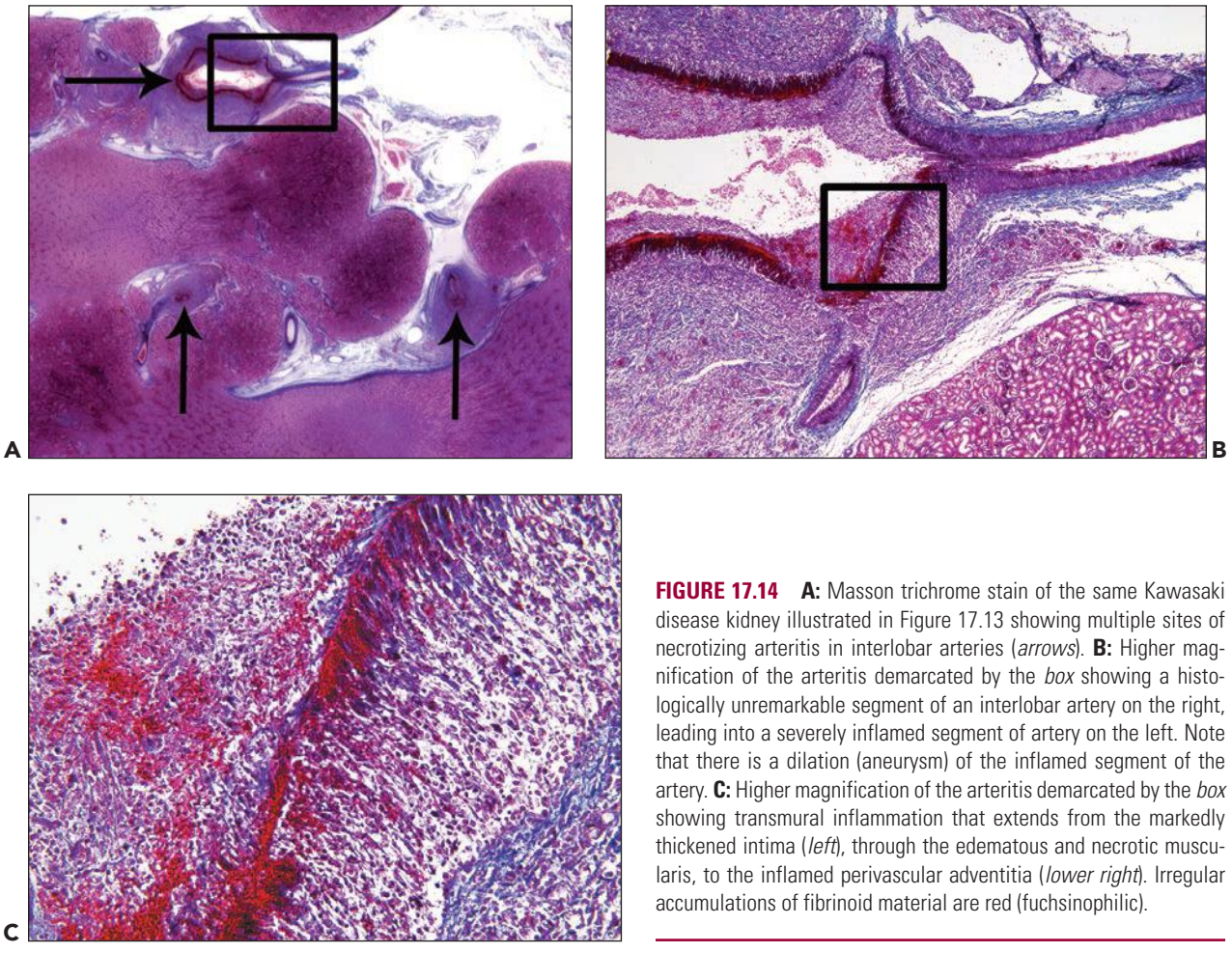


**FIGURE 17.14** **A:** Masson trichrome stain of the same Kawasaki disease kidney illustrated in Figure 17.13 showing multiple sites of necrotizing arteritis in interlobar arteries (*arrows*). **B:** Higher magnification of the arteritis demarcated by the *box* showing a histologically unremarkable segment of an interlobar artery on the right, leading into a severely inflamed segment of artery on the left. Note that there is a dilation (aneurysm) of the inflamed segment of the artery. **C:** Higher magnification of the arteritis demarcated by the *box* showing transmural inflammation that extends from the markedly thickened intima (*left*), through the edematous and necrotic muscularis, to the inflamed perivascular adventitia (*lower right*). Irregular accumulations of fibrinoid material are red (fuchsinophilic).

raises the possibility of an etiologic role for an infectious pathogen or environmental antigen or toxin, but no such agent has been identified. Bell et al. (78) carefully studied two epidemics in the United States, but these investigators were unable to identify evidence of person-to-person transmission or common exposures to potential etiologic agents.

Immune mechanisms have been implicated in the pathogenesis of KD because of several immunologic abnormalities, such as decreased circulating CD8 T lymphocytes, increased circulating CD4 T lymphocytes with activation markers, increased circulating CD14 monocytes/macrophages, increased circulating cytokines, and hyperactive B lymphocytes with increased immunoglobulin secretion (65,79,80). Circulating anti–endothelial cell antibodies have been reported in patients with KD (81–85). This finding has prompted the hypothesis that these autoantibodies cause the vascular injury of KD by producing in situ immune complex formation with endothelial cell antigens. Binding of these antibodies to endothelial cells may be able to induce monocyte adhesion (84), which would be in line with the observation of monocytes in the intima of early lesions in patients (76) and the reduction of circulating monocytes by intravenous immunoglobulin therapy that is known to be an effective therapy for KD (80).

The presence or absence of ANCA in patients with KD has been controversial. The current predominant view is that no significant association exists between KD and ANCA, and this has been our experience as well with samples from 25 patients with KD. The first of two publications on this issue by Savage et al. (86) concluded that the frequency of ANCA in patients with KD was high, but a subsequent publication by Nash et al. (87) using more refined methodology concluded that no significant occurrence of ANCA is seen in patients with KD.

Takahashi et al. (88) created an animal model of arteritis that has pathologic features that are remarkably similar to the arteritis of KD by injecting an extract of *Candida albicans* into the peritoneum of mice. All susceptible strains of mice develop an arteritis with a distribution and histologic appearance that mimics KD. Duong et al. (89) have created a similar model by injecting extracts from *Lactobacillus casei* into mice. These animal models raise the possibility that the human disease is the result of a genetic predisposition to develop a pathogenic innate or acquired immune response to a pathogenic organism. The predominance of monocytes rather than T cells in the early lesions favors an innate rather than an acquired immune response.

## Differential Diagnosis

The vasculitis of KD shares many pathologic features with other forms of systemic vasculitis; however, it has a number of distinctive characteristics that clearly set it apart. These include (a) a unique association with the MCLNS although MCLNS may not be completely expressed in all patients, (b) a strong

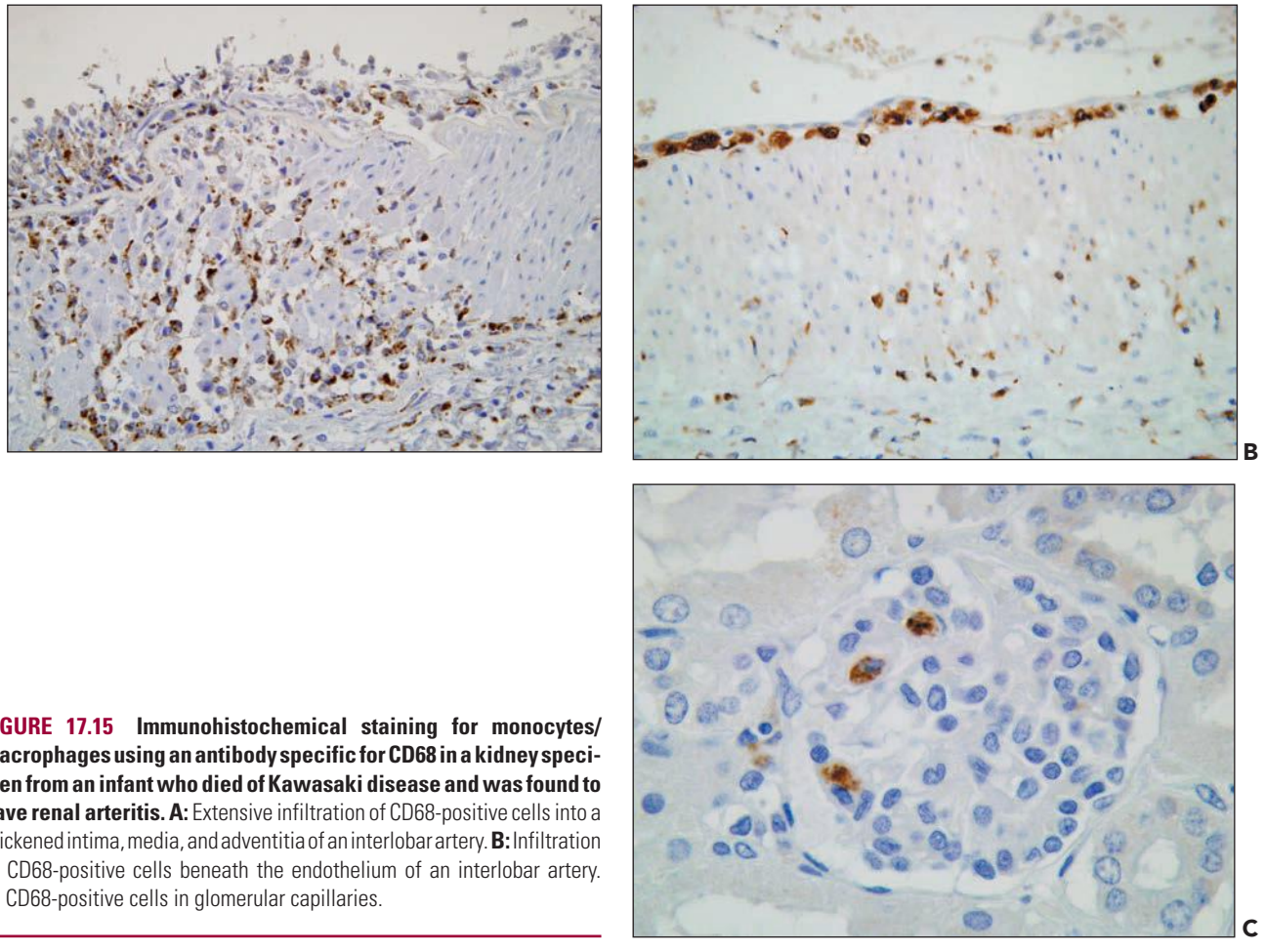

**FIGURE 17.15 Immunohistochemical staining for monocytes/macrophages using an antibody specific for CD68 in a kidney specimen from an infant who died of Kawasaki disease and was found to have renal arteritis. A:** Extensive infiltration of CD68-positive cells into a thickened intima, media, and adventitia of an interlobar artery. **B:** Infiltration of CD68-positive cells beneath the endothelium of an interlobar artery. **C:** CD68-positive cells in glomerular capillaries.

predilection for the coronary arteries although many other arteries may be involved, and (c) a pattern of inflammation in arteries that shares features with other forms of necrotizing arteritis but has distinctive characteristics of its own.

KD arteritis and PAN both cause systemic arteritis. Before KD was clearly recognized, some patients with this disease were diagnosed as "infantile polyarteritis nodosa." However, most cases reported in the literature as infantile PAN are, in fact, KD (37). Necrotizing arteritis also may occur in MPA, GPA, and EGPA. Although these vasculitides share some pathologic features with KD arteritis, the overall clinical manifestations, as well as some characteristics of the pathologic lesions, allow confident differentiation of KD from these systemic vasculitides. For example, MPA, GPA, and EGPA often have clinical and pathologic evidence for involvement of capillaries (e.g., glomerulonephritis and pulmonary capillaritis) and venules (e.g., dermal leukocytoclastic venulitis) that does not occur with KD. The acute arteritis of PAN, MPA, GPA, and EGPA are indistinguishable from each other histologically but are distinct from KD in that the acute lesions of KD have less neutrophil infiltration, more mononuclear leukocyte infiltration, more edema of the muscularis, and less fibrinoid necrosis.

There is no problem distinguishing acute KD arteritis from the two major forms of large-vessel vasculitides, that is, Takayasu arteritis and GCA. However, the chronic sclerotic sequelae of KD arteritis are more difficult to distinguish from the sclerotic phase of large-vessel vasculitis. Distinguishing chronic sclerotic KD arteritis from chronic Takayasu arteritis is most problematic because both occur in children. A careful clinical history is most helpful in making this distinction.

## Clinical Course, Prognosis, and Therapy

KD usually is an acute process with an excellent prognosis if treated appropriately (64–66). KD usually is effectively controlled with aspirin and intravenous γ-globulin therapy (64–66). Aspirin reduces systemic manifestations of inflammation (e.g., fever) and inhibits platelet aggregation, but does not prevent the development of coronary arteritis or aneurysms (65). Intravenous γ-globulin therapy, especially if given during the 10 days after the onset of fever, reduces the frequency and severity of coronary artery disease (64–66). If not treated early in the course of the disease, approximately 15% to 25% of patients will have serious cardiovascular damage such as myocardial infarction or sudden death (64). Less than 5% of children with KD who receive therapy with intravenous gamma globulin develop coronary artery aneurysms (64). Nevertheless, KD has surpassed rheumatic fever as the leading cause of cardiac disease in children in the United States. If disease manifestations do not improve with intravenous γ-globulin therapy, corticosteroids, immunosuppressive drugs, and plasma exchange may be of value (64–66).

# TAKAYASU ARTERITIS

## Clinical Presentation

Takayasu arteritis is a large-vessel vasculitis characterized by granulomatous inflammation that most often affects the aorta and its major branches, but it also can affect the pulmonary arteries (38,46,90–92). The disease also has been called aortic arch syndrome and pulseless disease because it often affects the aortic arch and the arteries arising from it and frequently causes diminished or absent pulses, especially in the upper extremities, because of arterial narrowing. Takayasu arteritis has been reported most often in Asia, but it occurs worldwide. Takayasu arteritis has a strong female predilection, with a female-to-male ratio of approximately 9:1. Affected individuals are usually between 10 and 20 years old, and it rarely occurs after 50 years of age. This age distribution is in marked contrast to GCA, which rarely occurs before 50 years of age. In fact, patient age is the most useful distinguishing feature between Takayasu arteritis and GCA because the other clinical manifestations and pathologic changes overlap (1). The differences in age distribution, demographic characteristics, and vascular distribution warrant considering these to be different forms of vasculitis.

Takayasu arteritis can be arbitrarily divided into four types: type I, limited to the aortic arch and its branches; type II, confined to the descending thoracic and abdominal aorta; type III, with combined features of types I and II; and type IV, with pulmonary artery involvement with or without combined features of other types (90). Type III is most common, occurring in 65% of 107 patients with Takayasu arteritis studied by Lupi-Herrera et al. (93).

In addition to nonspecific constitutional symptoms such as fever, arthralgias, and weight loss, the major clinical manifestations of Takayasu arteritis are reduced pulses, vascular bruits, claudication, and renovascular hypertension (46,88,90,91,93,94). Hypertension is a common mode of presentation, and Takayasu arteritis is the most common cause of renovascular hypertension in children and young adults in some parts of Asia and the Middle East (94–96). Of the 107 patients studied by Lupi-Herrera et al. (93), 96% had reduced peripheral pulses, 94% had vascular bruits, and 72% had hypertension, which was attributed to renal arterial involvement in 62% of patients. In an American College of Rheumatology study of 63 patients (46), 98% had reduced pulses, 98% had claudication, and 90% had subclavian or abdominal bruits.

Renovascular hypertension is a major cause of morbidity and mortality in patients with Takayasu arteritis (10,95–97). The major cause is renal ischemia secondary to renal artery stenosis, aortic coarctation, or both, although reduced aortic elasticity and impairment of carotid artery baroreceptors may play a role in some patients (10). Adequate control of renovascular hypertension may not be possible medically, and these patients may require surgical vascular repair (10,97) or transluminal angioplasty (98).

## Pathologic Findings

### Gross Pathology

Gross aortic lesions include segmental stenosis (80% of patients), dilation without overt aneurysm (60%), aneurysm (20%), and dissection (10%) (94). The abdominal aorta is the most common segment of the aorta involved. Arteries affected by Takayasu arteritis typically have segments with thickened, firm walls and narrowed lumens (90). In some instances, the lumen may be completely obliterated, usually by firm pale fibrous material rather than acute thrombus. Kidneys may show signs of hypertensive nephrosclerosis (94).

### Light Microscopy

Active Takayasu arteritis has transmural inflammation with a predominance of mononuclear leukocytes, often with admixed multinucleated giant cells histologically indistinguishable from GCA (38,90,94). Takayasu arteritis often results in marked intimal thickening and resultant stenosis of the lumen (Fig. 17.16), which is the basis for the ischemic clinical manifestations. The active phase of Takayasu arteritis is characterized by irregular focal areas of infiltration of predominantly mononuclear leukocytes, sometimes with a granulomatous appearance, in the adventitia, media, and intima (Figs. 17.17 and 17.18) (90). The inflammatory infiltrates contain predominantly lymphocytes and macrophages with varying numbers of multinucleated giant cells. In some specimens, apparently in earlier phases of the disease, the inflammation is more pronounced in the media and adventitia. In the aorta, there is patchy destruction of medial elastic lamellae by the inflammation.

In the chronic phase of the disease, the inflammation is replaced by fibrous scars with only a few scattered chronic inflammatory cells. The sclerotic changes, including marked intimal thickening, often result in luminal narrowing, which can be made worse acutely by thrombosis. Quiescent disease may be difficult to identify histologically. Residual destruction of the elastica supports earlier active arteritis (Fig. 17.19). Exacerbation of active granulomatous inflammation may be superimposed on sclerotic lesions (94).

The kidney parenchyma may show histologic evidence for chronic hypertensive nephrosclerosis or, rarely, malignant hypertensive nephropathy (94,95).

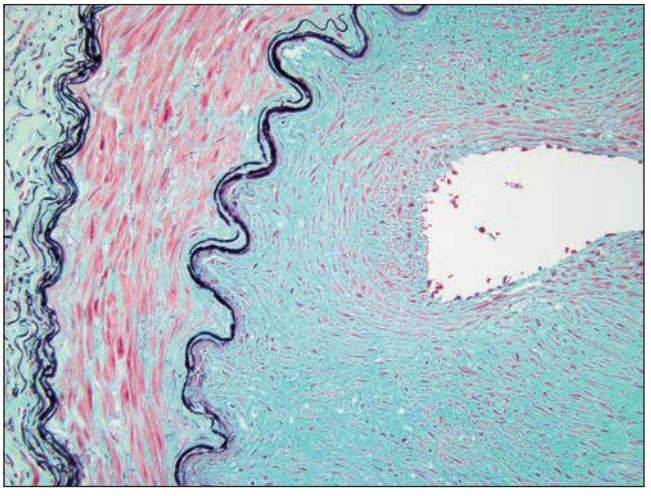

**FIGURE 17.16 Elastic tissue stain of a renal artery from a nephrectomy specimen taken from a patient with Takayasu arteritis showing a grossly thickened intima and markedly narrowed lumen.** At the time of nephrectomy, there is minimal inflammation. There is slight focal fragmentation and fraying of the internal elastic lamina; however, important in the differentiation from primary hypertensive arteriosclerosis, there is no replication of silver-positive laminae within the thickened intima.

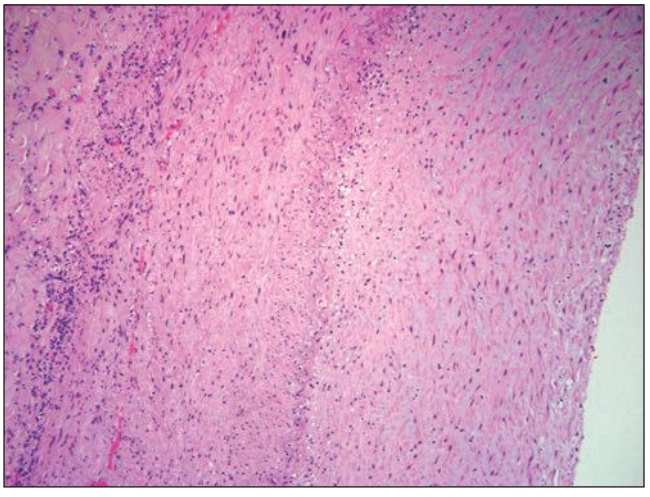

**FIGURE 17.17 Renal artery from a nephrectomy specimen affected by Takayasu arteritis.** The internal elastica is running vertically near the center of the image with a markedly thickened intima on the right and the lumen at the far right. There is a sprinkling of mononuclear leukocytes throughout the artery wall with concentrations of leukocytes near the internal elastic lamina and around the boundary between the media and the adventitia at the left of the image. (H&E stain.)

If Takayasu arteritis has caused persistent renal artery stenosis, the renal cortex typically will have a characteristic pattern of ischemic atrophy. The cortical tubulointerstitial compartment is markedly reduced in volume by shrinkage of the tubules resulting in what is called "endocrinization" because the tubules take on the appearance of endocrine acini. Unlike arterionephrosclerosis, there is little or no interstitial fibrosis or interstitial inflammatory cell infiltration. Glomeruli are crowded together because of the reduction in the tubulointerstitial volume.

There are reports of various glomerular lesions in patients with Takayasu arteritis (99–103). The diverse patterns of glomerular disease observed at least raise the possibility that they are coincidental or idiosyncratic occurrences. The most commonly described lesion is mesangial hypercellularity (103) and the most distinctive lesion that has been reported is nodular mesangial matrix expansion and mesangiolysis (99).

No definitive data are available on the evaluation of the arteritis of Takayasu arteritis by immunohistology or electron microscopy.

## Etiology and Pathogenesis

The etiology and pathogenesis of Takayasu arteritis are unknown (90,91). A relationship with tuberculosis has been suspected but not substantiated. As with many idiopathic inflammatory diseases, current pathogenic theories incriminate genetically determined autoimmune mechanisms, but no data strongly support this possibility. One theory of pathogenesis proposes that the inflammation begins in the adventitia and secondarily extends into the media and intima (91); however, there is inconclusive evidence to support this.

## Differential Diagnosis

The differential diagnosis of renal involvement by Takayasu arteritis includes atherosclerotic disease, fibromuscular dysplasia, and GCA. The histologic features of Takayasu arteritis are indistinguishable from those of GCA (1,92); thus, clinical data, especially patient age, presence or absence of polymyalgia rheumatica, and the distribution of the vasculitis, must be taken into consideration when deciding whether chronic granulomatous and sclerosing aortitis and arteritis are more likely to represent GCA or Takayasu arteritis. Arteriosclerotic changes in arteries can mimic the sclerotic changes of Takayasu arteritis and giant cell arteritis, especially in older patients many years after the initial active phase of the disease. Extensive fragmentation of the internal and external elastica rather than extensive

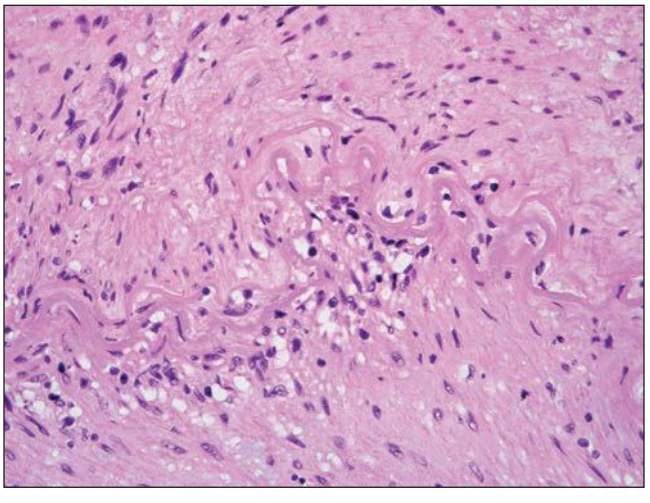

**FIGURE 17.18 Higher magnification of the specimen illustrated in Figure 17.15 showing infiltration of mononuclear leukocytes around the boundary between the intima and media.** The internal elastic lamina can be seen as a corrugated hyaline line running horizontally through the center of the field of view. (H&E stain.)

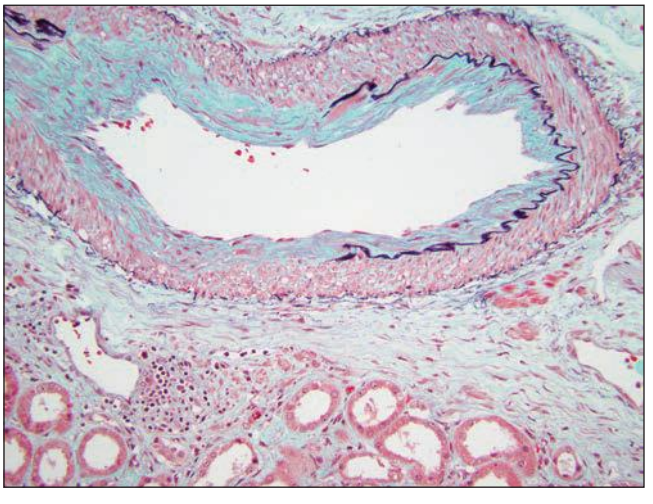

**FIGURE 17.19** Interlobar artery from the same nephrectomy specimen illustrated in Figures 17.14 to 17.16 showing modest fibrotic thickening of the intima and extensive focal destruction of the internal elastic lamina. This indicates earlier arteritis that was quiescent at the time of nephrectomy.

replication of the internal elastica supports arterial sclerosis caused by arteritis versus hypertension or aging. Syphilitic aortitis and arteritis can mimic Takayasu arteritis and should be excluded by clinical and serologic data.

### Clinical Course, Prognosis, and Therapy

The most dangerous clinical outcome is central nervous system involvement caused by involvement of the carotid or vertebral arteries resulting in transient ischemic attacks, blindness, amaurosis fugax, or stroke (91). Up to half of patients develop aortic insufficiency. Renal artery stenosis with renovascular hypertension develops in 30% to 75% of patients (91,94).

Corticosteroids are the standard first-line therapy for Takayasu arteritis (91). More than half of patients will enter remission with steroid therapy although half will relapse with discontinuation of treatment. Addition of methotrexate to the regimen allows most of these patients to be tapered off of steroids. If disease still persists, additional immunosuppressive agents may be required, such as cyclophosphamide. Approximately 20% of patients will have only one episode of disease that does not recur after treatment. The remainder requires repeated therapy.

Control of renovascular hypertension is an important element of management (9,91,103). However, reduction of hypertension should be approached cautiously because rapid reduction may cause organ damage, including renal failure, because of reduced perfusion through stenotic arteries. Hypertension that is recalcitrant to medical management may require angioplasty or surgical intervention. This should be carried out during a period of disease quiescence. Surgical intervention with bypass or reconstruction probably is superior to angioplasty (88). Autotransplantation may be required in severe cases (104).

## GIANT CELL ARTERITIS

### Clinical Presentation

Giant cell arteritis often affects the aorta and its major branches (1). It has a predilection for the extracranial branches of the carotid artery, but it can affect arteries in almost any organ. GCA is primarily confined to arteries with elastic laminae. For example, Wilkinson and Russell (105) noted that involvement of the internal carotid and vertebral arteries ended at the site where they penetrated the dura mater, which is also the site at which the elastic laminae end. GCA also has been called temporal arteritis, but this designation is not appropriate because all patients do not have temporal artery involvement, and patients with other types of vasculitis, such as PAN, GPA, and MPA, can have involvement of the temporal arteries (1). This latter problem accounts for some of the reported examples of necrotizing glomerulonephritis associated with "temporal arteritis," which in fact are examples of small-vessel vasculitis, such as GPA or MPA, affecting the temporal artery.

More than 95% of patients with GCA are older than 50 years of age, and more than 50% have polymyalgia rheumatica, which is characterized by stiffness and aching in the neck and the proximal muscles of the shoulders and hips (91,105–107). The mean age at onset is approximately 75 years old. The disease is most common in persons of northern European ancestry. The incidence of GCA in the population older than 50 years of age is approximately 17/100,000 in northern Europe and northern United States, but only approximately 1/100,000 in southern United States. Women are affected twice as often as men. The most common initial symptom is headache. Approximately one half of patients have temporal artery tenderness, nodularity, or decreased pulsation. Involvement of other extracranial branches of the carotid artery causes blindness, deafness, jaw claudication, and tongue dysfunction. Involvement of arteries to the extremities causes claudication and reduced pulses.

Clinically significant renal disease is rare in patients with GCA. In a study by Klein et al. (108) of 248 patients with GCA, 34 patients had evidence of involvement of arteries in addition to the arteries of the head and neck. Six patients had hematuria with erythrocyte casts that correlated with systemic disease activity, but none had proteinuria or renal insufficiency. Three of the thirty-four patients died and underwent postmortem examination. All three had involvement of intrarenal arteries by GCA. None of the patients had glomerulonephritis. Although the number of patients studied is small, this evaluation suggests that the proportion of patients with systemic GCA involving the renal arteries is high, even though one usually sees no clinical manifestations of renal dysfunction. This study also demonstrates that involvement of renal arteries by GCA in the absence of glomerulonephritis can cause hematuria. Vanderschueren et al. (109) detected microscopic hematuria with dysmorphic red blood cells in 48% of 42 patients with GCA. None of the patients with hematuria had hypertension, renal insufficiency, or significant proteinuria, and only one had red blood cell casts. The absence of these common features of glomerulonephritis suggests that the hematuria was caused directly by the arteritis rather than by glomerulonephritis. The hematuria cleared in 71% of patients following corticosteroid treatment.

In a review of organ involvement in patients with arteritis in the temporal arteries, Sonnenblick et al. (8) compiled anecdotal reports of glomerulonephritis in patients with temporal artery vasculitis. As noted earlier, not only GCA but also other forms of vasculitis can affect temporal arteries, including forms of vasculitis known to cause glomerulonephritis. For example, Canton et al. (110) reported a patient who had necrotizing inflammation of the temporal artery in a temporal artery biopsy and pauci-immune necrotizing and crescentic glomerulonephritis and necrotizing arteritis in a renal biopsy. This case was published as "renal failure in temporal arteritis." Such patients probably do not have GCA, but more likely have necrotizing vasculitis, such as GPA or MPA. Other reports of "giant cell arteritis" with extensive involvement of renal parenchymal arteries have atypical features that are difficult to categorize, such as the case described by Elling and Kristensen (111) with extensive necrotizing arteritis and arteriolitis with giant cells in a patient with polymyalgia rheumatica. Even manifestations of polymyalgia rheumatica are not enough to warrant a diagnosis of GCA because similar symptoms occasionally occur in patients with ANCA small-vessel vasculitis.

In summary, typical GCA can affect the arteries of the kidneys. This rarely causes clinically significant disease although microscopic hematuria occurs in more than a third of untreated patients.

### Pathologic Findings

#### Gross Pathology

The characteristic but nonspecific gross abnormality in involved arteries is segmental firm thickening of the vessel wall that gradually tapers into more normal segments. Vessel lumens

are narrowed or even obliterated at the sites of mural thickening. When it can be visualized grossly, the endothelial surface lacks the yellowish plaques and ulcerations typical for atherosclerosis, unless there is concurrent atherosclerosis. Aneurysm formation is rare except in the aorta (108). The main renal artery is affected most often, but any of the extrarenal or intrarenal arteries may be affected.

## Light Microscopy

The lesions of GCA, as is true of all vasculitides, are segmental (106,108,112). The lesions appear to begin in the media or adventitia but extend to involve the intima, thus producing panarteritis. The intramural inflammatory infiltrates consist primarily of mononuclear leukocytes, with lymphocytes and macrophages predominating (Figs. 17.20 to 17.22). Multinucleated giant cells are present in approximately half to two thirds of specimens (Fig. 17.23). They are more often absent after corticosteroid treatment and in the chronic sclerotic phase of the disease. Eosinophils occasionally are prominent (112). Focal fibrinoid necrosis may occur, but it is usually mild. If necrosis is severe, the possibility of vasculitis other than GCA should be considered, for example, GPA, MPA, or PAN. The granulomatous inflammation with giant cells often is centered on the internal elastic lamina and less often occurs on the external elastic lamina (112). Involved elastica often has fragmentation (see Fig. 17.21), and the fragments sometimes can be identified within the cytoplasm of macrophages, especially multinucleated giant cells. Medial as well as intimal expansion causes luminal narrowing. Unlike in the necrotizing vasculitides, thrombosis is uncommon.

Although GCA often is thought of as a disease that involves principally the head and neck and major branches from the aorta, pathologic evidence for GCA can be observed in many organs, including the female genital tract (113), breast (114), heart (106,112), lungs (112), thyroid (115), liver (116), small bowel (117), gallbladder (118), pancreas (119), esophagus (111), prostate (111), bone marrow (120), spinal cord (121), peripheral nerves (122), and kidneys (106,108,123–125). Not only large and medium arteries but also small parenchymal arteries can be involved by GCA, including arteries small enough to be encountered in renal biopsy specimens (123,124) (see Fig. 17.22). Renal-limited GCA with typical pathologic changes in renal arteries may occur with no evidence for systemic involvement (123,125).

As with Takayasu arteritis, if GCA results in persistent renal artery stenosis, the renal cortex will have the characteristic pattern of ischemic atrophy caused by renal artery stenosis (see Fig. 17.23). Glomeruli are crowded together because of the reduction in the tubulointerstitial volume and tubules shrink resulting in "endocrinization."

Secondary AA amyloidosis can be a complication of GCA and may prompt renal biopsy (126,127). The pathologic findings in the renal tissue are those of typical amyloidosis.

Temporal artery biopsy often is performed when a diagnosis of GCA is suspected, and results may show pathologic evidence of GCA even in the absence of clinical evidence of temporal artery involvement (91,111). For adequate sensitivity, the biopsy specimen should be 1 to 3 cm long and should be sampled at multiple levels because of the segmental distribution of GCA. The diagnostic sensitivity is reduced by corticosteroid treatment (128). Overall, approximately a half to two thirds of temporal artery biopsies in patients thought to have

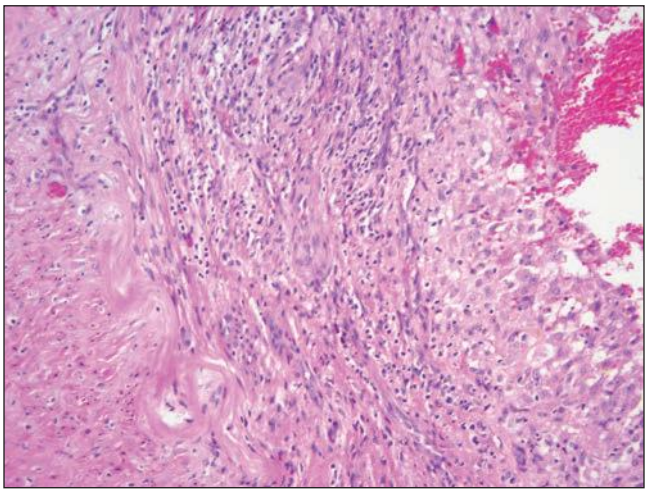

**FIGURE 17.20 Arteritis affecting the main renal artery in a nephrectomy specimen from a patient with giant cell arteritis.** The narrowed lumen is on the right, and the muscularis is on the left. The intima is markedly thickened and infiltrated by mononuclear leukocytes. (H&E.)

GCA show giant cells (91,112). However, other features often are present that are consistent with a diagnosis of GCA, such as intimal thickening with fragmentation of (gaps in) the internal elastic lamina (91). Together with the appropriate clinical findings, such changes allow a confident diagnosis of GCA.

## Immunofluorescence and Electron Microscopy

Limited reports of immunofluorescence microscopy of kidneys affected with GCA describe no glomerular, arterial, or tubulointerstitial staining for immunoglobulins or complement (123,124).

Electron microscopy is of little value in the pathologic evaluation of GCA. In essence, the same features are seen at the ultrastructural level that are seen by light microscopy. In an ultrastructural analysis of one specimen, Shiiki et al. (129)

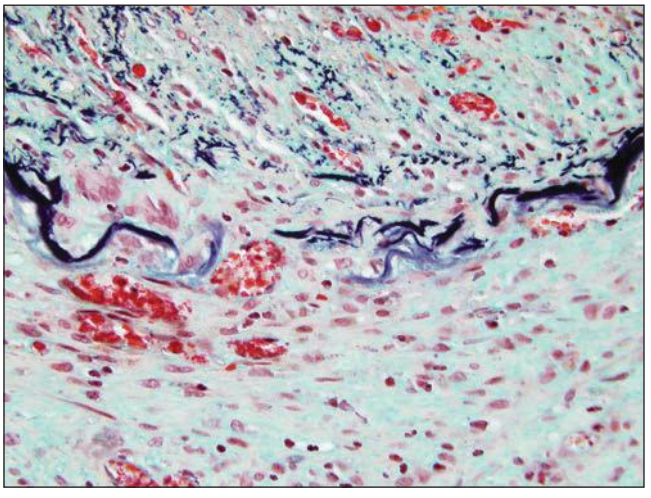

**FIGURE 17.21** Elastic tissue stain of the same renal artery shown in Figure 17.18 showing marked fragmentation of the internal elastic lamina. The thickened intima is above the elastica.

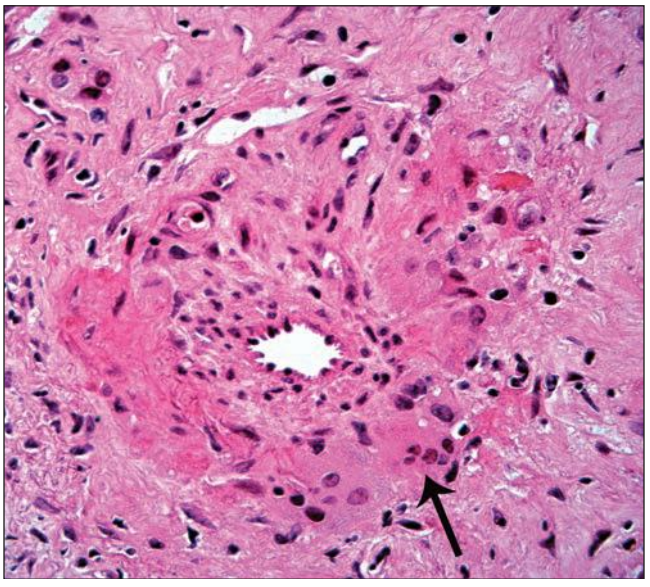

**FIGURE 17.22 Granulomatous inflammation of an arcuate artery in the kidney biopsy from a patient with giant cell arteritis.** The demarcation between intima and muscularis is obliterated by fibrosis and infiltrating macrophages, including a few multinucleated giant cells (*arrow*). (H&E.)

observed macrophages and giant cells closely opposed to injured smooth muscle cells in the media.

## Etiology and Pathogenesis

The etiology and pathogenesis of GCA are unknown (91, 129–132). Based in part on the histologic appearance of the vascular inflammation, cell-mediated immunity has been incriminated most often. This concept has been supported further by immunophenotypic analysis of the infiltrating leukocytes (123,129–132). For example, in a lymphocyte phenotyping analysis of 24 temporal artery biopsy specimens from patients with GCA, Cid et al. (130) determined that the infiltrating leukocytes were predominantly CD4 T lymphocytes and HLA-DR–expressing macrophages. Antigen-presenting interdigitating reticulum cells were observed in many specimens. Interleukin-2 receptor expression was observed in 88% of specimens from patients with up to 4 days of corticosteroid treatment, but in only 14% of patients who had been treated for longer, even though the cellular composition of the infiltrates had not changed. Cid et al. (130) concluded that the presence of CD4 lymphocytes with activation markers (interleukin-2 receptors) and associated interdigitating reticulum cells suggests an autoimmune cell-mediated immune response to an autoantigen in the vessel wall. The disruption of the elastic laminae, the concentration of inflammatory infiltrates (especially the macrophages and giant cells) at the sites of disrupted elastic laminae, and the predilection for involvement of arteries with elastic laminae have raised the possibility of an autoimmune response to the elastica material. Papaioannou et al. (118), however, were unable to demonstrate an in vitro cell-mediated or antibody-mediated response to elastin or other arterial wall antigens derived from the arteries of patients with GCA. The presence of activated T cell and macrophages also is consistent with a cell-mediated response to a foreign antigen that has become planted in the vessel wall or an innate inflammatory response, for example, mediated by activation of toll-like receptors by microbial products.

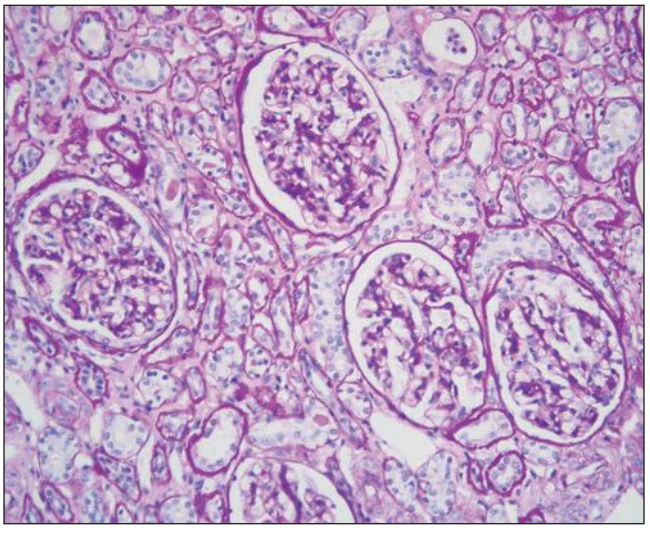

**FIGURE 17.23 Renal cortex from the same nephrectomy specimen with giant cell arteritis shown in Figures 17.18 and 17.19 showing classic changes of renal artery stenosis.** The tubulointerstitial compartment is diminished, resulting in crowding of glomeruli. The tubules show "endocrinization" and resemble small acini rather than tubules. There is minimal interstitial fibrosis and inflammation. (PAS.)

Weyand et al. (132) have proposed that the inflammation of GCA begins in the adventitia with the interaction of CD4-positive T lymphocytes with stimulated dendritic cells. However, the etiology of this stimulation has not been identified. According to this hypothesis, the inflammation then extends into the media and intima where induction of oxidative injury and inflammatory cytokines causes the vessel wall injury. For example, macrophage-derived growth factors could stimulate the intimal proliferation that results in vascular stenosis (131).

## Differential Diagnosis

The American College of Rheumatology proposes that a patient can be classified as having GCA if he or she has any three of the following criteria: (a) artery biopsy from any site that shows arteritis with infiltration of predominantly mononuclear leukocytes or granulomatous inflammation (giant cells are not required), (b) temporal artery tenderness or decreased pulsation unrelated to arteriosclerosis, (c) new onset of headache, (d) elevated erythrocyte sedimentation rate, and (e) age at disease onset 50 years or older (106). The Chapel Hill Nomenclature System defines GCA as arteritis, often granulomatous, usually affecting the aorta and/or its major branches, with a predilection for the branches of the carotid and vertebral arteries, which often involves the temporal artery, and with onset usually in patients older than 50 and often associated with polymyalgia rheumatica (1). A major clinical and pathologic differential consideration is Takayasu arteritis, which can produce all the clinical and pathologic manifestations of GCA with the possible exception of polymyalgia rheumatica. Histologically, GCA and Takayasu arteritis cannot be distinguished (1,92). In fact, the feature that is most helpful

in differentiating GCA from Takayasu arteritis is the age of the patient (1). Thus, a patient who has clinical or pathologic features consistent with GCA or Takayasu arteritis who is older than 50 years of age should be considered to have GCA rather than Takayasu arteritis, and a patient who is younger than 50 years of age should be considered to have Takayasu arteritis.

Especially given the older age of patients with GCA, a major differential consideration is arteriosclerotic and atherosclerotic arterial disease. Pathologically, the presence of extensive replication of elastica in the intima supports the presence of arteriosclerosis, atheromatous changes support atherosclerosis, and fragmentation and mononuclear leukocyte or granulomatous mural inflammation support GCA.

Temporal artery inflammation is not diagnostic for GCA because other forms of systemic vasculitis can affect the temporal arteries, including PAN, MPA, and GPA. GPA can be particularly problematic if the inflammation contains multinucleated giant cells. Although focal fibrinoid material occasionally occurs in GCA, the presence of extensive fibrinoid necrosis should raise concern for the presence of a variant of vasculitis other than GCA, such as PAN, MPA, or GPA.

## Clinical Course, Prognosis, and Therapy

Corticosteroids are the mainstay of therapy for GCA (91,133). Prednisone at a dose of 40 to 60 mg for 2 to 4 weeks followed by tapering usually is adequate to induce clinical remission (91). Approximately one half to two thirds of patients experience one or more relapses that require retreatment. Serologic measurement of the erythrocyte sedimentation rate is useful in monitoring response to treatment and maintenance of remission. Untoward complications from the steroid therapy occur in approximately a quarter of patients and are a major cause for morbidity. GCA patients have frequent relapses, especially when daily doses of corticosteroids are reduced to less than 10 mg. Relapses and poorly responding disease require increased corticosteroids, and some patients need long-term corticosteroid therapy at moderate to high doses. Corticosteroid-sparing therapy that has been used includes methotrexate, azathioprine, dapsone, hydroxychloroquine, cyclophosphamide, and TNF-α blockers, with variable results (133).

An uncommon but life-threatening complication is aortic aneurysm or dissection caused by giant cell aortitis (91). There are rare reports of acute renal failure caused by renal GCA, which is quickly and completely reversed by corticosteroid therapy (123,124).

## REFERENCES


1. Jennette JC, Falk RJ, Bacon PA, et al. 2012 revised International Chapel Hill Consensus Conference Nomenclature of Vasculitides. *Arthritis Rheum* 2013;65:1–11.
2. Jennette JC, Falk RJ, Andrassy K, et al. Nomenclature of systemic vasculitides. Proposal of an international consensus conference. *Arthritis Rheum* 1994;37:187.
3. Jennette JC, Falk RJ. The pathology of vasculitis involving the kidney. *Am J Kidney Dis* 1994;24:130.
4. Jennette JC, Falk RJ. Small-vessel vasculitis. *N Engl J Med* 1997;337:1512.
5. Arkin A. A clinical and pathological study of periarteritis nodosa: a report of five cases, one histologically healed. *Am J Pathol* 1930;6:401.
6. Naoe S, Takahashi K, Masuda H, et al. Kawasaki disease: with particular emphasis on arterial lesions. *Acta Pathol Jpn* 1991;41:785.
7. Guillevin L, Lhote F. Distinguishing polyarteritis nodosa from microscopic polyangiitis and implications for treatment. *Curr Opin Rheumatol* 1995;7:20.
8. Sonnenblick M, Nesher G, Rosin A. Nonclassical organ involvement in temporal arteritis. *Semin Arthritis Rheum* 1989;19:183.
9. Munir I, Uflacker R, Milutinovic J. Takayasu's arteritis associated with intrarenal vessel involvement. *Am J Kidney Dis* 2000;35:950.
10. Lagneau P, Michel JB. Renovascular hypertension and Takayasu's disease. *J Urol* 1985;134:876.
11. Jennette JC. Implications for pathogenesis of patterns of injury in small- and medium-sized vessel vasculitis. *Cleve Clin J Med* 2002;69(Suppl 2):SII33.
12. Jennette JC. Antineutrophil cytoplasmic autoantibody-associated diseases: a pathologist's perspective. *Am J Kidney Dis* 1991;18:164.
13. Kussmaul A, Maier R. Uber eine bisher nicht beschreibene eigenthumliche Arterienerkrankung (Periarteritis nodosa), die mit Morbus Brightii und rapid fortschreitender allgemeiner Muskellahmung einhergeht. *Dtsch Arch Klin Med* 1866;1:484.
14. Willan R. *On Cutaneous Diseases*. Philadelphia: Kimber & Conrad, 1808:452.
15. Rokitansky K. Ueber einige der wichtigsten Krankheiten der Arterien. *Denkschriften der Kais Akad der Wissensch Besonders Abgedruckt* 1852;4:49.
16. Ferrari E. Ueber Polyarteritis acuta nodosa (sogenannte Periarteritis nodosa), und ihre Beziehungen zur Polymyositis and Polyneuritis acuta. *Beitr Pathol Anat* 1903;34:350.
17. Dickson WEC. Polyarteritis acuta nodosa and periarteritis nodosa. *J Pathol Bacteriol* 1908;12:31.
18. Churg J, Strauss L. Allergic granulomatosis, allergic angiitis and periarteritis nodosa. *Am J Pathol* 1951;27:277.
19. Godman GC, Churg J. Wegener's granulomatosis: pathology and review of the literature. *Arch Pathol Lab Med* 1954;58:533.
20. Wohlwill F. On the only microscopically recognizable form of periarteritis nodosa. *Virchows Arch Pathol Anat Physiol Klin Med* 1923;246:377–411.
21. Gruber GB. Zur Frage der Periarteriitis nodosa, mit besonderer Berucksichtigung der Gallenblasen-und Nieren-Beteiligung. *Virchows Arch Pathol Anat Physiol Klin Med* 1925;258:441.
22. Davson J, Ball J, Platt R. The kidney in periarteritis nodosa. *Q J Med* 1948;17:175.
23. Zeek PM, Smith CC, Weeter JC. Studies on periarteritis nodosa. III. The differentiation between the vascular lesions of periarteritis nodosa and of hypersensitivity. *Am J Pathol* 1948;24:889.
24. Zeek PM. Periarteritis nodosa: a critical review. *Am J Clin Pathol* 1952;22:777.
25. Heptinstall RH. Polyarteritis (periarteritis) nodosa, Wegener's syndrome, and other forms of vasculitis. In: *Pathology of the Kidney*, 4th ed. Boston: Little, Brown and Company, 1992:1100.
26. Guillevin L, Lhote F, Amouroux J, et al. Antineutrophil cytoplasmic antibodies, abnormal angiograms and pathological findings in polyarteritis nodosa and Churg-Strauss syndrome: Indications for the classification of vasculitides of the polyarteritis Nodosa Group. *Br J Rheumatol* 1996;35:958.
27. Klinger H. Grenzformen der Periarteritis nodosa. *Frankfurt Ztschr Pathol* 1931;42:455.
28. Wegener F. Uber eine eigenartige rhinogene Granulomatose mit besonderer Beteiligung des Arterien systems under der Nieren. *Beitr Pathol Anat* 1939;102:36.
29. Falk RJ, Gross WL, Guillevin L, et al. "Granulomatosis with polyangiitis (Wegener's)": An alternative name for "Wegener's granulomatosis". A joint proposal of the American College of Rheumatology, the American Society of Nephrology, and the European League Against Rheumatism. *Ann Rheum Dis* 2011;70:704, *J Am Soc Nephrol* 2011;22:587–588, *Arthritis Rheum* 2011;63:863–864.
30. Carrington CB, Liebow A. Limited forms of angiitis and granulomatosis of Wegener's type. *Am J Med* 1966;41:497.
31. Lhote F, Guillevin L. Polyarteritis nodosa, microscopic polyangiitis, and Churg-Strauss syndrome: clinical aspects and treatment. *Rheum Dis Clin North Am* 1995;21:911.
32. Kirkland GS, Savige JA, Wilson D, et al. Classical polyarteritis nodosa and microscopic polyarteritis with medium vessel involvement: a comparison of the clinical and laboratory features. *Clin Nephrol* 1997;47:176.
33. Kawasaki T. Acute febrile mucocutaneous syndrome with lymphoid involvement with specific desquamation of the fingers and toes in children. *Arerugi* 1967;16:178.

34. Tanaka N, Sekimoto K, Naoe S. Kawasaki disease: relationship with infantile periarteritis nodosa. *Arch Pathol Lab Med* 1976;100:81.
35. Kawasaki T. Clinical signs and symptoms of mucocutaneous lymph node syndrome (Kawasaki disease). *Jpn J Med Sci Biol* 1979;32:237.
36. Tanaka N, Naoe S, Kawasaki T. Pathological study on autopsy cases of mucocutaneous lymph node syndrome. *J Jpn Red Cross Century Hosp* 1971;2:85.
37. Landing BH, Larson EJ. Are infantile periarteritis nodosa with coronary artery involvement and fatal mucocutaneous lymph node syndrome the same? Comparison of 20 patients from North America with patients from Hawaii and Japan. *Pediatrics* 1977;59:651.
38. Churg J. Large vessel vasculitis. *Clin Exp Immunol* 1993;93(Suppl 1):11.
39. Savory WS. Case of a young woman in whom the main arteries of both upper extremities and of the left side of the neck were throughout completely obliterated. *Med Chir Trans* 1856;39:205.
40. DiGiacomo V. A case of Takayasu's disease occurred over two hundred years ago. *Angiology* 1984;35:750.
41. Takayasu M. Case with unusual changes of the central vessels in the retina. *Acta Soc Ophthalmol* 1908;12:554.
42. Hutchinson J. Diseases of the arteries: on a peculiar form of thrombotic arteries of the aged which is sometimes productive of gangrene. *Arch Surg* 1890;1:323.
43. Gilmour JR. Giant-cell chronic arteritis. *J Pathol* 1941;53:263.
44. Paulley JW. Anarthritic rheumatoid diseases. *Lancet* 1956;2:946.
45. Hamrin B. Polymyalgia arteritica. *Acta Med Scand Suppl* 1972;533:1.
46. Arend WP, Michel BA, Bloch DA, et al. The American College of Rheumatology 1990 criteria for the classification of Takayasu arteritis. *Arthritis Rheum* 1990;33:1129.
47. Agard C, Mouthon L, Mahr A, et al. Microscopic polyangiitis and polyarteritis nodosa: how and when do they start? *Arthritis Rheum* 2003;49:709.
48. Handa R, Wali JP, Gupta SD, et al. Classical polyarteritis nodosa and microscopic polyangiitis: a clinicopathologic study. *J Assoc Physicians India* 2001;49:314.
49. Mahr A, Guillevin L, Poissonnet M, et al. Prevalences of polyarteritis nodosa, microscopic polyangiitis, Wegener's granulomatosis, and Churg-Strauss syndrome in a French urban multiethnic population in 2000: a capture-recapture estimate. *Arthritis Rheum* 2004;51:92.
50. Jennette JC, Milling DM, Falk RJ. Vasculitis affecting the skin: a review. *Arch Dermatol* 1994;130:899.
51. Tureyen A, Kayacetin E, Saritas U. Spontaneous kidney rupture due to polyarteritis nodosa presenting as acute abdomen. *Clin Rheumatol* 2002;21:539.
52. Rose GA, Spencer H. Polyarteritis nodosa. *Q J Med* 1957;26:43.
53. Minardi D, Dessi-Fulgheri P, Sarzani R, et al. Massive spontaneous perirenal hematoma and accelerated hypertension in a patient with polyarteritis nodosa. *Urol Int* 2003;70:227.
54. Agarwal A, Bansal M, Pandey R, et al. Bilateral subcapsular and perinephric hemorrhage as the initial presentation of polyarteritis nodosa. *Intern Med* 2012;51:1073–1076.
55. Smith DL, Wernick R. Spontaneous rupture of a renal artery aneurysm in polyarteritis nodosa: critical review of the literature and report of a case. *Am J Med* 1989;87:464.
56. Bakkaloglu SA, Ekim M, Tumer N, et al. Severe renal impairment in the case of classic polyarteritis nodosa. *Pediatr Nephrol* 2001;16:148.
57. Coblyn JS, McCluskey RT. Case records of the Massachusetts General Hospital. Weekly clinicopathological exercises. Case 3—2003. A 36-year-old man with renal failure, hypertension, and neurologic abnormalities. *N Engl J Med* 2003;348:333.
58. Gower RG, Sausker WF, Kohler PF, et al. Small vessel vasculitis caused by hepatitis B virus immune complexes: small vessel vasculitis and HBsAG. *J Allergy Clin Immunol* 1978;62:222.
59. Janssen HL, van Zonneveld M, van Nunen AB, et al. Polyarteritis nodosa associated with hepatitis B virus infection: the role of antiviral treatment and mutations in the hepatitis B virus genome. *Eur J Gastroenterol Hepatol* 2004;16:801.
60. Guillevin L. Treatment of classic polyarteritis nodosa in 1999. *Nephrol Dial Transplant* 1999;14:2077.
61. Saadoun D, Terrier B, Semoun O, et al. Hepatitis C virus-associated polyarteritis nodosa. *Arthritis Care Res (Hoboken)* 2011;63:427–435.
62. Seshan S, Jennette JC. Renal disease in systemic lupus erythematosus with emphasis on classification of lupus glomerulonephritis: advances and implications. *Arch Pathol Lab Med* 2009;133:233–248.
63. Mutabagani KH, Samad WL, Henry ML. Successful repair of a large renal artery aneurysm in a child with polyarteritis nodosa using renal autotransplantation techniques. *Saudi Med J* 2003;24(Suppl):S56.
64. Newburger JW, Fulton DR. Kawasaki disease. *Curr Opin Pediatr* 2004;16:508.
65. Burns JC, Glode MP. Kawasaki syndrome. *Lancet* 2004;364:533.
66. Kuo HC, Yang KD, Chang WC, et al. Kawasaki disease: an update on diagnosis and treatment. *Pediatr Neonatol* 2012;53:4–11.
67. Gribetz D, Landing BH, Larson EJ. Kawasaki disease: mucocutaneous lymph node syndrome (MCLS). In: Churg A, Churg J, eds. *Systemic Vasculitides*. New York: Igaku-Shoin, 1991:257.
68. Amano S, Hazama F, Hamashima Y. Pathology of Kawasaki disease. I. Pathology and morphogenesis of the vascular changes. *Jpn Circ J* 1979;43:633.
69. Amano S, Hazama F, Hamashima Y. Pathology of Kawasaki disease. II. Distribution and incidence of the vascular lesions. *Jpn Circ J* 1979;43:741.
70. Salcedo JR, Greenberg L, Kapur S. Renal histology of mucocutaneous lymph node syndrome (Kawasaki disease). *Clin Nephrol* 1988;29:47.
71. Foster BJ, Bernard C, Drummond KN. Kawasaki disease complicated by renal artery stenosis. *Arch Dis Child* 2000;83:253.
72. Subramaniam R, Lama T, Chong CY. Pelviureteric junction obstruction as sequelae of Kawasaki disease. *Pediatr Surg Int* 2004;20:553.
73. Fujiwara H, Hamashima Y. Pathology of the heart in Kawasaki disease. *Pediatrics* 1978;61:100.
74. Hirose S, Hamashima Y. Morphological observations on the vasculitis in the mucocutaneous lymph node syndrome: a skin biopsy study of 27 patients. *Eur J Pediatr* 1978;129:17.
75. Landing BH, Larson EJ. Pathological features of Kawasaki disease (mucocutaneous lymph node syndrome). *Am J Cardiovasc Pathol* 1987;1:218.
76. Jennette JC, Sciarrotta J, Takahashi H, et al. Predominance of monocytes and macrophages in the inflammatory infiltrates of acute Kawasaki disease arteritis. *Pediatr Res* 2002;53:94.
77. Rowley AH, Shulman ST, Mask CA, et al. IgA plasma cell infiltration of proximal respiratory tract, pancreas, kidney, and coronary artery in acute Kawasaki disease. *J Infect Dis* 2000;182:1183–1191.
78. Bell DM, Brink EW, Nitzkin JL, et al. Kawasaki syndrome: Description of two outbreaks in the United States. *N Engl J Med* 1981;304:1568.
79. Rider LG, Wener MH, French J, et al. Autoantibody production in Kawasaki syndrome. *Clin Exp Rheumatol* 1993;11:445.
80. Furukawa S, Matsubara T, Jujoh K, et al. Reduction of peripheral blood macrophages/monocytes in Kawasaki disease by intravenous gammaglobulin. *Eur J Pediatr* 1990;150:43.
81. Leung DY, Geha RS, Newburger JW, et al. Two monokines, interleukin 1 and tumor necrosis factor, render cultured vascular endothelial cells susceptible to lysis by antibodies circulating during Kawasaki syndrome. *J Exp Med* 1986;164:1958.
82. Leung DY, Collins T, Lapierre LA, et al. Immunoglobulin M antibodies present in the acute phase of Kawasaki syndrome lyse cultured vascular endothelial cells stimulated by gamma interferon. *J Clin Invest* 1986;77:1428.
83. Leung DY, Cotran RS, Kurt-Jones E, et al. Endothelial cell activation and high interleukin-1 secretion in the pathogenesis of acute Kawasaki disease. *Lancet* 1989;2:1298.
84. Grunebaum E, Blank M, Cohen S, et al. The role of anti-endothelial cell antibodies in Kawasaki disease: in vitro and in vivo studies. *Clin Exp Immunol* 2002;130:233.
85. Kaneko M, Ono T, Matsubara T, et al. Serological identification of endothelial antigens predominantly recognized in Kawasaki disease patients by recombinant expression cloning. *Microbiol Immunol* 2004;48:703.
86. Savage CO, Tizard J, Jayne D, et al. Antineutrophil cytoplasm antibodies in Kawasaki disease. *Arch Dis Child* 1989;64:360.
87. Nash MC, Shah V, Reader JA, et al. Anti-neutrophil cytoplasmic antibodies and anti-endothelial cell antibodies are not increased in Kawasaki disease. *Br J Rheumatol* 1995;34:882.
88. Takahashi K, Oharaseki T, Wakayama M, et al. Histopathological features of murine systemic vasculitis caused by *Candida albicans* extract: an animal model of Kawasaki disease. *Inflamm Res* 2004;53:72.

89. Duong TT, Silverman ED, Bissessar MV, et al. Superantigenic activity is responsible for induction of coronary arteritis in mice: an animal model of Kawasaki disease. *Int Immunol* 2003;15:79.
90. Lie JT. Takayasu arteritis. In: Churg J, ed. *Systemic Vasculitides*. Tokyo: Igaku-Shoin, 1991:59.
91. Maksimowicz-McKinnon K, Hoffman GS. Large-vessel vasculitis. *Semin Respir Crit Care Med* 2004;25:569.
92. Hall S, Barr W, Lie JT, et al. Takayasu arteritis: a study of 32 North American patients. *Medicine* 1985;64:89.
93. Lupi-Herrera E, Sanchez-Torres G, Marcushamer J, et al. Takayasu's arteritis: clinical study of 107 cases. *Am Heart J* 1977;93:94.
94. Sharma BK, Jain S, Radotra BD. An autopsy study of Takayasu arteritis in India. *Int J Cardiol* 1998;66(Suppl 1):S85.
95. Sharma BK, Singh G, Sagar S. Malignant hypertension in northwest India: a hospital based study. *Jpn Heart J* 1994;35:601.
96. el Reshaid K, Varro J, al Duwairi Q, et al. Takayasu's arteritis in Kuwait. *J Trop Med Hyg* 1995;98:299.
97. Kieffer E, Piquois A, Bertal A, et al. Reconstructive surgery of the renal arteries in Takayasu's disease. *Ann Vasc Surg* 1990;4:156.
98. Bongard O, Schneider PA, Krahenbuhl B, et al. Transluminal angioplasty of the aorta, renal and mesenteric arteries in Takayasu arteritis: report of two cases. *Eur J Vasc Surg* 1992;6:567.
99. Cavatorta F, Campisi S, Trabassi E, et al. IgA nephropathy associated with Takayasu's arteritis: report of a case and review of the literature. *Am J Nephrol* 1995;15:165.
100. Lai KN, Chan KW, Ho CP. Glomerulonephritis associated with Takayasu's arteritis: report of three cases and review of literature. *Am J Kidney Dis* 1986;7:197.
101. Logar D, Rozman B, Vizjak A, et al. Arteritis of both carotid arteries in a patient with focal crescentic glomerulonephritis and anti-neutrophil cytoplasmic autoantibodies. *Br J Rheumatol* 1994;33:167.
102. Yoshimura M, Kida H, Saito Y, et al. Peculiar glomerular lesions in Takayasu's arteritis. *Clin Nephrol* 1985;24:120.
103. de Pablo P, García-Torres R, Uribe N, et al. Kidney involvement in Takayasu arteritis. *Clin Exp Rheumatol* 2007;25(1 Suppl 44):S10–S14.
104. Beale PG, Meyers KE, Thomson PD. Management of renal hypertension in children with Takayasu's arteritis using renal autografting or allograft transplantation in selected circumstances and total lymphoid irradiation. *J Pediatr Surg* 1992;27:836.
105. Wilkinson IM, Russell RW. Arteries of the head and neck in giant cell arteritis: a pathological study to show the pattern of arterial involvement. *Arch Neurol* 1972;27:378.
106. Hamilton CR Jr, Shelley WM, Tumulty PA. Giant cell arteritis: including temporal arteritis and polymyalgia rheumatica. *Medicine* 1971;50:1.
107. Hunder GG, Bloch DA, Michel BA, et al. The American College of Rheumatology 1990 criteria for the classification of giant cell arteritis. *Arthritis Rheum* 1990;33:1122.
108. Klein RG, Hunder GG, Stanson AW, et al. Large artery involvement in giant cell (temporal) arteritis. *Annu Rev Med* 1975;83:806.
109. Vanderschueren S, Depoot I, Knockaert DC, et al. Microscopic haematuria in giant cell arteritis. *Clin Rheumatol* 2002;21:373.
110. Canton CG, Bernis C, Paraiso V, et al. Renal failure in temporal arteritis. *Am J Nephrol* 1992;12:380.
111. Elling H, Kristensen IB. Fatal renal failure in polymyalgia rheumatica caused by disseminated giant cell arteritis. *Scand J Rheumatol* 1980;9:206.
112. Rochelle N, Allen NB. Giant cell arteritis. In: Churg A, Churg J, eds. *Systemic Vasculitides*. New York: Igaku-Shoin, 1991:43.
113. Lhote F, Mainguene C, Griselle-Wiseler V, et al. Giant cell arteritis of the female genital tract with temporal arteritis. *Ann Rheum Dis* 1992;51:900.
114. McKendry RJ, Guindi M, Hill DP. Giant cell arteritis (temporal arteritis) affecting the breast: Report of two cases and review of published reports. *Ann Rheum Dis* 1990;49:1001.
115. How J, Bewsher PD, Walker W. Giant-cell arteritis and hypothyroidism. *Br Med J* 1977;2:99.
116. Dickson ER, Maldonado JE, Sheps SG, et al. Systemic giant-cell arteritis with polymyalgia rheumatica, reversible abnormalities of liver function. *JAMA* 1973;224:1496.
117. Srigley JR, Gardiner GW. Giant cell arteritis with small bowel infarction: A case report and review of the literature. *Am J Gastroenterol* 1980;73:157.
118. Papaioannou CC, Hunder GG, McDuffie FC. Cellular immunity in polymyalgia rheumatica and giant cell arteritis: Lack of response to muscle or artery homogenates. *Arthritis Rheum* 1979;22:740.
119. Hamrin B, Jonsson N, Landberg T. Arteritis in polymyalgia rheumatica. *Lancet* 1941;1:397.
120. Enos WF, Pierre RV, Rosenblatt JE. Giant cell arteritis detected by bone marrow biopsy. *Mayo Clin Proc* 1981;56:381.
121. Engelke WD, Dorstelmann D. Highly located transverse lesion of the cord with quadriplegia caused by giant cell arteritis. *Fortschritte der Neurologie, Psychiatrie und Ihrer Grenzgebiete* 1979;47:91.
122. Merianos P, Smyrnis P, Tsomy K, et al. Giant cell arteritis of the median nerve simulating carpal tunnel syndrome. *Hand* 1983;15:249.
123. Medvedev G, Al Shamari AE, Copland MA, et al. Isolated renal giant cell arteritis. *Am J Kidney Dis* 2002;40:658.
124. Scarpioni R, Poisetti PG, Cristinelli L, et al. Isolated renal giant cell arteritis, not so rare a cause of renal failure? *Am J Kidney Dis* 2003;41:720.
125. Watanabe R, Nakaya I, Kudo H, et al. Isolated granulomatous renal arteritis: A variant form of giant cell arteritis with few macrophages. *Intern Med* 2010;49:191–194.
126. Monteagudo M, Vidal G, Andreu J, et al. Giant cell (temporal) arteritis and secondary renal amyloidosis: Report of 2 cases. *J Rheumatol* 1997;24:605.
127. Strasser F, Hailemariam S, Weinreich T, et al. Giant cell arteritis "causing" AA-amyloidosis with rapid renal failure. *Schweiz Med Wochenschr* 2000;130:1606.
128. Allison MC, Gallagher PJ. Temporal artery biopsy and corticosteroid treatment. *Ann Rheum Dis* 1984;43:416.
129. Shiiki H, Shimokama T, Watanabe T. Temporal arteritis: cell composition and the possible pathogenetic role of cell-mediated immunity. *Hum Pathol* 1989;20:1057.
130. Cid MC, Campo E, Ercilla G, et al. Immunohistochemical analysis of lymphoid and macrophage cell subsets and their immunologic activation markers in temporal arteritis: influence of corticosteroid treatment. *Arthritis Rheum* 1989;32:884.
131. Andersson R. Immunological studies in giant cell arteritis. *Baillieres Clin Rheumatol* 1991;5:405.
132. Weyand CM, Ma-Krupa W, Goronzy JJ. Immunopathways in giant cell arteritis and polymyalgia rheumatica. *Autoimmun Rev* 2004;3:46.
133. de Boysson H, Boutemy J, Creveuil C, et al. Is there a place for cyclophosphamide in the treatment of giant-cell arteritis? A case series and systematic review. *Semin Arthritis Rheum* 2013;43(1):105–112.

CHAPTER 18

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# Thrombotic Microangiopathies

Thrombotic microangiopathy (TMA) is the pathologic term for a condition characterized by microvascular changes including thrombosis in association with laboratory abnormalities of microangiopathic hemolytic anemia (MAHA) and thrombocytopenia. In addition to the two flagship diseases of TMA, hemolytic-uremic syndrome (HUS) and thrombotic thrombocytopenic purpura (TTP), there is a broad spectrum of other disorders and conditions with diverse etiology and overlapping clinical manifestations that can also present with TMA. Such diseases and conditions include systemic infections, autoimmune diseases, exposure to certain drugs, pregnancy/postpartum states, eclampsia-preeclampsia, HELLP (hemolytic anemia, elevated liver enzymes, and low platelets) syndrome, solid organ and hematopoietic stem cell transplantation (HSCT), various glomerulopathies, malignancies, malignant hypertension, scleroderma renal crisis, and radiation nephropathy. This chapter discusses all major forms of TMA including those of systemic sclerosis (SSc) with the exception of eclampsia-preeclampsia that are covered in Chapter 19.

## HISTORICAL BACKGROUND AND NOMENCLATURE

In 1925, Moschcowitz (1) published a case report of a 16-year-old girl who had a sudden onset of fever, anemia, and central nervous system involvement. After an episode of pulmonary edema, she lapsed into coma and died. She did not have renal failure, and clinical findings were limited to traces of albumin, hyaline casts, and granular casts in the urine. At autopsy, hyaline thrombi were present in the capillaries and terminal arterioles of the heart, liver, and kidneys. This report was followed by the observations that thrombocytopenia was an additional feature in Moschcowitz disease and that the thrombi were composed predominantly of platelets (2). Singer et al. (3) were the first to use the term *thrombotic thrombocytopenic purpura*. The term *thrombotic microangiopathy* was introduced in 1952 by Symmers (4) for the vascular lesions of TTP.

In 1955, Gasser et al. (5) coined the term *hemolytic uremic syndrome* to describe a syndrome encountered in five children that consisted of hemolytic anemia, thrombocytopenia, and acute renal failure. The patients had cortical necrosis of the kidneys, and cerebral symptoms were also present. HUS was considered a new syndrome distinct from TTP for the following reasons: (a) HUS affected mostly infants, whereas TTP was primarily recognized as a disease of adults; (b) all patients with HUS presented with acute renal failure, whereas renal involvement was not considered a characteristic feature of TTP; and (c) the dominating pathologic finding in HUS (renal cortical necrosis) was different from findings observed previously in TTP. However, renal cortical necrosis is present only in some patients with HUS, and the characteristic glomerular and arteriolar changes of HUS were subsequently described by Habib et al. in 1958 (6). Shortly thereafter, Habib's group proposed the term *thrombotic microangiopathy*, which they borrowed from Symmers (4), to consolidate the vascular lesions of HUS and TTP. Since the original description of HUS, it has become apparent that HUS may also be seen in adults and that the severity of renal involvement may vary from patient to patient. In addition to TMA, Symmers (4) also used the term *thrombotic microangiopathic hemolytic anemia* for the cases he described. The term has now been shortened to *microangiopathic hemolytic anemia* and is used to describe the special form of anemia in both TTP and HUS.

The first comprehensive description of TTP with a review of all published cases until 1966 was published by Amorosi and Ultmann (7). This paper establishes the diagnostic criteria for TTP consisting of a "pentad" of characteristic clinical and laboratory features, including fever, MAHA, thrombocytopenia, neurologic abnormalities, and renal failure, which occurred in 88% to 98% of the 271 patients. The next major milestone was the documentation that plasma exchange is a highly effective treatment for TTP improving mortality from 90% to 22% (7,8). Since the disease can be rapidly fatal, there is an urgency to initiate treatment, which led to the implementation of the current, less stringent clinical diagnostic criteria for TTP that includes only MAHA and thrombocytopenia with no alternative etiology (9,10). In contrast, HUS is currently defined by the diagnostic "triad" of MAHA, thrombocytopenia, and renal impairment in the absence of associated diseases and TTP (11).

The microscopic features of HUS and TTP are similar to those seen in other conditions featuring TMA such as scleroderma renal crisis, some additional forms of autoimmune diseases, malignant hypertension, eclampsia or preeclampsia, postpartum renal failure, HSCT, antibody-mediated transplant rejection, HIV infection, and exposure to drugs, among others. Therefore, *thrombotic microangiopathy* is an appropriate term to describe the pathologic findings in HUS, TTP, and related conditions and is in widespread use.

## CLASSIFICATION

The classification of TMAs is quite complex mostly because there are multiple terms in use to describe overlapping clinical syndromes with diverse etiologies and major differences in

prognosis and therapy. Current clinical classification is based primarily on the patient's age, clinical presentation, and anticipated treatment rather than etiology. This is because there is an urgency to initiate treatment usually well before etiologic diagnosis is typically available. Once etiology is determined, treatment can be adjusted, if needed. However, the lack of etiologic information at the time of presentation is not the only barrier for the implementation of a purely etiologic classification. Etiologic diagnoses based on some of the key laboratory parameters, such as ADAMTS13 activity, may not be appropriate for the critical initial decision needed for treatment. For example, abnormally low ADAMTS13 activity does not identify all patients who will respond to plasma therapy (9). There is no uniformly accepted classification of TMAs in use. The clinical classification discussed below has four major diagnostic categories (Table 18.1), that is, classic HUS, atypical HUS (aHUS), TTP, and "other" TMAs. Because of historical as well as practical reasons, this classification also includes the secondary forms of aHUS (i.e., those other than familial and idiopathic forms) and the secondary forms of TTP (i.e., those other than congenital and idiopathic forms), under the aHUS and TTP categories, respectively. It should be emphasized that clinical recognition of various subsets, including the secondary forms, within the major categories at the onset of the disease is often difficult or impossible. Therefore, the rule of thumb is that at the time of presentation, patients are presumptively assigned into one of the four major diagnostic categories to guide initial treatment. MAHA, thrombocytopenia, and renal impairment with a diarrhea prodrome caused by Shiga toxin (Stx)-producing *Escherichia coli* (*E. coli*) are the diagnostic hallmarks of classic HUS. Most of these patients are children under the age of 5. However, adults with a history suggesting Stx-producing *E. coli* (STEC) infection are also treated with plasma exchange because the etiology cannot be certain when decision about plasma exchange needs to be made (12). Diagnosis of aHUS relies on the presence of MAHA, thrombocytopenia, and renal impairment, and exclusion of associated disease(s), Stx-associated HUS, and TTP (11). In most instances, however, aHUS cannot be distinguished from TTP at the time of presentation. Until recently, this had only limited therapeutic ramifications since the first line of treatment for aHUS has been plasmapheresis, similar to that of TTP. If additional clinical and laboratory data do not confirm the presumptive clinical diagnosis, the diagnosis is revised and treatment is readjusted, as needed. Those patients who fulfill the diagnostic criteria of TTP (MAHA, thrombocytopenia, with or without renal or neurologic abnormalities, and exclusion of systemic infections and other causes of TMA) are diagnosed as such and treated accordingly with plasmapheresis (9). The majority of patients diagnosed with TTP are adults; however, children without renal impairment can also be diagnosed with TTP. Those cases that do not fit into any of the aforementioned categories will be classified as "other TMAs" with a designation of associated disease(s) or condition(s). The current definition of aHUS limits the subgroups within the aHUS category to those familial and idiopathic forms (13), and all secondary forms are delegated to the "other TMA" category and designated as such (Tables 18.2 and 18.3). Some investigators further narrow the idiopathic group of aHUS to only those cases with complement abnormalities (11) and refer to them, along with the familial forms with complement abnormalities, as "primary" HUS or "complement"-HUS. HUS caused by *Streptococcus pneumoniae* is classified as a form of aHUS by some and, as a separate category, often designated as *S. pneumoniae*–associated HUS by others (14–16). The term TTP is applied to only those cases in the congenital and idiopathic groups, while all other cases formerly considered as various subgroups of TTP ("secondary" TTPs) are now designated as "TMAs" or "other TMAs." In this modified classification, the clinical diagnoses are better aligned with etiology in the HUS and TTP groups; however, the "other TMA" group is still quite heterogeneous. To avoid potential confusion due to terminology, some experts prefer the all-inclusive morphologic term TMA instead of HUS and TTP, while others use the hybrid term HUS/TTP.

**TABLE 18.1 Clinical classification of TMA**

1. **Classic HUS**
2. **Atypical HUS**
   a. Familial
   b. Sporadic (idiopathic)
   c. *Streptococcus pneumoniae* associated
   d. Cobalamin C (cblC) disorder
   e. Secondary forms
      i. Infections
      ii. Autoimmune disorders
      iii. Drugs
      iv. Pregnancy/postpartum
      v. HELLP syndrome
      vi. Other
3. **TTP**
   a. Congenital
   b. Idiopathic
   c. Secondary forms
      i. Infections
      ii. Autoimmune disorders
      iii. Drugs
      iv. Pregnancy/postpartum
      v. HELLP syndrome
      vi. Other
4. **Other TMAs**
   a. Glomerulopathies
   b. Malignant hypertension
   c. Malignancies
   d. Solid organ transplantation
   e. Scleroderma renal crisis
   f. Radiation nephropathy
   g. HSCT

## Classic HUS, Also Known as Diarrhea-Positive (D+) or Epidemic HUS

Classic or typical HUS is associated with prodromal bloody diarrhea. This form occurs mainly in young children, accounts for most cases (90% to 95%) seen in North

**TABLE 18.2 Classification of HUS and TTP[a]**

| Forms | Subgroups | Etiology | Age at onset |
|---|---|---|---|
| Classic HUS | | Shiga and verocytotoxin (Shiga-like toxin)-producing bacteria | Mostly in children under the age of 5 |
| Atypical HUS[b] | Familial | Genetic disorders of complement regulation | In both children and adults |
| | Sporadic (former idiopathic subset) | Genetic and acquired disorders of complement regulation | |
| *Streptococcus pneumoniae*–associated HUS[c] | | *Streptococcus pneumoniae* infection | Mostly in children |
| Defective cobalamin metabolism–associated HUS[c] | | Cobalamin C (cblC) disease (hereditary defect of cobalamin metabolism) | Mostly in infants |
| TTP | Congenital (Upshaw-Schulman syndrome) | Inherited via ADAMTS13 mutations | Neonatal onset |
| | Idiopathic | Acquired, secondary to anti-ADAMTS13 autoantibodies, present in ~50% of cases | Mostly in adults |

[a]TMAs in these forms have no associated underlying diseases or conditions, and the etiology is well defined in the majority of cases.

[b]The term "primary" HUS is also used for a subset of these cases with disorders of complement dysregulation.

[c]In some publications, these forms are classified under the atypical category of HUS.

America and Europe, and develops in isolated cases or as outbreaks occurring mostly in the summer (17). Although the designation "epidemic" HUS is also used for the classic form of HUS, the majority of cases are indeed sporadic. Furthermore, gastroenteritis is a common trigger for the atypical form of the disease, and therefore the term D+ HUS can be misleading. In North America and Western Europe, most of the classic forms are associated with O157:H7 serotype of Shiga toxin–producing *E. coli* (STEC) infection (18–21). However, many other serotypes of STEC have also been linked to classic HUS (20,22–26). Infection with Shiga toxin–producing *Shigella dysenteriae* serotype 1 has been a common cause of classic HUS in developing countries in Asia (27) and Africa (11,28–30), but not in industrialized countries (31). The annual incidence of classic HUS is estimated to be 21 per 1 million with a peak incidence in children under the age of 5 years (61 per 1 million) and the lowest rate in adults in the age group of 50 to 59 years (5 per 1 million) (32). TMA in the classic form due to STEC infection is most often confined to the glomeruli, with a consequently good prognosis.

**TABLE 18.3 Thrombotic microangiopathies other than classic and aHUS and TTP[a]**

- Infections
  - Systemic infections, human immunodeficiency virus infection, H1N1 influenza, *Salmonella typhi*, others
- Autoimmune diseases
  - SLE, APS, others
- Drugs
  - Quinine, ticlopidine, clopidogrel, anti-VEGF agents, oral contraceptives, mitomycin C, interferon, gemcitabine, CNIs, sirolimus, and others
- Pregnancy/postpartum
- HELPP syndrome
- Glomerulopathies
- Malignant hypertension
- Malignancies
- Solid organ transplantation
- Scleroderma renal crisis
- Radiation nephropathy
- HSCT

[a]These are TMAs associated with underlying diseases and conditions. The precise etiology for many of these forms remains uncertain.

## Atypical HUS

Since prodromal bloody diarrhea, characteristic of classic (D+) HUS, is typically absent in aHUS, this type has also been designated as nonenteropathic or diarrhea-negative (i.e., postdiarrhea negative) (D–) HUS. It should, however, be pointed out that gastroenteritis is a common trigger for at least some forms of aHUS (33,34), and therefore the term diarrhea-negative HUS is inaccurate. The atypical form affects both children and adults and in various reports accounts for approximately 5% to 12% of all cases of HUS (11,35). The onset is usually insidious, and marked proteinuria and hypertension are characteristic features. The majority of cases are sporadic, and approximately 20% to 30% are familial (34,36). Those classified as "idiopathic" (same as "sporadic") are the nonfamilial cases with no apparent association with underlying diseases. Genetic or acquired abnormalities of the complement regulatory proteins have been identified as the most common etiology in the aHUS group (11,37–42). Although the precise incidence of aHUS with complement abnormalities is not known, the best available data indicate that genomic abnormalities account for 17% to 60% (34,36,43) and autoantibodies for 6% to 10% of cases (44–46). However, in a significant proportion of patients, the etiology is still unknown.

Historically, the term aHUS also encompasses the "secondary" forms of HUS related to a wide variety of triggers and etiologic agents, such as infections other than STEC, including *S. pneumoniae*, human immunodeficiency virus, and H1N1 influenza A, autoimmune diseases, drugs, pregnancy, HELLP syndrome, hematopoietic stem cell or solid organ transplantation, various glomerulopathies, malignant hypertension, malignancies, ionizing radiation, and, in children, methylmalonic aciduria with homocystinuria and cblC type, a rare hereditary defect of cobalamin metabolism (47–59). Complement regulatory abnormalities, both genetic and acquired, are also seen in a variable but usually a small proportion of patients within various subgroups of "secondary" aHUS (34,36), currently classified in the "other TMA" category. In general, cases with "secondary" aHUS have no well-characterized specific etiology identified except those developing in association with *S. pneumoniae* infection and perhaps those rare cases with concurrent complement regulatory abnormalities.

Although most of the recent publications still include at least some "secondary" forms of HUS under the umbrella of aHUS (34–36,43), some limit the term of aHUS specifically to those cases related to complement regulatory abnormalities (11,60). However, sometimes it might be difficult to define the secondary forms and separate them from the idiopathic (sporadic) forms of aHUS. For example, a significant number of aHUS cases are associated with pregnancy; however, complement regulatory abnormalities are very common in these patients (61). Therefore, the cause is likely complement dysregulation, and pregnancy serves only as a trigger. The European Pediatric Research Group for HUS proposed a new simplified classification for HUS and TTP with only two major categories: one with well-defined underlying etiology and the other with clinical associations but unknown etiology (47). The evolving changes in the classification are the reflection of our better understanding of these complex diseases with the pendulum shifting from purely clinical toward clinical-etiologic classifications.

The prognosis of aHUS is poor; the mortality rate is 10% to 15% during the acute phase (35), and up to 50% of patients develop end-stage renal disease (ESRD) (11,34). Although the renal morphologic findings of TMA are similar to those seen in the classic form, vascular (i.e., arterial and arteriolar) involvement is more common in the atypical forms.

Based on the clinical presentation, etiologic factors, and underlying diseases, the following subgroups of aHUS are distinguished:

## Familial Forms

Familial occurrence of HUS was recognized nearly four decades ago by Kaplan (62). These forms represent approximately 20% to 30% of cases with aHUS (34). They occur in more than one member of the same family, may occur at any age, and may follow a recurrent pattern. The transmission is either autosomal recessive or autosomal dominant (34). Abnormalities in the complement regulatory proteins with underlying hereditary genetic disorder(s) have been identified in approximately 70% of the affected patients and also in some asymptomatic family members (34,63). Changes are common in the renal arteries, hypertension may be present, and the disease is usually severe.

## Sporadic (Noninfectious) Forms

Genetic or acquired deficiencies of some of the complement regulatory proteins have been identified in 41% to 80% of patients with these forms of aHUS (11,34). The morphologic changes are similar to those seen in the familial form, and the disease is, just like in the familial form, usually severe (11,34,36).

## *Streptococcus pneumoniae* Infection–Associated Form (Pneumococcal HUS)

This form affects mostly children under the age of 2 (15,35). Pneumococcal HUS is a relatively rare complication of invasive *S. pneumoniae* infection. Of the 435 children with culture-confirmed invasive pneumococcal disease in Utah from 1997 through 2008, only 7 patients (1.7%) developed HUS (64). The incidence of pneumococcal HUS was 0.015/100,000 child years averaged over the time period of the study, accounting for 5.6% of total HUS cases in Utah children (64). The relatively low incidence of pneumococcal HUS from 1997 to 2008 still represents a significant rise in the number of cases compared to those from 1971 to 1996 when no pneumococcal HUS cases were identified (64). A similar rise in the rate of incidence for pneumococcal HUS was also reported from other countries, including the United Kingdom, during the same time period (65). The rise in the incidence of pneumococcal HUS might be related to the introduction of heptavalent pneumococcal conjugate vaccine (PCV-7) in 2000 (64), which changed considerably the epidemiology of invasive pneumococcal infections (66). Marked reductions in invasive pneumococcal disease because of vaccine serotypes and the emergence of nonvaccine serotypes might have influenced the epidemiology of pneumococcal HUS as well (64,66). In the most recent and largest series of pneumococcal HUS reported from North America, of the 37 cases between 1997 and 2009, 76% of patients had completed their heptavalent pneumococcal conjugate vaccination (PCV7) series (15). Among 24 serotyped isolates in this series, 96% were non-PCV7 serotypes, most commonly 19A (50%) (15). In another study also from the United States, the pneumococcal HUS accounted for approximately 38% of all nonenteropathic cases of HUS (i.e., of all nonclassic forms) and 4.7% of all HUS cases (35). One study reported pneumococcal HUS as the most common form of pediatric HUS in Taiwan (16). This form of HUS carries an increased risk of mortality and renal morbidity compared with classic HUS according to some studies (15,67–69) but good long-term prognosis according to others (35).

## Forms Associated With Cobalamin C (cblC) Disorder

This form of aHUS occurs in association with a rare genetic abnormality of cobalamin metabolism (cblC) with autosomal recessive inheritance. Most of the patients present during early infancy; however, late-onset disease up until teenage years has also been documented. Only a small number of cases have been described in the literature with mostly poor prognosis and high mortality rate (56,70). However, a few cases with early treatment and renal functional recovery have also been reported recently (56,70).

## Thrombotic Thrombocytopenic Purpura

The great majority of patients diagnosed with TTP are adults featuring MAHA and thrombocytopenia, with or without renal or neurologic abnormalities (9). Children can also be affected, although rarely (71). In the rare congenital form (Upshaw-Schulman syndrome), the onset is during the neonatal period in about 75% of cases (71). The clinical diagnosis of TTP requires exclusion of other etiologies, such as systemic infection or another cause of TMA. TTP defined as such, that is, without an alternate cause that mimics TTP, is also referred to as idiopathic TTP. Severe deficiency of the activity of ADAMTS13 (A disintegrin-like and metalloprotease with a thrombospondin type 1 motif 1), a protease that cleaves von Willebrand factor (vWF), has been detected in 48% and 69% of idiopathic cases in two large registries from the United States and Japan, respectively (9,72). Since deficiency of ADAMTS13 results in elevated plasma levels of ultralarge vWF multimers (ULvWF), which are prone to induce platelet aggregation, significantly deficient ADAMTS13 activity is believed to play a key role in the development of at least some forms of TTP.

Similar to that of aHUS, historically the term TTP also incorporated the secondary forms of TTP, such as those associated with hematopoietic stem cell and solid organ transplantation, pregnancy/postpartum period, exposure to drugs, autoimmune diseases, infections, malignant hypertension, malignancy, and multiorgan failure. Severe ADAMTS13 deficiency is less common in the secondary forms than those with the idiopathic or congenital forms of the disease. In the Oklahoma Registry, the incidence of severe ADAMTS13 deficiency among the secondary forms of TTP varied from 0% to 21% (9).

Since renal involvement in TTP is typically mild, the renal prognosis is good. However, the mortality rates, although significantly improved since the introduction of plasma therapy, are still in the range of approximately 8.5% to 30% (73,74).

## Other TMAs (Formerly Secondary HUS and Secondary TTP)

These forms represent the most diverse group of all TMAs (see Table 18.3). Patients in this category develop morphologic features of TMA with or without the characteristic clinical and laboratory manifestations in the background of other diseases and conditions, such as infections other than STEC, autoimmune diseases, exposure to drugs, pregnancy/postpartum states, HELLP syndrome, solid organ and HSCT, various glomerulopathies, malignant hypertension, malignancies, scleroderma renal crisis, and ionizing radiation. The etiology of TMA in most of these cases is unknown; however, ADAMTS13 deficiency or abnormality in complement regulation is suspected in some cases with such abnormalities present. These, along with other factors with potential pathogenetic significance, are discussed under pathogenesis.

## Relationship Between Hemolytic-Uremic Syndrome and Thrombotic Thrombocytopenic Purpura

Although the morphologic lesions of these two conditions are thought by many to be virtually identical, certain clinical differences have been enumerated. Chief among them are that TTP (a) occurs among adults, (b) affects the central nervous system more commonly, (c) exhibits less frequent and less severe involvement of the kidney, and (d) involves multiple organs and has a poorer prognosis. However, these differences are not absolute, and some differences are less consistent than others.

First, with regard to age, TTP is not restricted to adults, and appreciable numbers of cases are seen in infants and children (71). Similarly, HUS is not confined to childhood, as was originally thought. Although the classic form of HUS is most common in infancy and childhood, many cases are reported in adults (17,75). The onset of the atypical form of HUS is more common during childhood (34,76); however, a significant proportion of cases with aHUS manifest in adults and the majority of adult patients with HUS have the atypical form of the disease (11).

Second, severe central nervous system involvement can also be encountered in HUS. In a recent major outbreak of classic HUS in Europe caused by *E. coli* O104:H4 during the summer of 2011, severe neurologic involvement developed in 26% of children (77), an incidence comparable to that reported by some prior series (18,78). In a subset of adult patients with HUS during the same outbreak, of 63 patients admitted to intensive care units, 12 patients (19%) developed severe neurologic symptoms (79). One study from the United States and one from Norway reported an even higher incidence of neurologic involvement in outbreaks caused by *E. coli* O111 (80) and O103:H25, respectively (80,81). However, the French national survey that included all classic HUS cases in France between 1993 and 2007 found a significantly lower prevalence of severe neurologic complications affecting approximately 3% of patients (82). Differences in the inclusion criteria for neurologic involvement and differences in the type of bacterial infections causing HUS may account, at least in part, for the differences in the reported incidence for neurologic involvement in various studies. In one of the largest reported series of 52 patients with classic HUS and severe neurologic complications, 17% of the patients died due to neurologic complications, 23% survived with severe sequelae, and 50% had full neurologic recovery (82). In the Oklahoma Registry, the incidence of major neurologic abnormalities was 37% among idiopathic TTP cases with no difference in the incidence between those with and without severe ADAMTS13 deficiency (9).

Third, clinical and morphologic renal abnormalities can be found in TTP and may occasionally be severe (9). Interestingly, the severity of renal involvement seems to cluster with ADAMTS13 activity. In a large series of 107 patients with idiopathic TTP, acute renal failure was present only in 6 out of 51 (12%) patients with severe ADAMTS13 deficiency (9). In contrast, acute renal failure was observed in 50 out of 56 (89%) patients with normal ADAMTS13 activity (9).

Fourth, extrarenal involvement can be seen in HUS, as in TTP, including the colon, liver, pancreas, heart, and brain, as shown in two autopsy series (83,84).

In addition, a historical review of a series of patients with TTP showed that the classic pentad of symptoms, as defined by Amorosi and Ultmann (7) and consisting of fever, thrombocytopenic purpura, MAHA, neurologic manifestations, and renal dysfunction, was present in only 40% of patients during the course of their illness (85). The frequent clinical presentation of TTP lacking the features of the classic pentad and the urgency to initiate treatment early in the disease led to the

implementation of MAHA and thrombocytopenia as the new diagnostic criteria for TTP in a proper clinical setting (86). In a recent publication relevant to the current clinical practice, the diagnostic pentad of TTP was only present in 3 out of 69 patients (5%) with severe ADAMTS13 deficiency at the time of presentation (9). This change in the diagnostic criteria of TTP over time has further narrowed the differences between HUS and TTP. However, distinction between TTP and HUS has gained ground with the recognition that a significant proportion of patients with TTP have very low ADAMTS13 activity, while many of the patients with the atypical form of HUS have complement regulatory factor abnormalities. Although these findings proved to be very helpful to our understanding of the pathogenesis, abnormal ADAMTS13 activity is not diagnostic of TTP since patients with other conditions, such as systemic infections, might also have significantly diminished ADAMTS13 activity. Furthermore, the activity is normal in approximately 50% of cases with idiopathic TTP, and abnormal ADAMTS13 activity does not identify all patients who will benefit from plasmapheresis (9,72). In regard to complement regulatory factor abnormalities, since penetrance is only 50% in those family members who carry the mutations, the mutations seem to be risk factors rather than unique causes of the disease. In addition, mutations are only identified in approximately 50% of patients with idiopathic aHUS, further limiting the diagnostic and prognostic utility of the test. Finally, since the prevalence of the complement regulatory factor mutations in the general population is unknown, the finding of a mutation in an asymptomatic patient is difficult to interpret.

Therefore, to many investigators and clinicians, the distinction between HUS and TTP appears to be less clear than has been generally claimed. Indeed, with the exception of classic and *S. pneumoniae*–associated HUS, there are no specific diagnostic criteria to distinguish TTP from HUS. Yet, demonstration of severely diminished ADAMTS13 activity or complement dysregulation in proper clinical context is strongly supportive of the diagnosis of TTP and aHUS, respectively.

### Microangiopathic Hemolytic Anemia and Thrombocytopenia

MAHA and thrombocytopenia are the two principal laboratory abnormalities of TMA required for the clinical diagnosis. MAHA is a nonimmune hemolytic anemia (hemoglobin less than 10 g/dL) characterized by deformed and fragmented erythrocytes (helmet cells, burr cells, schistocytes) in the peripheral circulation; the Coombs test is usually negative. Thrombocytopenia (platelets less than 150,000/mm$^3$) is secondary to platelet consumption; small blood vessels, particularly the renal arterioles and glomerular capillaries, show fibrin deposition in their walls and lumina. The microthrombotic lesions are "platelet rich" in TTP and "fibrin rich" in HUS. Investigators have suggested that the bizarre morphologic features of the red cells and the hemolytic anemia are due to mechanical red-cell fragmentation by shearing by fibrin strands at the site of vascular lesions. However, it has also been pointed out that in the early, and sometimes later, stages of HUS, thrombi in the glomeruli and arterioles are either absent or too scanty to be responsible for the mechanical destruction of red blood cells (87,88). Neuraminidase, secreted by bacteria or viruses, has been invoked as a possible cause of the damage to the red blood cells by its action on the cell membrane (89).

There are also a number of well-documented cases of TMA in the literature with no evidence of MAHA and/or thrombocytopenia (11,90).

## HEMOLYTIC-UREMIC SYNDROME

### Epidemiology

#### Classic HUS

The reported mean annual incidence of classic HUS was 7.1 cases per 1 million children less than 15 years of age and 18.7 cases per 1 million children less than 5 years of age in a recent French study for years 1996–2006 (91). A similar incidence rate of 6.7 cases per 1 million children was reported from California for 1994–1999 for children of 17 years of age or less (92). In New Mexico, the incidence rate was 17 cases per 1 million population in 2007 (93). Standardized incidence rates of HUS in 3 geographic areas from the 1990s were also comparable 2.7 per million person-years in the United States, 2.1 per million person-years in the United Kingdom, and 2.1 per million person-years in Saskatchewan (Canada) (94). However, the annual incidence rate of classic HUS in 2008 was substantially higher (170 cases per 1 million children less than 5 years of age) in Argentina, a country where classic HUS is considered to be endemic (95). At least some data indicate that the incidence is higher in females than in males (77,78,94,96). The prognosis is relatively good with most patients fully recovering, and the reported mortality rates are less than 5% for most studies (17,77,91,96,97).

#### Atypical HUS

The precise incidence of aHUS is not known. In the United States, it is estimated to be 2 per million, derived from the incidence of aHUS in children, including those with pneumococcal HUS (11,35). However, according to the most recent data more than 1000 cases of aHUS have been reported mostly from Europe and also from the United States (11,33,34,43,46,76,98–102). In general, the outcome is significantly worse, and the mortality is higher than in classic HUS (34). However, the outcome is closely related to the specific etiology of aHUS and is discussed below.

### Clinical Presentation

#### Classic HUS

Although classic HUS is considered to be most prevalent in infants and young children, it can occur at any age (75,77,103,104). In a systematic review of the literature on classic (diarrhea-associated) HUS that included 49 studies conducted between 1950 and 2001 with 3476 patients enrolled, the mean age of patients was 2.4 years (range 0.1 to 18 years at recruitment into studies) (105). The age of patients with classic HUS was comparable in more recent studies from France, Germany, Austria, Great Britain, and the United States with an average age of less than 5 years (18,78,106,107). Data from two large *E. coli* O157:H7 outbreaks in Japan in 1990 and in 1996 revealed slightly higher median age for patients who developed HUS (3.7 and 7 years, respectively) (108,109). In sharp contrast to these historical data, of the 845 patients with classic HUS in the most recent and largest ever *E. coli* outbreak during the summer of 2011 in Europe, 88% of patients were adults (i.e., older than 17 years of age) (75). The median age

of all patients with HUS during this outbreak was 44 years, and among those 101 pediatric patients (i.e., 17 years of age or younger), the median age was 11 years (75). It remains unclear whether the atypical age distribution in this most recent outbreak reflects the pattern of sprout consumption (the most likely vehicle for the infection) or is attributable to the specific properties of the serotype (O104:H4) of the causative *E. coli* strain (75).

The classic form of HUS usually begins with watery diarrhea and may progress rapidly to bloody diarrhea with hemorrhagic colitis. Clinically, the colitis may mimic appendicitis, diverticulitis, ulcerative colitis, regional enteritis, or intussusception. However, concurrent appendicitis, intussusception, and volvulus may occur as a complication (95,109). In severe cases, pseudomembranous enterocolitis, necrosis, and perforation of the colon with subsequent peritonitis can also be seen. Some evidence suggests that the colonic changes are part of the TMA, with ischemia as the underlying mechanism. Vascular microthrombi have been described in surgically removed segments of colon, as well as in autopsy material (83,110,111). However, microthrombotic lesions are seen only in a few small vessels even when hemorrhagic colitis is extensive; therefore, Shiga toxin–producing *E. coli* (STEC) possibly cause direct endothelial damage in the colon leading to mucosal hemorrhage. It is also possible that the thrombi are secondary to tissue ischemia and/or necrosis. The natural history of the colitis is usually favorable; it can, however, be fatal (95).

The second phase of classic HUS caused by STEC infection occurs abruptly within 3 to 4 days of the onset of gastrointestinal symptoms and is characterized by various combinations of acute renal failure, proteinuria, hematuria, anemia, bleeding abnormalities, central nervous system disorders (e.g., headache, altered consciousness, paresis, aphasia, syncope, seizures, visual changes, and dysarthria), and cardiovascular changes (e.g., hypertension and congestive heart failure). However, during a recent outbreak in Germany caused by a new hybrid strain of *E. coli* O104:H4, the incubation period from the onset of diarrhea to the development of HUS was longer than usual at 8 days (75).

The characteristic renal manifestations include oliguria or anuria, hematuria, hemoglobinuria, proteinuria, various types of casts in the urine, hyperkalemia, and elevated blood urea nitrogen (BUN) and serum creatinine level. Approximately one half of the pediatric patients with classic HUS require dialysis; however, some series reported significantly higher rates of dialysis requirement mostly in association with non-*E. coli* O157 infections (77,78,80,97). Hematuria is usually microscopic, hemoglobinuria is present in a few cases, and proteinuria may be severe.

Manifestations of both anemia and a tendency to bleed include intense pallor, weakness, melena, hematemesis, hematuria, petechiae, and ecchymoses in the skin. Hemoglobin levels are low—sometimes as low as 2 to 3 g/dL—with increased plasma bilirubin levels. The morphologic features of the red blood cells are abnormal, with the presence of helmet cells, burr cells, and fragmented cells (schistocytes) in the peripheral blood; reticulocytes are increased. The Coombs test is almost invariably negative. Leukocytosis is common in the early stages. Platelets are decreased to varying degrees, but megakaryocytes in the bone marrow are usually present in normal numbers. Additional laboratory findings include marked elevation in the serum lactic dehydrogenase level and concomitant reduction in the serum haptoglobin level, and in some cases, especially those with delay in diagnosis, hyperkalemia (≥6 mmol/L), acidosis (serum bicarbonate less than 15 mmol/L), and hyponatremia (less than 125 mmol/L) may be observed. The results of coagulation studies in HUS are not strikingly abnormal; a slight prolongation in prothrombin time occurs in approximately 50% of patients. Prolongation of the partial thromboplastin time is less common; plasma fibrinogen levels are variable, and accelerated fibrinolysis with fibrin split products in the circulation has been recorded. Various tests also indicate functional platelet impairment (112) and destruction (113).

Central nervous system disturbances such as irritability, restlessness, tremors, and ataxia occur. In more severe cases, coma, stupor, and decerebrate rigidity can also be seen. In most series, significant neurologic complications are reported in less than one third of the patients (18,77,78,80,81,97). Cardiovascular disturbances include hypertension and congestive heart failure. Hypertension is present in many patients, but clinically apparent cardiac involvement is infrequent (114). Pancreatic involvement with the clinical features of acute pancreatitis is relatively frequent and has been recorded in 26% of cases in one series from Japan (109). Diabetes mellitus can also be seen (114).

In addition to the classic clinical presentation, incomplete forms of classic HUS have also been described in children (115). These patients present either with bloody diarrhea, hemolytic anemia, and thrombocytopenia without renal failure or with bloody diarrhea, anemia, hematuria, or proteinuria but without azotemia or thrombocytopenia.

### Verotoxin-Producing *Escherichia coli* Infection

In the mid-1980s, verotoxin-producing *E. coli* (VTEC) clearly emerged as the major etiologic factor in the pathogenesis of classic HUS. The term VTEC refers to various strains of *E. coli* that produce one or two distinct bacteriophage-mediated protein exotoxins, VT1 and VT2. Both VT1 and VT2 are composed of a single A subunit of 32-kDa and five 7.7-kDa B subunits (116). These toxins are closely related to Shiga toxin (Stx), the exotoxin produced by *S. dysenteriae* (117), and therefore the terms VT and Stx are used interchangeably. VTEC bacteria are also referred to as STEC. Cattle and other farm and wild animals are the main reservoirs of many STEC serotypes, and cattle are considered to be the most important source of human infections (118). Human infection typically occurs through acquisition of the bacteria via consumption of contaminated food, water, or by person-to-person transmission (17). Most but not all STEC bacteria causing HUS have the capacity to colonize the intestinal mucosa with an attaching-and-effacing (A/E) mechanism (119). The A/E lesion is mediated by proteins encoded within a large pathogenicity island called the *locus of enterocyte effacement* or LEE, and this subset of STEC bacteria is referred to as enterohemorrhagic *E. coli* (EHEC) (120). The bacteria also possess mobile genetic elements carrying additional virulence genes such as plasmids, phages, and pathogenicity islands (e.g., O-I 122) (119). Unlike all other STEC strains linked to HUS, humans are the only known natural reservoir for the enteroaggregative hemorrhagic *E. coli* (EAHEC) O104:H4 responsible for a major HUS outbreak in Germany in 2011 (25). EAHEC is a highly

pathogenic hybrid organism possessing features common to both enteroaggregative *E. coli* (EAEC) and STEC (121–123). EAHEC strains have evolved from EAEC that cause watery diarrhea in children and travelers' diarrhea, by acquiring genes for Stx2a and antibiotic resistance (121,122). Except for Stx2a, no other EHEC-specific virulence markers including the locus of enterocyte effacement (LEE) are present in EAHEC strains. EAHEC O104:H4 colonizes the bowel through aggregative adherence fimbrial pili encoded by the EAEC plasmid. The aggregative adherence fimbrial colonization mechanism substitutes for the LEE functions for bacterial adherence and delivery of Stx2a into the colonic mucosa, ultimately resulting in HUS (25,121,122).

The clinical significance of STEC was first recognized by Riley et al. (124) in 2 outbreaks of hemorrhagic colitis and subsequently by Karmali et al. (125,126) in 11 of 15 cases of sporadic HUS. It is now well established that STEC, especially the O157:H7 serotype, cause enteric illness that results in a spectrum of outcomes including asymptomatic infection, uncomplicated diarrhea, hemorrhagic colitis, and HUS. Data from Western Europe and North America indicate that about 90% of children with HUS have some evidence of STEC infection, and the O157 serogroup is the most commonly involved, seen in up to 83% of the cases (18,114,127). Among various serotypes, the O157:H7 is the most common; however, geographic differences in the occurrence of various serotypes are apparent. In the United States, 63% of 558 patients with diarrhea-associated HUS in 1997–2009 tested for evidence of STEC were positive with the great majority belonging to the O157 serogroup (96). However, an emerging role of non-O157 serogroups has been reported from Europe (20,75,78) and non-O157:H7 STEC strains predominate in Australia (97). In the United States, serogroup STEC O111 was the second most common cause of classic HUS after STEC O157:H7 in 1983–2002 (128). A large outbreak in the United States in 2008 caused by STEC O111 infection affected mostly adults (80). The largest ever outbreak in Europe during the summer of 2011 was caused by the unusual STEC O104:H4 strain and also affected mostly adults (75). Worldwide, the serogroups O26, O103, O111, O118, O121, O145, and O157 are responsible for the majority of HUS cases (24). It should, however, be emphasized that compared with STEC O157 infections, identification of non-O157 STEC infections is more complex. Currently, there are limited public health surveillance data on the occurrence of such infections, and many of the non-O157 STEC infections may go undiagnosed or unreported (http://www.cdc.gov/ecoli/2012/O145-06-12/index.html).

The number of outbreaks of *E. coli* 0157 infections reported to the United States Centers for Disease Control and Prevention has increased dramatically, from 4 in 1992 to 46 in 2002 (17). Out of the total of 350 outbreaks between 1982 and 2002, there were 8598 cases of *E. coli* 0157 infection and 354 (4.1%) patients developed HUS (17). The largest reported outbreak of STEC 0157 in North America affected 501 patients with 45 cases of HUS (approximately 9%) and 3 deaths (129). This outbreak was traced to undercooked hamburgers from a fast food restaurant chain in Washington State. One of the largest ever outbreaks of STEC O157 infection occurred during the summer of 1996 in Japan and affected a total of 12,680 patients (109). The probable source of the infection was lunch food supplied to elementary school children; 121 patients developed HUS (0.9%) and 3 children died. A survey report from the United Kingdom showed that 15% of 1275 patients with STEC 0157 infection developed HUS (130). The largest ever outbreak in Europe during the summer of 2011 caused by the enteroaggregative STEC O104:H4 strain affected 3816 patients of which an unusually high proportion (22%) developed HUS and 54 patients (1.4%) died (75). Contaminated sprouted fenugreek seeds were the suspected primary vehicle of transmission (25,131).

Higher initial leukocyte count and antibiotic use are reported as risk factors for the development of oliguric HUS among those children with STEC O157:H7 infection (132). Antibiotics may potentiate the synthesis and release of Stxs from EHEC (133).

The type of Stx produced by bacteria may also play a significant role in the pathogenicity. Bacteria can possess one or more different *stx* alleles, and the different *stx* subtypes are associated with different clinical outcomes of infections (134). Certain Stxs, such as Stx2, Stx2c, and $Stx2d_{activatable}$, are associated with severe disease such as HUS and bloody diarrhea, while other Stx2 variants have been mostly identified from patients with uncomplicated diarrhea or from asymptomatic shedders (134). However, in spite of the significant progress made in our understanding the biology of STEC infection, the virulence and the evolution of the different STEC serotypes have only been partially unraveled (119).

### Transmission

The main reservoir of the STEC is the intestinal tract of healthy cattle. Most outbreaks in the United States have resulted from transmission of the organism through the consumption of undercooked ground beef or dairy products including raw milk (17). Various other sources and modes of transmission have also been reported, including swimming in infected water, drinking water, consumption of lettuce, apple cider and apple juice, coleslaw, spinach, raw milk, melons, cookie doughs, and grapes (17,104,135–138). Outbreaks due to contaminated drinking water tended to be much larger than all other outbreaks (17). Outbreaks due to secondary transmission of the organisms from person to person by the fecal-oral route occurred at child day care centers, homes, and communities (17,136,139,140). Outbreaks due to animal contact are one of the more recently recognized transmission routes; they occurred at various settings including farms, county fairs, and petting zoos (17).

### *Shigella Dysenteriae* Infection

In addition to STEC, infection with *S. dysenteriae* type 1 has also been identified as an etiologic factor for classic (D+) HUS. In 1978, Koster et al. (141) reported a series of HUS cases from Bangladesh in association with shigellosis, severe colitis, and endotoxemia. HUS associated with *S. dysenteriae* type 1 infection is clinically and morphologically similar, but not identical, to the classic (i.e., VTEC-associated) form of HUS (142). Morphologically, renal necropsy specimens from eight of nine patients with *Shigella*-associated HUS showed renal cortical necrosis, extensive glomerular thrombosis, or arterial thrombosis (142). Subsequently, several other reports confirmed the association of *S. dysenteriae* infection with HUS in both children and adults (28,30,143,144).

## Atypical HUS

The clinical manifestations of aHUS are similar to those of classic HUS except that they typically develop without prior diarrhea and/or hemorrhagic colitis. The onset is usually sudden with general distress, fatigue, vomiting, and drowsiness. In most patients, the diagnostic triad of MAHA, thrombocytopenia, and renal impairment are present. Arterial hypertension is common sometimes accompanied by cardiac failure or neurologic complications. Extrarenal manifestations are observed in approximately 20% of patients (33,34) with central nervous system involvement (10% of patients) being the most frequent. Less frequent complications include myocardial infarction in approximately 3% of patients, distal ischemic gangrene, and multiorgan failure secondary to localized or diffuse microvascular thrombosis (33,34,145,146). Sudden death due to cardiac involvement has also been reported (34,145).

In approximately one fifth of the patients with aHUS, the clinical onset is characterized by subclinical anemia and fluctuating thrombocytopenia for weeks or months with preserved renal function at diagnosis (33). The course may alternate between remissions and acute relapses with hypertension and renal impairment developing over several weeks or months. Since anemia and thrombocytopenia are not uniformly present, arterial hypertension, proteinuria, or progressive deterioration of renal function may be the only manifestations of renal TMA in these incomplete forms. Among patients with aHUS, approximately half of the children and the majority of adults need dialysis at admission (11).

### Hereditary Aspects—Familial Forms

Familial occurrence of HUS has been well documented, both in siblings and in related family members of different generations (34,62,147). Familial forms represent a subset accounting for approximately 20% to 30% of cases of aHUS in various reports (33,34,36). In a series of 82 patients with the familial forms of aHUS, gene mutations, mostly of complement regulatory proteins, were identified in 74% of patients (34). When compared with the sporadic forms of aHUS, the familial forms had a worse prognosis (34). In contrast with the 74% of patients in the familial group, only 49% of patients in the sporadic group died or developed ESRD at 3 years (34). Both autosomal dominant and recessive patterns of inheritance have been reported (11,62,148).

Patients with the autosomal recessive pattern of inheritance have gradual clinical onset of disease, frequent relapses, and renal disease characterized by vascular lesions; ESRD and death are common (149). The clinical and morphologic features of patients with autosomal dominant pattern of inheritance are similar to those of the patients with autosomal recessive inheritance, except the onset is typically in adults, and death occurs even more frequently in patients with the autosomal dominant pattern of inheritance (greater than 90%) (149).

### Recurrences

Recurrent attacks of HUS in the same patient occur mostly in those with the atypical form of the disease. They have been described in patients with various forms of genetic abnormalities of the alternative complement regulatory proteins, thrombomodulin and C3 (34), in association with anti-factor H autoantibodies (34,44). Typical HUS may recur following reinfection with STEC (150). In addition, rare cases of atypical (D–) recurrences in patients with an initial episode of classic HUS have been reported (151). In one recent study, recurrences were more frequent in patients with membrane cofactor protein (*MCP*) mutations than in patients either with *CFI* mutations or without mutations (34). However, despite the more frequent recurrences, patients with *MCP* mutations had a better outcome than those in the other groups (34).

### Triggering Events

In a significant proportion of patients with aHUS, the onset of HUS is preceded by a triggering event or disease. Infections, mainly upper respiratory tract infections or diarrhea/gastroenteritis, are the most common, reported in 50% to 80% of patients in various cohorts (33,34,152). Since diarrhea and gastroenteritis are present in up to 28% of patients preceding aHUS (33,34), the presence or absence of diarrhea cannot be used to distinguish the classic form of HUS from aHUS. Other infections, including H1N1 influenza, varicella, and interestingly STEC, have also been reported in association with aHUS (51,153–155). The possibility of H1N1 influenza as a cause of aHUS rather than a trigger has also been entertained (51). Pregnancy is also a well-established risk factor for triggering aHUS (156). In approximately 20% of women with aHUS, the disease is linked to pregnancy, with the onset being most often during the third trimester or postpartum period (61,156). The presence of complement abnormalities in a significant proportion of these patients is a strong argument for the pregnancy being a trigger rather than a specific cause of the disease.

## THROMBOTIC THROMBOCYTOPENIC PURPURA

TTP is a rare disease with a reported incidence of 6 cases per million in the United Kingdom (73). The age-sex-race standardized annual incidence rate of idiopathic TTP with ADAMTS13 activity of less than 5% in the Oklahoma TTP-HUS registry was 1.7 cases per million (86). The relative incidence of acquired TTP is significantly higher among females than in males and also higher among African Americans and obese patients. The reported age-sex-race standardized incidence rate ratio for African Americans to non-African Americans was 9.3 and that for women to men was 2.7 (86,157), similar to the demographics of systemic lupus erythematosus (SLE) (158). The clinical features of TTP are similar to those of aHUS, and in most instances, TTP cannot be distinguished from aHUS at the time of presentation.

The congenital form of the disease (159,161) is due to an inherited deficiency of ADAMTS13, while the more common acquired (idiopathic) TTP (71,163) is due to the reduction of ADAMTS13 activity by anti-ADAMTS13 autoantibodies. Although common, severe deficiency in ADAMTS13 activity is not a universal feature of the acquired (idiopathic) form of the disease. The reported incidence of severe ADAMTS13 deficiency among patients with idiopathic TTP varies from 48% to 69% in three large registries, from the United States, South East England, and Japan (9,72,73). Importantly, measurements of ADAMTS13 levels are not required for initial management decision to begin or not begin plasma exchange (9). Furthermore, since severe ADAMTS13 deficiency can also be seen in various conditions other than TTP, the presence of severe ADAMTS13 deficiency may not conclusively confirm the diagnosis of TTP (9).

With the current clinical practice and revised diagnostic criteria that require only thrombocytopenia and MAHA to consider the diagnosis of TTP (10), the classic pentad (fever, MAHA, thrombocytopenia, neurologic abnormalities, and renal failure) is rarely seen and was present only in 5% of patients in the Oklahoma TTP-HUS registry (9). Importantly, major neurologic abnormalities, once considered to be one of the cornerstones of the diagnosis of TTP, may only be present in a minority of patients (164).

Mild renal manifestations with elevation of serum creatinine, proteinuria, and hematuria are not uncommon in TTP (73,164,165); however, acute renal failure is present only in a small proportion of patients (73,164,166). Additional clinical signs and symptoms at presentation include pallor, jaundice, fatigue, arthralgia or myalgia, chest pain, heart failure, hypotension, and abdominal pain (165).

Some patients respond quickly to plasma exchange therapy, while others may have a prolonged course with multiple exacerbations and complications. Relapses occur in up to 50% of patients who survive the first episode of TTP (167,168). However, recurrences are generally milder than the first episodes with fewer neurologic symptoms, lower mortality rates, and higher platelet count and hemoglobin levels (168). Although triggering events in TTP are considered to be relatively uncommon, underlying conditions, such as infections (169), acute pancreatitis (170), or pregnancy (156), may promote the onset of the disease. Some data indicate that pregnancy might be the initiating event in as many as 25% of cases with TTP including late-onset congenital forms and the idiopathic forms (73,171). Although historical data show an approximately 90% mortality rate for TTP (7), the current mortality rates of 8.5% to 30% are much lower due to the efficiency of plasma exchange therapy (73,74).

## THROMBOTIC MICROANGIOPATHY IN ASSOCIATION WITH OTHER RENAL OR SYSTEMIC DISEASES

Historically, most forms of TMA developing in the background of other diseases or conditions were classified as secondary HUS or TTP. Such secondary forms of HUS and TTP may be seen in association with a wide array of renal and systemic diseases and conditions, such as infections other than STEC, autoimmune diseases, exposure to drugs, pregnancy/postpartum states, HELLP syndrome, solid organ and HSCT, various glomerulopathies, malignancies, malignant hypertension, scleroderma renal crisis, and ionizing radiation. In the current schema of TMA classification, most of these secondary forms of HUS and TTP are listed under the category of "other TMAs" (see Tables 18.2 and 18.3). However, it should be acknowledged that in some of these forms, such as those associated with pregnancy, distinction between "primary" and "secondary" may be difficult. It should also be emphasized that some forms of TMA, such as those associated with HSCT, malignant hypertension, scleroderma renal crisis, radiation nephropathy, antibody-mediated transplant rejection, as well as malignancies should always be designated as TMAs rather than secondary HUS or TTP. This is because names (i.e., diagnoses) have therapeutic ramifications and the diagnosis of HUS or TTP (even secondary forms) may prompt the implementation of unnecessary and potentially harmful therapies.

SSc, scleroderma renal crisis, radiation nephropathy, and HSCT are discussed in this chapter on pages 775–786. Hypertension and malignant hypertension are discussed in further detail in Chapter 20.

### Systemic Infections

Severe systemic infections can mimic the clinical features of TTP or HUS. Out of the 451 patients in the Oklahoma TTP-HUS Registry, 31 (7%) had severe infections, 16 of which (52%) presented with the classic pentad of TTP, and 4 of them had severe ADAMTS13 deficiency with demonstrable inhibitor activity in 2 of them (172). A review of the literature conducted in conjunction with the analysis of the Oklahoma TTP-HUS Registry revealed a wide array of systemic infections in association with the clinical presentation of TTP. Although some of these infections, such as brucellosis, streptococcal infection with acute glomerulonephritis, angioinvasive fungal infections, cytomegalovirus (CMV), HIV, ehrlichiosis, and Rocky Mountain spotted fever, may cause microvascular injury, in the majority of the cases, the etiology of the MAHA is uncertain. The possibility that some of these cases may have severe systemic infections with concurrent TTP or HUS, triggered by infection, has also been entertained (173). Well-documented association of certain infections and also inflammatory conditions with TTP supports the role of severe infections as potential triggers for TTP (169,174). Since TTP can never be excluded with certainty in patients who are acutely ill and present with MAHA and thrombocytopenia, these cases represent a difficult differential diagnostic and therapeutic challenge.

### Human Immunodeficiency Virus

The reported frequency of HIV infection–associated TMA varies greatly, from 0% to 83% in various series (175–187). Some of the very high frequencies of TMA in the background of HIV infection may reflect the high regional prevalence of HIV infection. Some studies seem to indicate that the frequency of HIV-associated TMA decreased since the advent of HAART (highly active antiretroviral) era (179,180). The clinical manifestations are those of TTP, or less frequently HUS. It can occur in patients with full-blown AIDS, as well as in patients with asymptomatic HIV infection.

The prognosis also varies significantly with reported mortality as high as 100% in some series (180), and as low as 4%, in others (187). A study from France identified two distinct subsets of HIV-associated TMA on the basis of ADAMTS13 activity (186). Those with severe ADAMTS13 deficiency were associated with less profound immune deficiency and a good prognosis. The possibility that a detectable ADAMTS13 may be associated with a less favorable prognosis was raised. In a cohort of HIV-associated TTP patients from South East England, the mortality rate was only 4%, significantly better than in those with idiopathic TTP (187). The majority of the patients in this study had severely diminished ADAMTS13 activity. However, the clinical laboratory manifestations of advanced HIV infection can be similar to that of TTP, including those of MAHA, thrombocytopenia, abnormal renal function, and sometimes even severely diminished ADAMTS13 activity (181,185,188,189). Therefore, the clinical diagnosis of TTP is often uncertain in these patients. It has been suggested that in some patients, HIV infection can trigger TTP, similar to that seen in association with other infections, inflammatory

disorders, and pregnancy (185). In some, and perhaps a significant proportion of patients, TTP can be mimicked by AIDS-related conditions, comorbidities, and multiple drugs in use. Also, the possibility of a specific HIV-associated form of TMA (other than HUS or TTP) has been hypothesized (190). Findings that may support the existence of such form of TMA include the presence of endothelial dysfunction in association with HIV infection (191), and also human herpesvirus 8 infection involving endothelial cells, common in HIV-infected patients (192). A number of other factors have also been implicated as possible triggers or predisposing factors for TMA in HIV-infected patients. These include CMV infection, cryptosporidiosis, and AIDS-related malignancies (180,181,193).

## Systemic Lupus Erythematosus

TMA may develop in patients with connective tissue diseases, among which SLE is the most common. TMA in patients with SLE can be seen in association with a variety of clinical-laboratory manifestations, including those of lupus nephritis, antiphospholipid antibody nephropathy/lupus anticoagulant syndrome, HUS, or TTP. TMA can develop in any class of lupus nephritis and can be the sole finding in a kidney biopsy. The morphologic changes are those of classic TMA with glomerular and arteriolar and less frequently arterial involvement. Those forms associated with antiphospholipid antibodies are discussed in a separate paragraph and further discussed in Chapter 14.

Some data indicate that severely diminished ADAMTS13 activity may correlate with the clinical features of TMA in patients with SLE. Severe ADAMTS13 deficiency was reported in 3 patients with SLE who had clinical evidence of TMA (194,195); however, none of the 36 patients with SLE but without clinical signs of TMA had severely diminished ADAMTS13 activity (196). Unfortunately, no morphologic data were available in either of these studies. In contrast, another study documented laboratory features of TMA with normal ADAMTS13 activity in 3 patients with biopsy-proven severe proliferative lupus nephritis, but there was no morphologic evidence of TMA (197). It is unclear whether the lack of TMA in these biopsies was due to sampling error, timing of the biopsy, or perhaps some other factors. However, good clinical response to mycophenolate mofetil treatment and the lack of abnormal ADAMTS13 activity was interpreted as suggestive of lupus nephritis being the direct cause of clinically diagnosed TMA.

It is also important to emphasize that clinical distinction of TTP or HUS from SLE may be difficult since both disorders can present with similar clinical and laboratory manifestation (158).

## Drugs

A number of drugs have been implicated to induce TMA with the clinical features of HUS or TTP (164,198–201). Among these drugs, quinine, various chemotherapeutic and targeted cancer agents, antiplatelet drugs, and calcineurin inhibitors (CNIs) are the most common and best characterized (201–204).

In the Oklahoma TTP-HUS Registry, quinine was responsible for 88% of drug-associated TMAs (198,205). Most of the patients with quinine-associated TMA are older, white women (198). The onset is usually explosive, and the clinical course is typically severe with high acute mortality. Acute renal failure is common and there is also a high risk for chronic renal failure. Neutropenia, liver toxicity, and relapses after quinine reexposure are often seen. The pathogenesis is considered to be immune mediated by the demonstration of quinine-dependent antibodies reactive with platelets as well as other cells (198,200,205,206). The ADAMTS13 levels are not severely decreased consistent with a different pathogenetic mechanism from that of idiopathic TTP (202).

The association of TMA with the thienopyridine-derived antiplatelet agents ticlopidine and clopidogrel is also well documented (200,207,208). The clinical presentation is that of TTP or rarely HUS. In FDA safety databases, ticlopidine and clopidogrel are the two most common drugs associated with TTP (208,209). Laboratory studies indicate that most cases of thienopyridine-associated TTP involve autoantibodies to ADAMTS13, present with severe thrombocytopenia, and respond to plasma exchange (207,208,210). A minority of cases with thienopyridine-associated TMA present with severe renal insufficiency involve direct endothelial cell damage and are less responsive to plasma exchange (208,210).

Among various chemotherapeutic drugs, mitomycin C and gemcitabine are the most commonly reported (204,211–213). The development of TMA is likely dose dependent with an incidence of 2% to 15% in mitomycin C and 0.25% to 0.4% in gemcitabine-treated patients (201,204). The onset is usually insidious within a few weeks of the last dose of chemotherapy. The typical clinical presentation includes severe MAHA, thrombocytopenia, renal failure, elevated bilirubin, and pulmonary edema. The course is potentially progressive even after discontinuation of the drug, and the prognosis is poor. Most patients die within a few months of diagnosis, either from pulmonary or renal failure or from underlying malignant disease (211–213). However, less aggressive forms in association with mitomycin C have also been documented with renal functional recovery using either rituximab or corticosteroid treatment (214,215). The pathogenesis is not clear; however, the effects are dose dependent pointing to direct toxicity (212,216). The role of decreased levels of prostacyclin, secretion of vWF, or exposure of the subendothelium have all been postulated as potential pathogenetic factors (201).

Based on biopsy and autopsy reports, the renal changes in mitomycin-associated HUS are those seen in the more severe forms of HUS, with cellular intimal thickening of renal interlobular arteries and thrombotic glomerular lesions (217,218). Mesangiolysis can be severe. Autopsy studies indicate that the microvascular lesions are primarily found in the kidneys; however, fibrin thrombi in small vessels of the brain and fibrocellular intimal thickening of pulmonary arterioles and small arteries have also been described (218).

With the advent of targeted cancer agents, including those of immunotoxins, monoclonal antibodies, and tyrosine kinase inhibitors, TMA has emerged as a rare but potentially serious complication of these drugs (201,219). Of these, the antivascular endothelial growth factor (VEGF) antibody treatment-associated TMA is the best studied (54,55,201,220–224). The clinical presentation of TMA varies from mild renal involvement to severe disease with systemic manifestations (54,201,222). Improvement of the renal function was documented in at least some cases following discontinuation of the treatment (54). Cases have been observed with the use of various anti-VEGF agents that target the VEGF pathway by different mechanisms, suggesting a potential class-wide effect (55,201,224). Glomerular capillary endothelial cell damage with the loss of fenestration is postulated to be the underlying cause for TMA

in patients treated with anti-VEGF agents (54). This is also supported by experimental evidence showing that local genetic ablation of VEGF from podocytes in the mouse causes TMA similar to that seen in humans (54,219). TMA has also been described in association with various immunotoxins, such as Combotox (225), antibodies including Apolizumab (Hu1D10) (226), as well as the tyrosine kinase inhibitor Imatinib (Gleevec) (227).

There are a number of additional drugs that were documented in association with TMA, such as antibiotics, H-2 receptor antagonists, hormones, interferons, nonsteroidal anti-inflammatory drugs, vaccines, and various chemotherapy agents including bleomycin, cisplatin, daunorubicin, cytosine arabinoside, cyclophosphamide, doxorubicin, bortezomib, and vincristine (198,200,217,228). Based on a limited number of detailed pathologic descriptions in these cases, the renal morphologic features seem to be those of typical HUS.

TMA caused by the immunosuppressive agents, cyclosporine, tacrolimus, and sirolimus, is discussed below under Solid Organ Transplantation.

## Renal Transplantation

TMA with renal involvement is a well-known complication of solid organ transplantation including the kidney, lung, heart, liver, pancreas, and intestines. It is most commonly observed in renal transplants during the early posttransplant period (i.e., within the first 3 to 6 months); however, it may develop later. In renal transplants, it may occur either as a recurrent or as a de novo disease. De novo TMA in renal transplants has been linked to treatment with various immunosuppressant drugs, antibody-mediated rejection (AMR), viral infections, ischemia-reperfusion injury, and anticardiolipin antibodies. Recurrent posttransplant HUS is a complication of the posttransplant course of patients whose original renal disease was HUS, mostly the atypical form. Among immunosuppressant drugs, the ones that are most commonly associated with TMA in kidney transplant patients are CNIs and mTOR inhibitors. Similarly, in patients with transplanted organs other than the kidney who develop TMA in their native kidneys, association with CNI and mTOR inhibitors is the most frequent.

In a historical cohort study of 15,870 renal transplant recipients from the United States Renal Data System (USRDS) between January 1, 1998, and July 31, 2000, the incidence of de novo TMA was 0.8% with a 1.26-year mean follow-up (229). The risk of TMA was highest for the first 3 months after transplant. Risk factors for de novo TMA included younger recipient age, older donor age, female recipient, and initial use of sirolimus. Patient survival rate was approximately 50% at 3 years. In two more recent single-institution studies, the prevalence of de novo TMA among renal transplant recipients was 6.1% and 3.4%, respectively (230,231).

### Calcineurin Inhibitors

Cyclosporine-associated TMA was first recognized in bone marrow transplant patients, followed by descriptions in patients with solid organ transplants, including the kidneys (232–235). The precise incidence of cyclosporine-associated TMA is not well established. The frequency varies between 3% and 14% in various reports (236,237). The difference might, in part, be attributed to clinical variables, differences in biopsy practices, as well as differences in biopsy interpretation. Tacrolimus (FK506, Prograf), another CNI and mainstream immunosuppressant in transplant patients, has also been associated with de novo TMA in renal transplants (237–239). According to some studies, the incidence of tacrolimus-associated TMA is somewhat lower than that associated with cyclosporine. However, other studies showed no differences between the rates of TMA with the use of cyclosporine versus tacrolimus (229). De novo TMA has also been linked to mTOR inhibitors (Sirolimus and its novel derivative Everolimus) in renal transplant recipients (229,240–242). The incidence of de novo TMA in renal transplant patients treated with mTOR inhibitors is low; however, when mTOR inhibitors are used in combination with CNI, a much higher incidence of TMA has been reported (up to 20.7%) (243). The relative risk of TMA was highest in those patients treated with cyclosporine in combination with sirolimus and the lowest in those on tacrolimus and mycophenolate mofetil (243). The morphologic findings of CNI and mTOR inhibitor-associated TMA are similar to those seen in other forms of HUS with both glomerular capillary and arteriolar and arterial lesions present.

### Antibody-Mediated Rejection

AMR has emerged as a significant cause of de novo TMA post–renal transplantation (230,231,244,245). The reported incidence of de novo TMA in the background of AMR varies significantly from 3.2% to as high as 49.5% (230,231,245,246). Satoskar et al. (231) published a series of cases of de novo TMA in renal transplant recipients, in 55% of which TMA developed in association with C4d-positive AMR. The risk for de novo TMA was significantly greater in those patients with AMR. High panel reactive antibody status at the time of the biopsy also showed a strong correlation with the development of TMA. Interestingly, there were no significant differences in the outcome between the two groups. In contrast, only 16% of those cases with de novo TMA in a study by Meehan et al. (230) were associated with tubulointerstitial capillary C4d positivity. The incidence of de novo TMA in C4d-positive biopsies was also substantially different between the two studies with 3.3% in the study by Meehan et al. (230) and 13.6% in the study by Satoskar et al. (231). The probability of TMA with C4d positivity in early posttransplant biopsies (i.e., within the first 90 days) was significantly greater than in C4d-negative biopsies in the study by Meehan et al. (230). Both studies identified differences in the morphologic features between C4d-positive and C4d-negative cases with TMA (230,231). In early posttransplant biopsies, glomerular thrombi were more frequent in the C4d-positive group, while arteriolar lesions were seen with higher frequency in C4d-negative cases. At 1 year of follow-up after the biopsy, the median serum creatinine was significantly higher in C4d-positive patients with TMA compared with those without TMA (230).

### Complement Regulatory Abnormalities

Recent data indicating a potential link between mutations in genes encoding for some of the complement regulatory factors and de novo HUS in renal transplants add further complexity to this issue (247). Heterozygous mutations of the factor H and factor I were detected in 29% of patients with de novo TMA raising the possibility that genetic susceptibility may trigger TMA in this setting. TMA has also been described in a transplant patient with autoantibodies against factor H whose original disease was MPGN, with rapid recurrence in the first graft and TMA in the second graft (248).

### Viruses

Although less often than with CNIs or AMR, viral pathogenesis has also been implicated in the development of posttransplant HUS. Viral infections linked to de novo HUS include influenza, CMV, hepatitis C virus, and parvoviruses (249–255). Both CMV and parvoviruses can cause endothelial injury that can be the trigger of HUS in this setting (249,251).

### Recurrence

The recurrence rate of HUS in the transplanted kidneys is approximately 50% for those whose original disease was aHUS with an 80% to 90% risk for graft loss among them (34,256–260). However, the risk of recurrence varies greatly depending on the underlying cause, most frequently genetic abnormality of the complement system. The recurrence rates are 15% to 20% for patients with mutations in the gene encoding MCP and 50% to 100% in patients with mutations in the genes that encode the circulating regulators of complement: factor H and factor I (11). A recurrence rate of 40% to 100% has been documented in forms associated with C3 and complement factor B (CFB) mutations (11). Recurrence has also been described in at least one patient with thrombomodulin mutation and in those with high antibody titers against complement factor H (CFH). Some of these recurrences, especially those associated with factor H mutations, occur very early posttransplantation.

There are no specific morphologic features that can distinguish recurrent from de novo TMA in a kidney biopsy. The clinical history of HUS as the cause of ESRD in the native kidney should cause one to entertain the possibility of recurrence; however, the possibility that in a given renal transplant, TMA is related to causes other than recurrence can never be excluded with certainty.

### Clinical-Laboratory Features

The clinical-laboratory manifestations of TMA in renal transplant patients show remarkable differences. In some patients, TMA is "localized" to the kidney without systemic manifestations, that is, with no evidence of hemolysis and/or thrombocytopenia. In these patients, rising serum creatinine can be the only abnormal laboratory finding. Others might show the full or partial spectrum of classic laboratory manifestations with MAHA, thrombocytopenia, elevated LDH, and haptoglobin levels. Zarifian et al. (236) described 26 patients who developed biopsy-proven de novo HUS in renal transplants attributed to CNI toxicity; however, only 2 of them showed thrombocytopenia and MAHA with elevated LDH. However, some more recent studies reported a high incidence of MAHA and thrombocytopenia, along with high LDH and low haptoglobin and hemoglobin levels in various forms of TMA in transplant patients, including those associated with CNIs, AMR, and complement regulatory factor abnormality (231,247,261,262). In some of these series, the incidence of microangiopathic anemia and thrombocytopenia was as high as 100% and 75%, respectively (231,262).

## Other Solid Organ Transplants

Renal TMA is also a well-known complication of solid organ transplantation, other than the kidney, such as the lung (235,263,264), heart (265,266), liver (264,267), combined kidney and pancreas (268), and intestines (269). In a lung transplant cohort of patients with cystic fibrosis, nearly all of those who underwent native kidney biopsy due to worsening renal function showed evidence of CNI toxicity (93.3%) and almost half of them revealed features of TMA (235). Interestingly, none of those with TMA developed thrombocytopenia or signs of intravascular hemolysis. The incidence of renal TMA was 5.7% (5/67) in another study of lung transplant patients on combined cyclosporine and everolimus therapy (263). However, none of those treated with only CNI ($n$ = 445) developed TMA. The reported incidence of renal TMA was similar in patients with lung transplants (14%) versus those with liver transplants (13%) in a recent study from Germany (264).

Although the precise pathogenetic mechanisms of cyclosporine-induced TMA are not known, there have been a number of observations, both in vitro and in vivo, that link cyclosporine to microvascular thrombosis (270). Direct endothelial injury (271), shift of the endothelial anticoagulant properties to procoagulant phenotype (272), suppression of the protein C anticoagulant pathway (273), induced production of thromboplastin by mononuclear cells (274), and increased production and release of high molecular weight vWF multimers from the endothelial cells (272) have all been implicated as potential contributing factors. In addition, cyclosporine but not tacrolimus induces renal hypoperfusion that can further perpetuate thrombosis in renal microcirculation (275).

## Pregnancy

Eclampsia and preeclampsia, pregnancy-related TTP, and pregnancy-related aHUS represent the spectrum of various forms of TMAs that develop during or soon after pregnancy. Although these are historically classified as secondary forms of aHUS and TTP (i.e., without specific underlying etiology), recent data indicate that in a significant proportion or perhaps in the majority of patients with pregnancy-related aHUS, genetic abnormalities of the alternative complement regulatory pathway and/or C3 convertase are present (61). Furthermore, severe ADAMTS13 deficiency is suspected to be the major cause of TTP precipitated by pregnancy (9,86,277–279). Therefore, it is widely accepted that pregnancy is a trigger rather than the primary cause of TMA, and genetic abnormalities of the complement system and severe ADAMTS13 deficiency are the most common underlying etiologies. In up to 21% of adult female patients with aHUS, the disease is pregnancy associated with 79% of cases developing during the postpartum period (61,76). The prevalence of genetic abnormalities of the complement system is very high, identified in up to 86% of patients in a recent study with pregnancy-associated aHUS (61). The risk of pregnancy-related aHUS was highest during the second pregnancy, and the outcome was poor with 76% of patients developing ESRD (61). Pregnancy-related TTP can also develop both antepartum and postpartum. However, in contrast to aHUS, the incidence of pregnancy-related TTP is highest during the second and third trimesters of pregnancy (279). Although maternal mortality of pregnancy-associated TTP has improved significantly since 1996, it is still relatively high (approximately 9%) (279). However, the renal involvement in pregnancy-associated TTP is less severe, and the renal outcome is substantially better than with pregnancy-associated aHUS (279). A good renal outcome and no mortality were reported in pregnancy-induced TTP in patients with the congenital form of the disease (i.e., Upshaw-Shulman syndrome) from Japan (276). However, TTP in patients with Upshaw-Shulman

syndrome during pregnancy was associated with a significant fetal loss (50%), an incidence substantially higher than that seen in the noncongenital forms of the disease (276,279). The presence of complement abnormalities during pregnancy is also a risk factor for fetal loss and preeclampsia; however, the incidence of such complications appears to be relatively low (4.8% and 7.7%, respectively) (61,280). There is a significant overlap between the clinical and laboratory manifestations of eclampsia-preeclampsia, pregnancy-associated TTP, and HELLP syndrome (hemolysis, elevated liver enzymes, and low platelets) (279). Since these conditions require different therapies, difficulty in differentiating these conditions from each other remains a significant clinical problem. Although abnormal ADAMTS13 activity is not specific for TTP, severe deficiency or absence of ADAMTS13 activity favors TTP over HELLP syndrome and preeclampsia-eclampsia (281).

Postpartum aHUS secondary to a genetic abnormality in factor H acquired through liver transplantation has also been reported (282). A new ADAMTS13 missense mutation (D1362V) in TTP diagnosed during pregnancy was described recently (277).

Mutations in complement regulatory proteins also predispose to preeclampsia (280).

### Glomerular Diseases

TMA has been described as a complication in a number of glomerular diseases, including IgA nephropathy, C1q nephropathy, membranous glomerulonephritis, postinfectious glomerulonephritis, antiglomerular basement membrane glomerulonephritis, pauci-immune glomerulonephritis, and cryoglobulinemic glomerulonephritis, among others (283–292). Although one recent study reported a high frequency of TMA in association with IgA nephropathy, TMA is a relatively rare occurrence in other glomerular diseases including those of lupus nephritis. Laboratory evidence of TMA may not be present in a significant proportion of patients with glomerulonephritis and superimposed TMA (284,292). In a series of 128 patients with IgA nephropathy 68 of whom had TMA, laboratory features of TMA were present only in a minority of patients and almost exclusively in those with hypertension or malignant hypertension (284). However, patients with both morphologic and laboratory features of TMA had significantly worse renal outcome than those with only morphologic evidence of TMA (292). The presence of TMA in biopsies (including those cases without laboratory features of TMA) was still associated with a significantly worse outcome versus those with IgA nephropathy but without TMA. Although TMA was more common in patients with hypertension or malignant hypertension, 33% of patients with TMA either were normotensive or had only mild hypertension arguing against a crucial role of severe hypertension in the etiology. This is in spite of the fact that the renal morphologic findings with mostly arteriolar and arterial lesions resembled those seen in malignant hypertension and scleroderma renal crisis.

TMA has also been described in a patient who presented first with membranoproliferative glomerulonephritis type 1 with C3 and IgM glomerular deposits followed by aHUS 2 years later (293). Interestingly, a novel heterozygous CFH mutation was identified in this patient along with three CFH polymorphisms, often associated with aHUS (294). This case exemplifies the etiologic-genetic complexity of aHUS and that certain genetic abnormalities can potentially manifest in various renal abnormalities in the same patient.

### Malignant Hypertension

The association between severe ("malignant") hypertension and TMA has been well documented (297–300). The term *primary malignant nephrosclerosis* (297) has also been used for TMA secondary to severe hypertension. The renal pathologic features of severe hypertension (i.e., *malignant-phase essential hypertension*, malignant nephrosclerosis, or malignant hypertension, historically) are virtually identical to those seen in other forms of TMA, including that of HUS and TTP except that arterial and arteriolar involvement is more prevalent than glomerular involvement in association with malignant hypertension (298). However, serum levels of vWF factor were normal, and thrombocytopenia was present only in 24% of patients in a study with biopsy-proven malignant hypertension–associated TMA (298). In contrast, a recent study of patients with malignant hypertension showed positive correlation between elevated serum levels of vWF, prothrombin fragment 1 + 2, and plasmin-antiplasmin complexes with markers of TMA (elevated LDH level, low platelet count, and presence of schistocytes), and renal dysfunction (301). Relevant to the pathogenesis, elevated levels of soluble P (sP)-selectin were not associated with markers of endothelial dysfunction (vWF and soluble tissue factor [TF]), and therefore sP-selectin more likely originated from activated platelets rather than endothelial cells (301).

### Malignancy

Rarely, the course of malignant diseases can be complicated by TMA with the classic laboratory findings of MAHA and thrombocytopenia. Clinically, this may pose a differential diagnostic challenge especially when the underlying malignancy is not readily apparent. Malignancies documented in association with TMA include mucin-producing metastatic adenocarcinomas of stomach, small bowel, breast, pancreas, prostate, and lung and also squamous cell carcinomas as well as non-Hodgkin lymphomas and leukemias (302,303). Clinically, the onset can be abrupt, and the severity of anemia, thrombocytopenia, and neurologic and renal abnormalities is similar to those of idiopathic TTP (302–304). ADAMTS13 activity is not severely deficient; however, it may be lower than normal in some patients due to high plasma levels of vWF (305). Usual autopsy findings are tumor emboli and/or fibrin microthrombi, mostly within small pulmonary vessels, including arterioles and capillaries (306).

## PATHOLOGIC FINDINGS OF TMA

The histologic features of HUS and TTP are quite similar and are described together. Although minor differences in distribution of renal lesions in HUS and TTP are well documented, the renal biopsy findings alone cannot distinguish between the two. Generally, the clinical presentation of classic HUS is quite characteristic, and hence the historical clinicopathologic data are quite relevant even today. However, there is significant overlap between atypical (D–) HUS and TTP presentations, and the etiologic and serologic features that help define these two entities have been better characterized only in the last decade or so. Hence, aHUS and TTP are often referred to in the literature as HUS/TTP syndrome.

The pathologic features of TMA have been studied in renal biopsies and autopsy material (87,110,111,297,307–309). The basic morphologic changes are similar in most cases regardless of cause. The severity of clinical disease rests mainly on the extent of involvement and, in particular, the presence of changes in renal arteries. Many data indicate that in aHUS (and TTP), the renal arterial involvement is more widespread and severe than in the classic form of HUS. This difference in the renal morphologic features between classic and atypical forms may explain the poorer prognosis seen in the atypical form. Certain morphologic features may be more pronounced in some forms of HUS, such as mesangiolysis in HUS associated with mitomycin and bone marrow transplantation. Sometimes, changes of HUS may be superimposed on those of other glomerular or vascular diseases (e.g., lupus nephritis or vascular lesions in chronic hypertension or arteriosclerosis).

The characteristic morphologic lesions of the kidney in childhood HUS most likely represent only the most severe end of the spectrum since the usual course of HUS in children is relatively mild, and only those patients with the most severe or lingering clinical symptoms undergo biopsy or autopsy (<5% to 10% of children with HUS). Most children with classic HUS do not require a biopsy and recover with no or only minor residual renal symptoms.

The microscopic features of TMA have been traditionally divided into early (acute, within 2 months from initial presentation) and late (chronic) changes (309,310) (Table 18.4). We follow this approach in the microscopic description; however, some overlap between acute and chronic features may occur.

## Gross Appearance

Renal cortical necrosis is a frequent finding in patients who die of HUS and is variable in its extent. Although large areas of necrosis can sometimes be seen, more often the necrosis is patchy and widespread, so the swollen kidney has a reddish, mottled appearance. Calcification may be apparent in the previously necrotic areas in patients who have survived for longer periods; this can be seen on x-ray of the abdomen. Other patients who die may have no apparent cortical necrosis, although petechial hemorrhages are seen in an enlarged, swollen kidney. The cortex is widened, with petechiae often visible on the pelvic mucosa as well. Bilateral nephrectomy specimens from patients who have developed irreversible renal failure with uncontrollable hypertension may be of normal or reduced size if the patient had an extended period of hemodialysis before nephrectomy. Reduction in size is partly a consequence of the damage inflicted by the disease itself, particularly if there has been extensive arterial narrowing; however, for patients who have undergone long-term dialysis, dialysis arteriopathy may also be observed. Focal scarring or areas of calcification may be seen corresponding to previous areas of necrosis.

## Light Microscopy

### Glomeruli

The glomerular morphologic features vary according to the severity and the duration of the disease and the presence or absence of arterial changes. The percentage of the glomeruli with pathologic changes may also vary; in some instances, only few glomeruli are involved, whereas in other cases, most of the glomeruli are affected.

**TABLE 18.4 Renal morphologic features of TMA**

| Early lesions | Late lesions |
|---|---|
| **Light microscopy** | |
| Fibrin thrombi in capillary lumens, mesangium, and subendothelium | Reduplication of GBMs |
| Endothelial swelling and subendothelial widening | Mesangiolysis |
| "Bloodless" glomeruli with capillary luminal narrowing | Arterial intimal fibrosis |
| Mesangiolysis | Organization and recanalization of luminal thrombi |
| Fragmented RBCs in the subendothelium and mesangium | Glomerulosclerosis |
| Glomerular capillary tuft collapse in the presence of predominant arterial involvement | Interstitial fibrosis |
| "Mucoid" intimal hyperplasia in arteries, fibrinoid necrosis | |
| **Immunofluorescence microscopy** | |
| Fibrin deposition in the glomeruli and arterioles | Nonspecific weak IgM staining in the glomeruli and arterioles; less frequent C3 and IgG |
| Nonspecific weak IgM staining in the glomeruli and arterioles; less frequent C3 and IgG | |
| **Electron microscopy** | |
| Electron-dense fibrin fibrils in capillary lumens, mesangium, and subendothelium | Reduplication of the GBMs |
| Subendothelial expansion by electron-lucent material/"fluff" | Electron-dense depolymerized fibrin |
| Endothelial cell swelling | Arterial intimal thickening |
| Fragmented RBCs in the subendothelium and mesangium | |
| Mesangiolysis | |

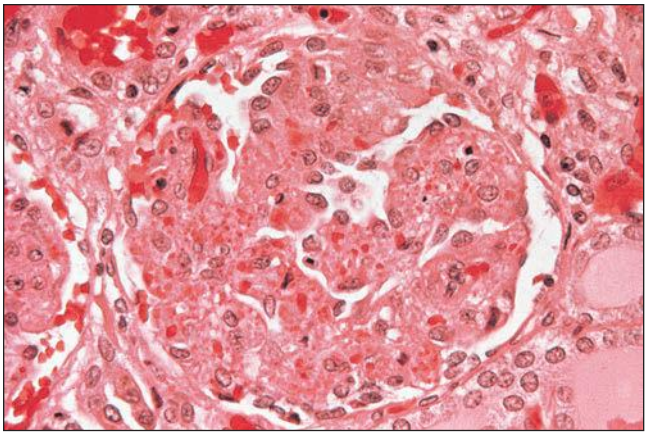

**FIGURE 18.1 Hemolytic-uremic syndrome, classic form.** The glomerulus is slightly hypocellular, and most of the glomerular capillary lumina are closed due to thickening of the capillary walls. Red blood cells and fragmented red blood cells are seen in the mesangial areas. The specimen is from a child with STEC infection. This type of glomerular change is typical during the acute stage of the disease. (H&E) (Courtesy of Dr. Vivette D'Agati.)

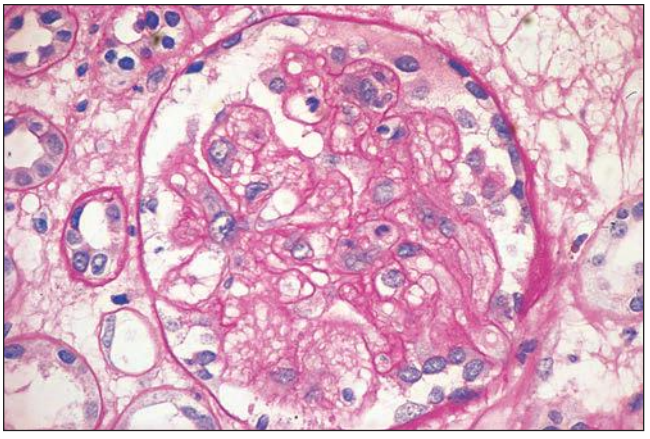

**FIGURE 18.3 Hemolytic-uremic syndrome, atypical form.** The mesangial areas of the glomerulus have fibrillary appearance. Focal reduplication of the glomerular capillary basement membranes is also seen. A few intracapillary polymorphonuclear leukocytes are present. The specimen is from an adult patient without known etiology of HUS. (Periodic acid-Schiff reaction.)

In the early stages, the glomeruli may show thickening of the capillary walls, caused mainly by expansion (swelling) of a thin layer between the endothelial cells and the underlying basement membrane. Severe swelling of the glomerular endothelial or interposed mesangial cells with subendothelial widening may occlude the capillary lumina (Fig. 18.1). The term *bloodless* is used to characterize the glomeruli with complete or near complete closure of the capillary lumina (Fig. 18.2). Separation of the endothelium from the underlying basement membrane and production of new basement membrane–like material by endothelial or interposed mesangial cells result in the occasional double contour appearance of the glomerular capillary walls best seen with silver or periodic acid-Schiff (PAS) stains (Fig. 18.3). Although double contours are generally considered to be a feature of a more advanced disease, they can also be seen during the relatively early stages of TMA. The glomerular capillary lumina may have fragmented red blood cells, fibrin, and platelet thrombi (Fig. 18.4). Fibrin is sometimes clearly detectable beneath the glomerular capillary endothelial cells. Larger localized areas of fibrin may be seen in the glomerular capillary tufts, particularly in continuity with thrombus or fibrinoid necrosis in the afferent arteriole as it enters the glomerulus (Figs. 18.5 and 18.6). The glomeruli may also be congested, containing red blood cells in dilated capillary loops, especially in cases with severe vascular involvement (Fig. 18.7). This feature is sometimes designated "glomerular paralysis." This change is typical in patients in the early stages of cortical necrosis; frank glomerular necrosis usually develops later. Small crescents may occasionally be present. An increased number

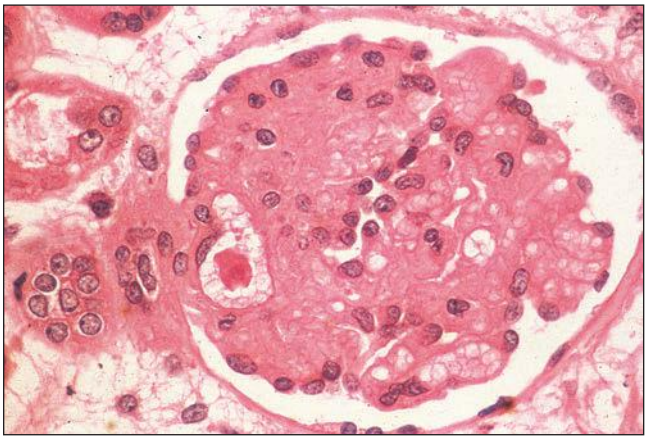

**FIGURE 18.2 Hemolytic-uremic syndrome, atypical form.** "Bloodless" glomerulus. The glomerular capillary walls are thickened, and the mesangial areas blend with the capillaries. (H&E) (From Kern WF, et al. *Atlas of Renal Pathology*. W.B. Saunders Company, Philadelphia, 1999, with permission.)

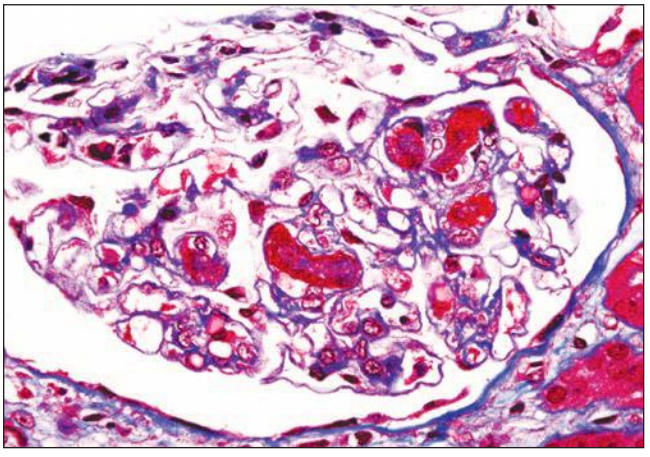

**FIGURE 18.4 Thrombotic microangiopathy, associated with cyclosporine administration.** Some of the glomerular capillary lumina are occluded by thrombi. No additional significant pathologic changes are present. (Masson trichrome.)

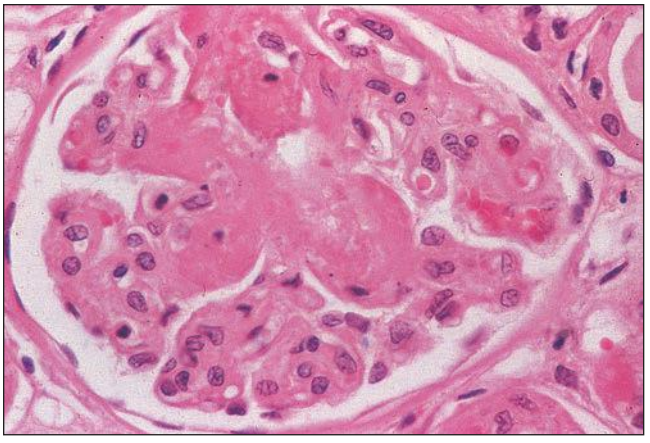

**FIGURE 18.5 Hemolytic-uremic syndrome, atypical form.** Some of the glomerular capillary tufts are permeated by eosinophilic acellular material. This change is often described as fibrinoid necrosis. Intraluminal thrombi are also present. (H&E) (From Kern WF, et al. *Atlas of Renal Pathology*. W.B. Saunders Company, 1999, with permission.)

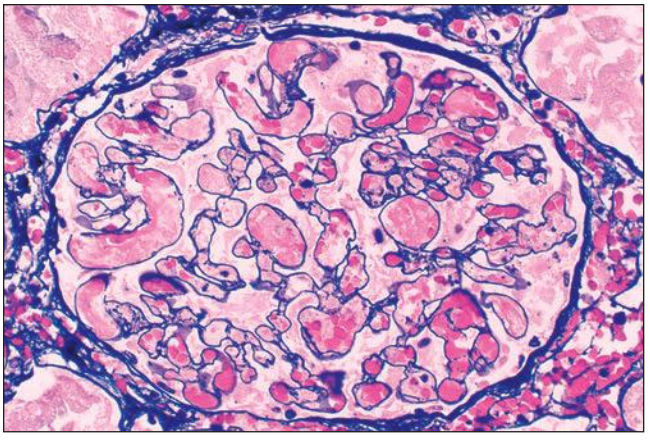

**FIGURE 18.7 Thrombotic microangiopathy in a patient with primary antiphospholipid antibody syndrome.** Some of the glomerular capillary lumina are occluded by fibrin thrombi; the rest of the capillaries are congested. Glomerular capillary congestion in HUS is often referred to as "glomerular paralysis." Mesangiolysis is also apparent. (Methenamine-silver.)

of polymorphonuclear leukocytes in the glomerular capillaries can be seen in some cases; this feature may be prominent in patients with classic HUS (311). Capillary thrombi, endothelial swelling, and congestion were identified as the typical glomerular findings in patients with severe classic HUS (311). The glomerular capillary thrombi in TTP are usually not very extensive (Fig. 18.8). On occasion, the majority of the glomeruli may be affected, but in general, the glomerular changes in TTP are usually not as dramatic as in HUS.

Mesangial abnormalities may also be present in TMA during the early stages. A fibrillar or spongiform appearance of the glomerular mesangium is a characteristic feature, particularly in patients with narrowed arteries and arterioles (see Fig. 18.3). The reason for this fibrillar appearance is not obvious; collapsed glomerular capillary walls and mesangial edema may be contributing factors. Fibrin and fragmented red blood cells can also be seen in the mesangium (see Fig. 18.1). Mesangial cells, although often swollen and hypertrophic, are usually not increased in number during the acute phase of the disease. If mesangial cell proliferation occurs, it is usually slight, focal, segmental, and late.

Occasionally, mesangiolysis can also be seen. The term *mesangiolysis* was first used by Yajima (312) in 1956 in patients with nephritis associated with subacute bacterial endocarditis. However, the glomerular capillary cysts, one of the most typical features of mesangiolysis, were described earlier by Pearce (313) in an experimental model of glomerular lesion induced by *Crotalus adamanteus* venom. The term *mesangiolysis* refers to partial or complete dissolution of the mesangial matrix and cells. The affected glomerular lobules of mesangiolysis stain

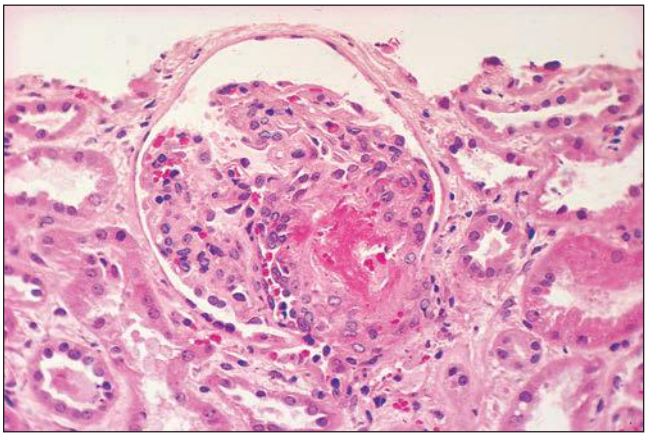

**FIGURE 18.6 Thrombotic microangiopathy, secondary to abruptio placentae.** The dilated vascular pole is occluded by a thrombus. The change is similar to that seen on Figure 18.10, but no significant chronicity with reduplication of the basement membranes is present. (H&E)

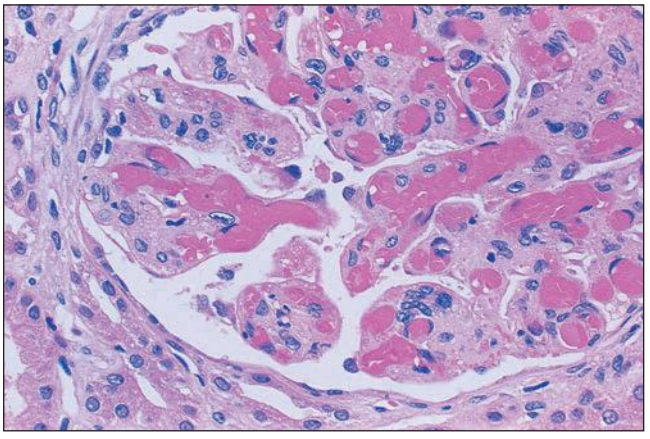

**FIGURE 18.8 Thrombotic thrombocytopenic purpura.** Most of the glomerular capillary lumina are occluded by homogenous eosinophilic thrombi. The extent of the glomerular capillary thrombosis in TTP is variable; however, it is often mild and patchy. The specimen is from a 40-year-old obese African American female who died a few days after initial presentation. Severe ADAMTS13 deficiency and a strong ADAMTS13 inhibitor were demonstrated in her serum. (H&E)

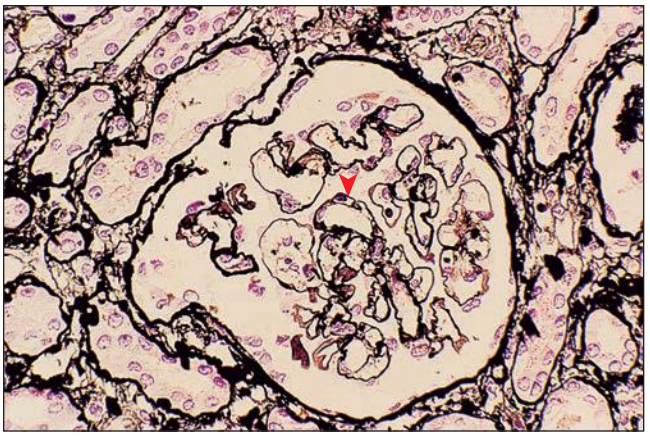

**FIGURE 18.9 Thrombotic microangiopathy, associated with mitomycin administration.** Ectatic glomerular capillary lumina are present as a result of mesangiolysis. Focal reduplication of the glomerular capillary basement membranes is also seen (arrowhead). (Methenamine-silver.)

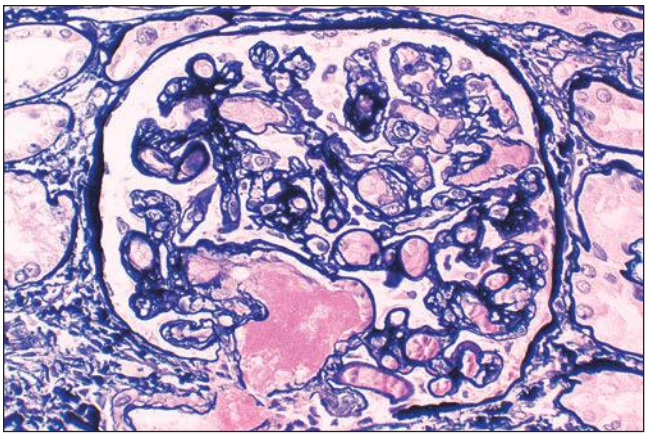

**FIGURE 18.10 Thrombotic microangiopathy, postpartum.** The dilated infundibulum is occluded by homogenous eosinophilic material (intraluminal thrombus). Extensive reduplication of the glomerular capillary basement membranes indicates developing chronicity. (Methenamine-silver.)

poorly because of mesangial edema. The borders of the dissolving mesangium are hazy, and the mesangial matrix is difficult to identify. No associated inflammatory reaction or fibrin deposition is usually seen (314). Eventually, the glomerular basement membranes become unanchored from the underlying dissolving mesangial mass, leading to markedly dilated, sometimes cystic capillaries (Fig. 18.9). A particularly severe and widespread form of mesangiolysis can occur in aHUS after bone marrow transplantation or mitomycin therapy. However, in classic HUS, mesangiolysis has rarely been described. Mesangiolysis can also be seen in diabetes mellitus, various forms of glomerulonephritis, and transplant glomerulopathy (315). "Healing" of mesangiolysis may lead to proliferating or sclerosing glomerular changes as the disease progresses. In the late stage of mesangiolysis, the mesangium may be thickened by pale fibrillary (sclerotic) material. This process of healing and sclerosing may lead to a distinctive pattern of glomerular sclerosis ("bland sclerosis") characterized by loss of glomerular cells and capillary lumina but with at least partial preservation of the lobular architecture.

Ischemic-type glomerular injury, characterized by collapse of the glomerular capillary tuft and thickening and wrinkling of the capillary basement membranes, during the acute stage of TMA usually indicates severe vascular lesions such as (a) arteriolar or arterial thrombi, (b) acute thickening of the arterial or arteriolar intima, or (c) coexistent chronic hypertensive vascular disease (arteriosclerosis). Focal necrotizing glomerular lesions can also be seen in TMA, albeit rarely. If present, they are usually small, affecting only a few capillary loops or a segment of the glomerulus. Sometimes, the necrotizing lesion is associated with arteriolar thrombosis and/or fibrinoid necrosis of the arteriolar wall.

In the more advanced stages of TMA, glomerular changes differ from those seen early in the course of the illness. This stage is characterized by mostly chronic-type glomerular changes such as mesangial widening resulting from matrix accumulation (mesangial sclerosis), thick capillary walls with occasional double contours, segmentally sclerotic lesions, and chronic ischemic glomerular injury. Activated mesangial cells can migrate along the glomerular capillary walls between the glomerular capillary endothelium and the lamina densa of glomerular basement membranes, with resulting mesangial cell interposition. Production of glomerular basement membrane–like material by glomerular capillary endothelial and migrating interposed mesangial cells results in a double contour ("tram-tracking") appearance of the glomerular capillary walls (Figs. 18.10 and 18.11; see Fig. 18.3). The double contour is composed of new (inner) basement membrane and the original (outer) basement membrane. In the more advanced stage of TMA, the double contours with the mesangial sclerosis and occasional mild hypercellularity may give rise to a pattern of glomerular injury reminiscent of a membranoproliferative glomerular lesion (see Fig. 18.11). Double contours of the glomerular capillary walls may apparently persist for several months or years.

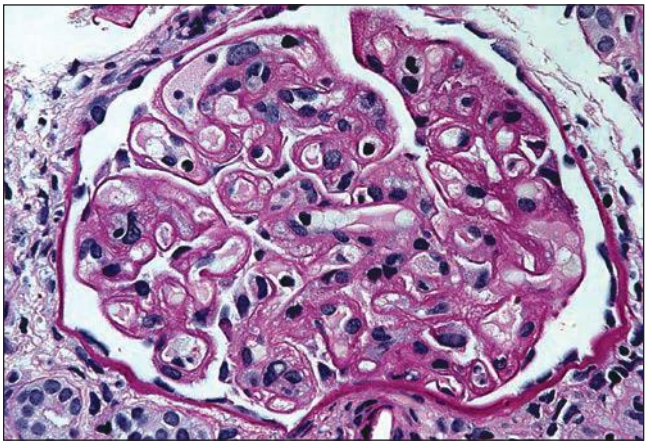

**FIGURE 18.11 Thrombotic microangiopathy with extensive reduplication of the glomerular capillary basement membranes.** This pattern is usually interpreted as chronic or advanced stage of TMA and may resemble a membranoproliferative pattern of glomerular injury. It should be emphasized that such extensive reduplication of the basement membranes as shown on the photograph is rarely seen. (Periodic acid-Schiff reaction.)

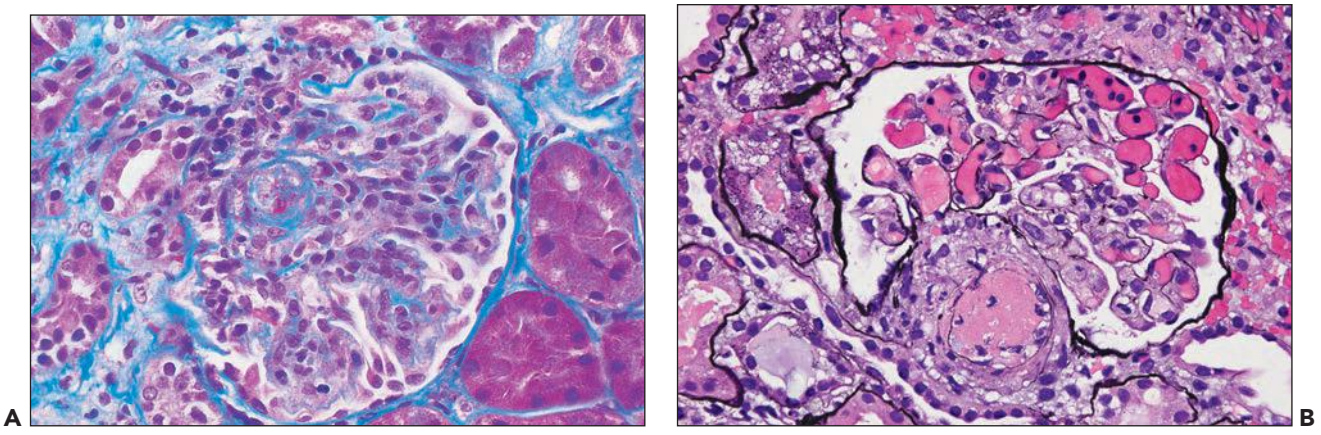


**FIGURE 18.12 Thrombotic microangiopathy superimposed on lupus nephritis. A:** The glomerulus is hypercellular with closure of some of the glomerular capillary lumina. There is a small thrombus in the arteriolar lumen. (Masson trichrome.) **B:** The arteriolar lumen is occluded by a thrombus. The glomerular capillary lumina are congested, but no capillary thrombi or cellular proliferation is present. Although this patient's serum was positive for antiphospholipid antibodies, a similar picture can also be seen in patients who are antiphospholipid antibody negative. (Methenamine-silver.)

Segmentally sclerotic glomerular lesions can occasionally be seen in cases with evolving chronicity of TMA. The lesions closely resemble those seen in idiopathic focal segmental glomerulosclerosis with focal and segmental collapse of the glomerular capillary lumina, mesangial matrix accumulation, and visceral epithelial cell hyperplasia overlying the sclerotic segments. These segmentally sclerotic changes may represent healed necrotizing glomerular lesions. Alternatively, chronic sclerosing-type glomerular injury with segmental features can also develop as part of evolving chronicity affecting the glomeruli.

Chronic ischemic-type glomerular injury is characterized by thickening and wrinkling of the glomerular capillary basement membranes, simplification of the glomerular tuft, widening of the Bowman space between the collapsed glomerular loops and the Bowman capsule, and collagen accumulation internal to the Bowman capsule replacing the Bowman space. The ischemic changes may affect the glomeruli globally or segmentally. Simplification refers to shrinkage of the glomerular capillary tuft accompanied by an apparent decrease in the number of normal glomerular lobules and by apparent loss of the mesangial matrix and cells. Accumulation of PAS-negative collagen material inside the Bowman capsule usually begins at the hilar region of the glomerulus, and eventually the collagen may involve the entire circumference of the Bowman space. If the changes progress to complete glomerular ischemic obsolescence, the glomeruli appear as small, hypocellular, compact eosinophilic masses ("tombstones" or globally sclerotic glomeruli). However, with PAS stain, the collapsed PAS-positive glomerular tuft can easily be distinguished from PAS-negative collagenization of the Bowman space. In TTP, focal glomerular capillary wall thickening, mild proliferation, and sclerotic changes may be observed, but overall the glomerular changes are minor when compared to classic HUS. In patients who have TMA superimposed on glomerulonephritis (e.g., lupus glomerulonephritis), the glomeruli may be markedly hypercellular in addition to the typical changes of HUS (Fig. 18.12).

## Arteries and Arterioles

Various changes occur in the renal arteries and arterioles. Arteriolar and arterial changes are more common in patients with aHUS and TTP (87,309). In the early stages, renal arterioles show swelling of the endothelial cells and subendothelial space. The arteriolar lumen may be severely narrowed and, sometimes, fragmented red blood cells are seen in the thickened arteriolar wall. Infiltration of the arteriolar wall by fibrin may occur, a change often referred to as *fibrinoid necrosis* (Fig. 18.13). The term *fibrinoid necrosis* is probably a misnomer because little evidence indicates that cellular necrosis is a constant feature of the lesion. Fibrinoid necrosis is thought to be related to increased vascular permeability and nonspecific trapping of plasma proteins, including fibrin in arteriolar walls. Fibrinoid

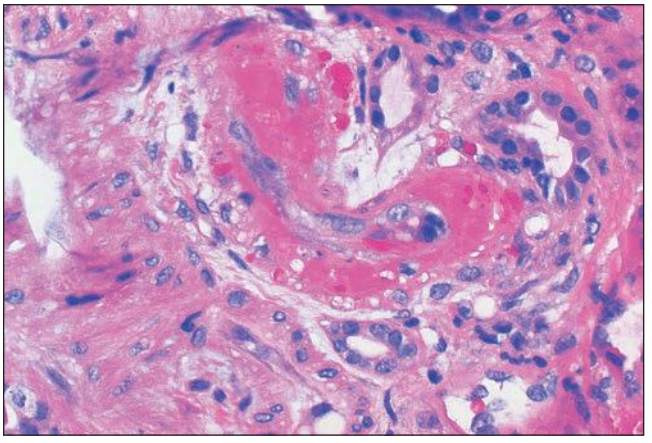

**FIGURE 18.13 Thrombotic microangiopathy with arteriolar fibrinoid necrosis in a patient with systemic lupus erythematosus (SLE).** In the setting of SLE, this finding is also referred to as lupus vasculopathy. Note the lack of inflammatory reaction in the vessel wall. Such finding can also be associated with intraluminal thrombosis affecting arterioles and/or glomerular capillary lumina. (H&E)

necrosis tends to occur only at the hilum of the glomerulus, and it may only involve the thickened intima of the arterioles. More often, however, the media of the arteriole is also affected. Unlike in true leukocytoclastic vasculitis, acute inflammatory cell infiltrate is rarely seen in fibrinoid necrosis with TMA. As the disease progresses, the involved arterioles tend to become hyalinized, losing the staining reactions for fibrin. Hyalinized arterioles show homogenous eosinophilic, refractile, strongly PAS-positive acellular material accumulated in the intima or media. Fibrin thrombi may also be seen in the afferent arterioles, and these may continue into the glomerular capillary tuft (see Figs. 18.10 and 18.12). One of the most conspicuous features of TTP is the presence of eosinophilic, platelet-rich granular thrombi in terminal renal interlobular arteries, or more commonly, in *afferent arterioles*. The most common site is the junction of the afferent arteriole and the glomerular tuft, sometimes called the infundibulum (Fig. 18.14). The thrombotic material in the lumen may merge with the arteriolar wall; therefore, it is often difficult to distinguish between fibrinoid necrosis of the arteriolar wall and the fibrin thrombus in the lumen. Sometimes both fresh and organizing thrombotic lesions can be recognized. Aneurysmal dilatation of arterioles sometimes takes place, particularly in the hilar region of the glomerulus, in patients with TTP (4,316). In addition, the arterioles may show proliferation of cells assumed to be endothelial cells; this change was first reported by Baehr et al. (2) and was later confirmed by several authors. The cellular proliferation in the arterioles is sometimes a prominent feature, and the collections of cells, often concentrically arranged, may attain the size of glomeruli. Capillary channels with or without an edematous extracellular matrix can sometimes be recognized. Lipid-containing macrophages may be present in the intima of small renal arteries undergoing proliferative changes. Because these proliferations resemble glomeruli, they are referred to as *glomera*, or *glomeruloid structures* (317). Glomeruloid structures are typically described in TTP but may also be seen in HUS, SLE, and various forms of glomerulonephritis (317).

Interlobular renal arteries may show two major changes. One of the early abnormalities is swelling of the intima, which may be accompanied by a suffusion of red blood cells (some of which may be fragmented) or fibrin. Fibrin may appear deep in the intima and may permeate the wall extensively; as in arterioles, this lesion is also called *fibrinoid necrosis*. The second lesion is intimal swelling, which is usually sparsely cellular, containing mainly lucent amorphous material with a mucoid appearance (Fig. 18.15). This change is usually designated *mucoid intimal hyperplasia*; it may be severe, with marked narrowing of the lumen. Often, one sees a rapid proliferation of cells in the intima that might be regarded as organization of the intimal edema. The proliferating intimal cells are myointimal cells and are responsible for the cellular intimal thickening later during the course of the disease (Fig. 18.16). The cellular intimal proliferation may give rise to a pattern of change referred to as an "onion skin" lesion, which consists of concentric, ring-like layers of myointimal

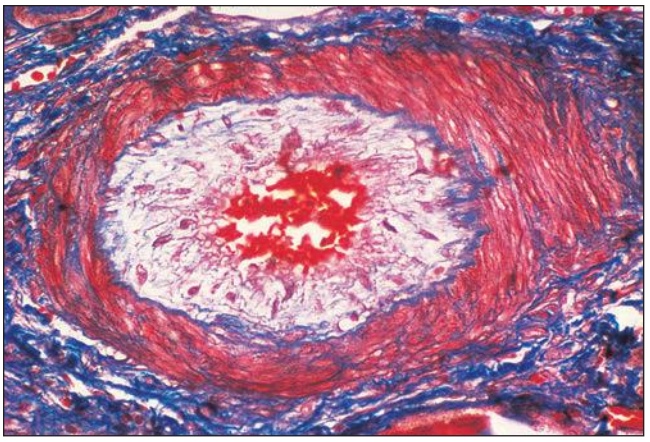

**FIGURE 18.15 Thrombotic microangiopathy secondary to malignant hypertension.** The small interlobular artery shows the edematous intima containing few myointimal cells ("mucoid intimal hyperplasia"). The patient presented with severe ("malignant") hypertension and acute renal failure. (Lendrum stain.)

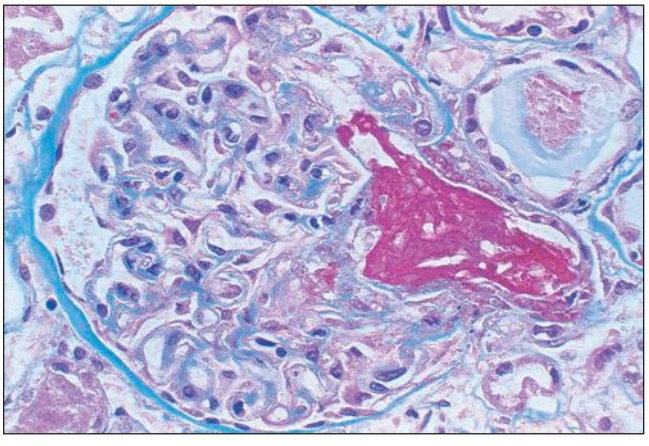

**FIGURE 18.14 Thrombotic thrombocytopenic purpura.** The infundibulum (i.e., vascular pole) is occluded by a large thrombus. The glomerular capillary walls are thickened; however, the glomerular capillary lumina are patent. (Masson trichrome.)

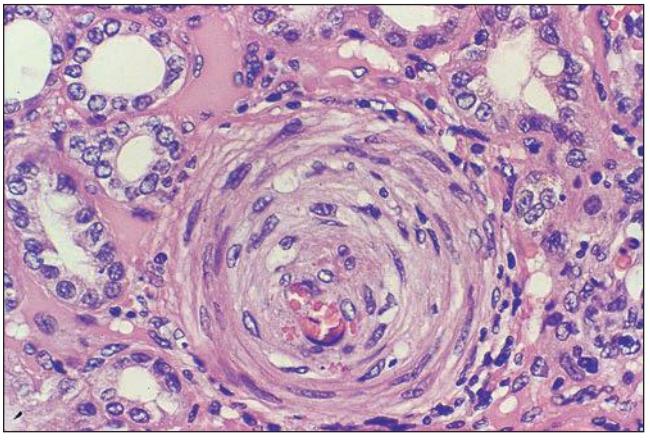

**FIGURE 18.16 Hemolytic-uremic syndrome, atypical form.** Prominent circumferential intimal cellular proliferation in a small interlobular artery in a nephrectomy specimen from a 5-year-old boy with aHUS. The child had severe hypertension. (H&E)

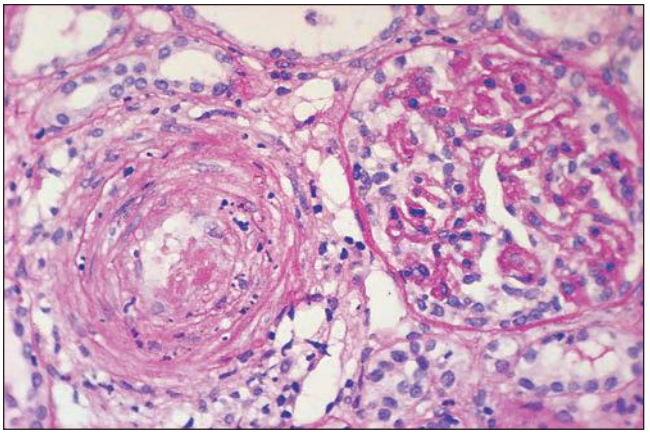

**FIGURE 18.17 Hemolytic-uremic syndrome, atypical form.** The interlobular artery shows luminal thrombus with nuclear debris in the arterial wall. The glomerulus exhibits ischemic features with thickening and wrinkling of the glomerular capillary basement membranes. The specimen is from an adult patient without known etiology of HUS. (Periodic acid-Schiff reaction.)

cells and delicate connective tissue fibrils. The arterial changes may result in severe narrowing or occlusion of the vascular lumen with consequent reduction in blood flow. These severe vascular changes are associated with a poor prognosis (318) and are responsible for the secondary ischemic glomerular changes such as shrinkage of glomerular capillary tufts and wrinkling and thickening of the capillary walls. Fibrous replacement of the thickened intima is a later change in the interlobular arteries. Occasionally, thrombosis and recanalization can also be seen in the interlobular arteries (Fig. 18.17). Changes similar to those seen in the interlobular arteries can also be present, although less frequently, in the larger (arcuate and intralobar) arteries. Especially in older patients with preexisting chronic hypertension, the acute vascular lesions of TMA may be superimposed on chronic vascular changes such as intimal fibroplasia, medial hypertrophy, or arteriolar or arterial hyalinosis.

### Tubules

The tubules frequently contain hyaline casts and red blood cells. Frank tubular epithelial necrosis may occur or acute tubular necrosis as is seen with ischemia may be present. Iron pigment and hyaline droplets in the cytoplasm of the proximal convoluted segment may be observed, with varying degrees of tubular loss. In later stages, tubular atrophy may be seen. In those cases with cortical necrosis, small patchy infarcts or larger necrotic areas are seen. Rarely, calcifications of the cortex can be widespread in chronic cases of cortical necrosis.

### Interstitium

The interstitium may be edematous or fibrous, and in some cases, it contains mild mononuclear cell infiltration. Large numbers of red blood cells are present in the interstitium in areas of cortical necrosis.

## Immunofluorescence Microscopy

In the *glomeruli*, the usual finding is the presence of fibrinogen or fibrin along the capillary loops in a continuous, broken linear or granular pattern; fibrinogen or fibrin is found less frequently in the mesangium (Fig. 18.18A). The deposits along the capillary walls may be accompanied by IgM, by C3, less frequently by IgG and only rarely by IgA (Fig. 18.19). Intracapillary thrombi also contain fibrinogen or fibrin and fibrin fragments.

*Arterioles and small arteries* often exhibit fibrinogen or fibrin in their walls, usually in a subendothelial position (see Fig. 18.18B). IgM positivity was described in vessel walls, as were C3, C1q, and IgG and IgA. Intravascular thrombi also show positive fluorescence for fibrinogen or fibrin.

Although the morphologic changes of TMA are similar in both TTP and HUS, some studies suggest that the composition of microthrombi in TTP might be different from those in HUS. On autopsy material from 23 patients with TTP, Asada et al. (319) showed strong immunohistochemical staining in the renal vascular thrombi as well as in the subendothelial hyaline deposits with factor VIII–related antigen, but only weak staining with fibrinogen or fibrin. This staining pattern was in contrast to the immunohistochemical results of thrombi in patients with disseminated intravascular coagulation in which strong fibrinogen

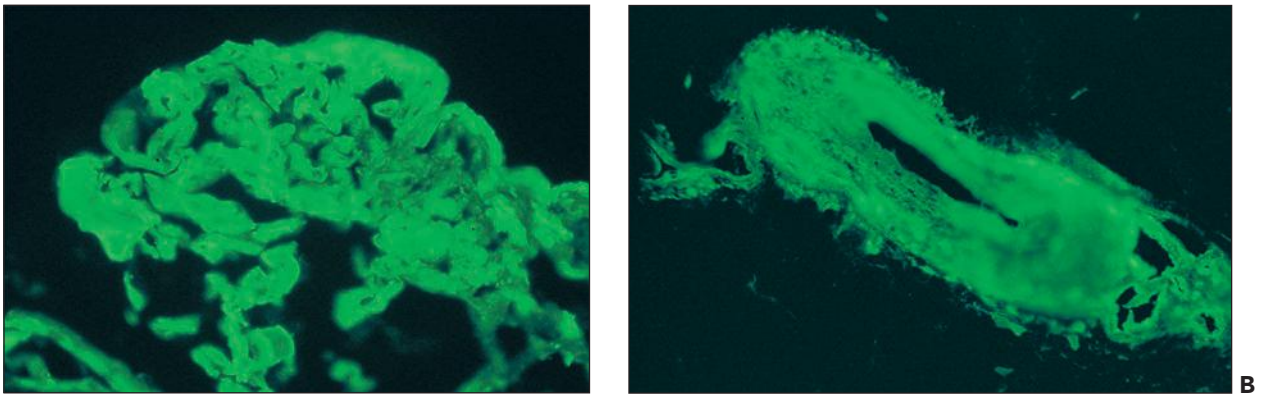


**FIGURE 18.18 Hemolytic-uremic syndrome (TMA).** The glomerular capillary walls and lumina **(A)** and the arterial wall **(B)** show strong fibrinogen positivity. The patient presented with severe ("malignant") hypertension, acute renal failure, MAHA, and thrombocytopenia. (Direct immunofluorescence.)

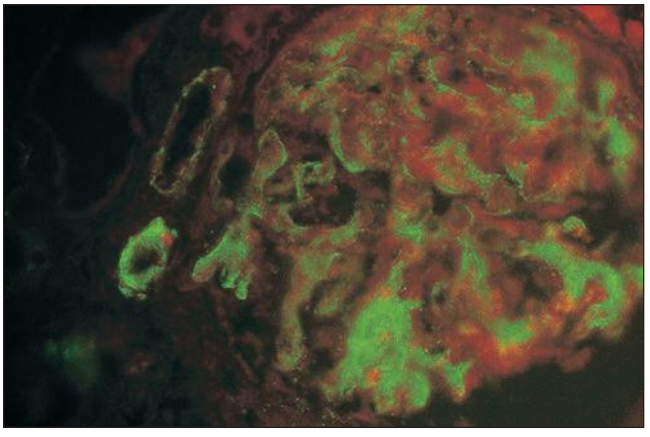

**FIGURE 18.19 Thrombotic microangiopathy.** IgM glomerular positivity in a patient with postpartum HUS. The arteriolar wall is also positive. (Direct immunofluorescence.)

or fibrin and weak factor VIII–related antigen staining was observed. Electron microscopic analysis showed numerous platelets in the glomerular capillary thrombi in TTP. The authors concluded that thrombi in TTP are composed of platelets. The strong subendothelial factor VIII–related antigen positivity was interpreted by these investigators as suggesting that the hyaline deposits are not a result of increased vascular permeability, but rather platelet thrombi incorporated into the vascular wall. Similarly, a more recent autopsy study of 25 patients with TTP and 31 patients with HUS demonstrated the histochemical and immunohistochemical differences in thrombi (320). HUS lesions contained a large component of fibrin, highlighted by phosphotungstic acid-hematoxylin stain, but only a few platelets or vWF (320,321). On the other hand, TTP-associated arterial thrombi had abundant platelets that stained for antibody to factor VIII. The TTP microthrombi were also rich in vWF but contained very little fibrin (322). Despite these apparent differences in HUS and TTP microthrombi, given the extensive clinical overlap between aHUS and TTP, the histologic accuracy and validity of these findings are unclear.

## Electron Microscopy

In the *glomeruli*, the most consistent change is a thickening of the capillary wall resulting from widening of the subendothelial space and swelling of the endothelial cells. The acellular subendothelial "fluff" is pale and rarefied and contains irregular collections of electron-dense material (Fig. 18.20). This subendothelial material in the lamina rara interna of the glomerular basement membrane is usually granular and has a variable electron density. Sometimes, it has a fibrillar or beaded appearance, but usually it lacks the periodicity and electron density of fully developed fibrin. The exact nature of the subendothelial material is unknown, but it is thought to represent the breakdown products of intravascular coagulation or cell debris organized into the capillary wall. Signs of endothelial damage may occur early during the course of TMA, with features such as swelling, localized areas of detachment of the endothelial cytoplasm from the basement membrane, and cytolysis. One may see intracapillary thrombi composed of amorphous osmiophilic material admixed with fibrin, platelets, and deformed red blood cells (Fig. 18.21). The platelets may be numerous and may fill the capillary lumen. Platelets can sometimes be seen between the endothelial cells; occasionally, the remnants of platelets are visible within the lamina densa of the glomerular capillary basement membrane. However, platelets are evanescent and may not be identified. Electron-dense fibrillar fibrin wisps are sometimes a conspicuous feature in the lumina and rarely in the subendothelial space. The swollen mesangial matrix appears as a meshwork filled with electron-dense, finely granular or fibrillar material similar to the subendothelial changes. Mesangiolysis may be seen, and this progressive disintegration results in capillary ectasia. One sees frequent visceral epithelial foot process effacement.

In the older lesions, wrinkling and collapse of the glomerular basement membranes may occur and are conspicuous in some cases. Multiple layers of material resembling basement membrane are often seen in the capillary wall with cellular (mesangial) interposition (Fig. 18.22). Small, rounded particles that are 40 nm in diameter and are limited by a thin membrane have been described in the cytoplasm of the endothelial cells.

*Arterioles and arteries* show changes in the endothelial cells similar to those seen in the glomeruli. One sees swelling, cytolysis, and detachment of the endothelium from the underlying structures with a widening of the intima. The intima has a lucent appearance with strands or granules of greater electron density (Fig. 18.23). Structures consistent with fibrin are found at various depths of the vessel wall; luminal thrombi made up of platelets, fibrin, and electron-dense material may be present. In the later stages, elongated myointimal cells abound in the thickened intima.

## Differential Diagnosis

The chronic stage of TMA with a membranoproliferative-type glomerular injury may raise differential diagnostic considerations, such as membranoproliferative glomerulonephritis, cryoglobulinemic glomerulonephritis, glomerular lesions with paraprotein deposition, and in a transplant setting, transplant glomerulopathy (323). Light microscopic differentiation of membranoproliferative glomerulonephritis from membranoproliferative-type glomerular injury of advanced TMA is usually straightforward; in TMA, the double contours of the glomeruli are usually sparse, and no or only minor mesangial hypercellularity is present. In addition, electron microscopy does not disclose discrete, electron-dense, immune-type deposits in advanced TMA.

There is a growing consensus among experts that transplant glomerulopathy may represent a chronic smoldering form of glomerular TMA due to long-standing antibody-mediated injury. In renal transplant patients, the histologic demarcation between glomerular manifestations of chronic AMR, i.e., transplant glomerulopathy, and hepatitis C–associated membranoproliferative glomerulonephritis might be blurred (254,324). In many instances, these entities coexist due to multiple pathways of injury in an allograft, causing diagnostic challenges. The presence of donor-specific antibodies and positive C4d staining favor humoral rejection; however, concurrent hepatitis C virus infection should raise consideration for hepatitis C–related glomerular disease. Hepatitis C–associated glomerular injury in an allograft may be altered due to immunosuppression permitting excessive viral replication, but limiting antibody production. In such circumstances, the characteristic immune complex deposits of membranoproliferative

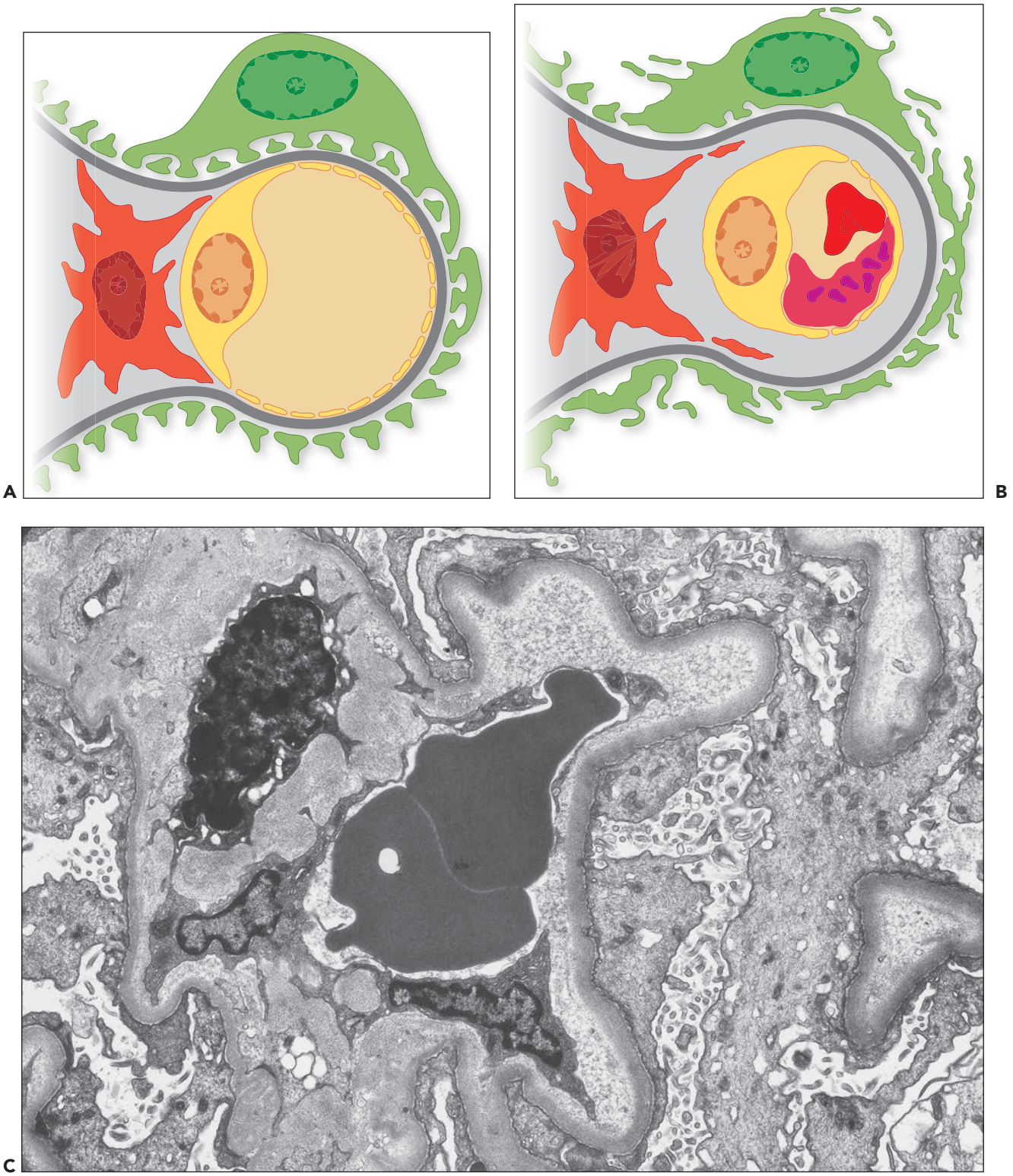


**FIGURE 18.20** **A:** Diagram depicting one normal glomerular capillary (green, podocyte; dark gray, GBM; yellow, endothelial cell; red, mesangial cell; light gray, mesangial matrix). **B:** Diagram depicting a glomerular capillary in HUS with expansion of a lucent subendothelial zone and a thrombus in the lumen containing platelets (*purple*). **C:** Electron micrograph of a glomerular capillary loop in HUS. The subendothelial zone is lucent and contains fluffy material (×6400).

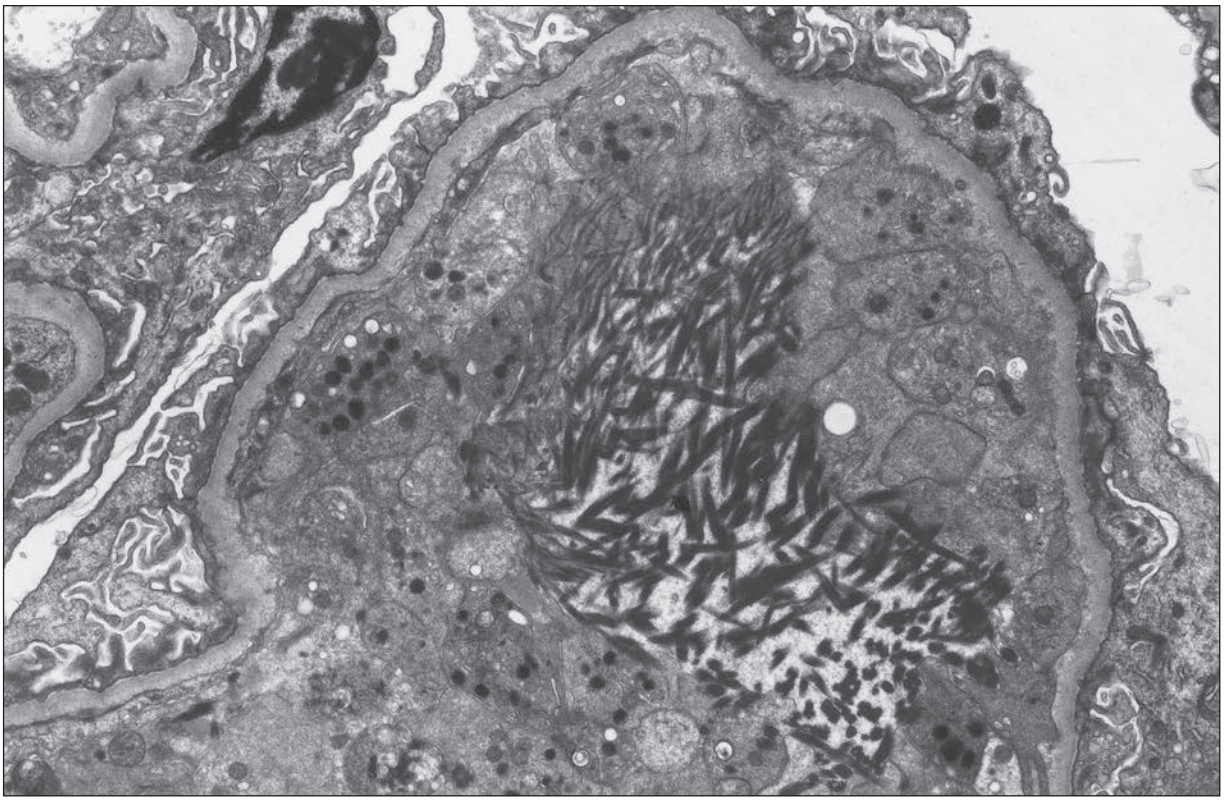

**FIGURE 18.21 Electron micrograph of a glomerular capillary loop from a renal transplant recipient with cyclosporine-induced TMA.** Fibrin and platelets in the capillary loop are clearly recognizable. The foot processes of the visceral epithelial cells reveal moderate effacement (×14,000). (From Laszik Z. Thrombotic microangiopathies. In: Silva FG, D'Agati V, Nadasdy T, eds. *Renal Biopsy Interpretation*. New York: Churchill Livingstone, 1996.)

glomerulonephritis may be absent or reveal aberrant features. The relatively high incidence of hepatitis C virus infection in association with transplant glomerulopathy pattern glomerular injury posttransplantation (254) is suggestive of a potentially significant etiologic role of hepatitis C in this process. The significance of hepatitis C infection in causing chronic TMA-like changes in an allograft may have been underestimated until recently (325,326).

## Changes in Other Organs

Arteriolar and arterial changes may be confined to the kidney in HUS, but sometimes, small thrombi may also be seen in capillaries and arterioles or small arteries of other organs. An autopsy study of 65 pediatric patients with classic HUS showed that extrarenal pathologic changes were most common in the large bowel, followed by the CNS, the heart, and the pancreas (84). An earlier study found extrarenal microthrombi in eight of nine autopsies of children (83). The thrombi were associated with hemorrhagic colonic necrosis in six patients and with pancreatic islet cell necrosis in three patients; microthrombi were also present in the brain, heart, adrenals, and lung. In the colon, it was not possible to determine whether the capillary thrombi resulted from the primary process of HUS or whether they were secondary to nearby tissue necrosis. In another study, small infarcts of the liver or microscopic areas of cell death were described in association with fibrin thrombi in sinusoids, and small areas of infarction of the brain were recorded.

The distribution of lesions is not as well characterized in aHUS, especially given the extensive clinical overlap with TTP. Nevertheless, the microthrombi are mostly confined to the kidney in aHUS, with less frequent (only in 6 of 31 cases) involvement of other organs such as the pancreas, adrenal gland, and rarely brain and heart (320). Occasional reports of extrarenal vascular involvement in postpartum HUS include brain, liver, and central retinal artery damage (327,328). TMA induced by monoclonal antibodies against VEGF appears to be localized to the kidney (54). The blockade of VEGF production by podocytes alters the local VEGF signaling needed for the integrity of glomerular endothelial cells and results in loss of endothelial fenestrations and other characteristic morphology of TMA. In an autopsy series of 8 patients with HSCT-associated TMA, limited extrarenal manifestations of TMA were present in 3 patients, involving the lung, heart, or the pancreas (738).

One of the features of TTP is the widespread distribution of microthrombi in arterioles throughout the body, particularly in the brain, myocardium, adrenals, pancreas, intestinal tract, and spleen (320). Myocardial damage and necrosis have been increasingly recognized in TTP, contributed to some extent with the introduction of highly sensitive troponin assays (329). Ischemic changes may be seen in relation to these lesions in various tissues. The thrombosed vessels may show remarkably few changes, although endothelial proliferation similar to that seen in the renal arteries may be present. Antiphospholipid antibody syndrome (APS) is frequently associated with widespread TMA

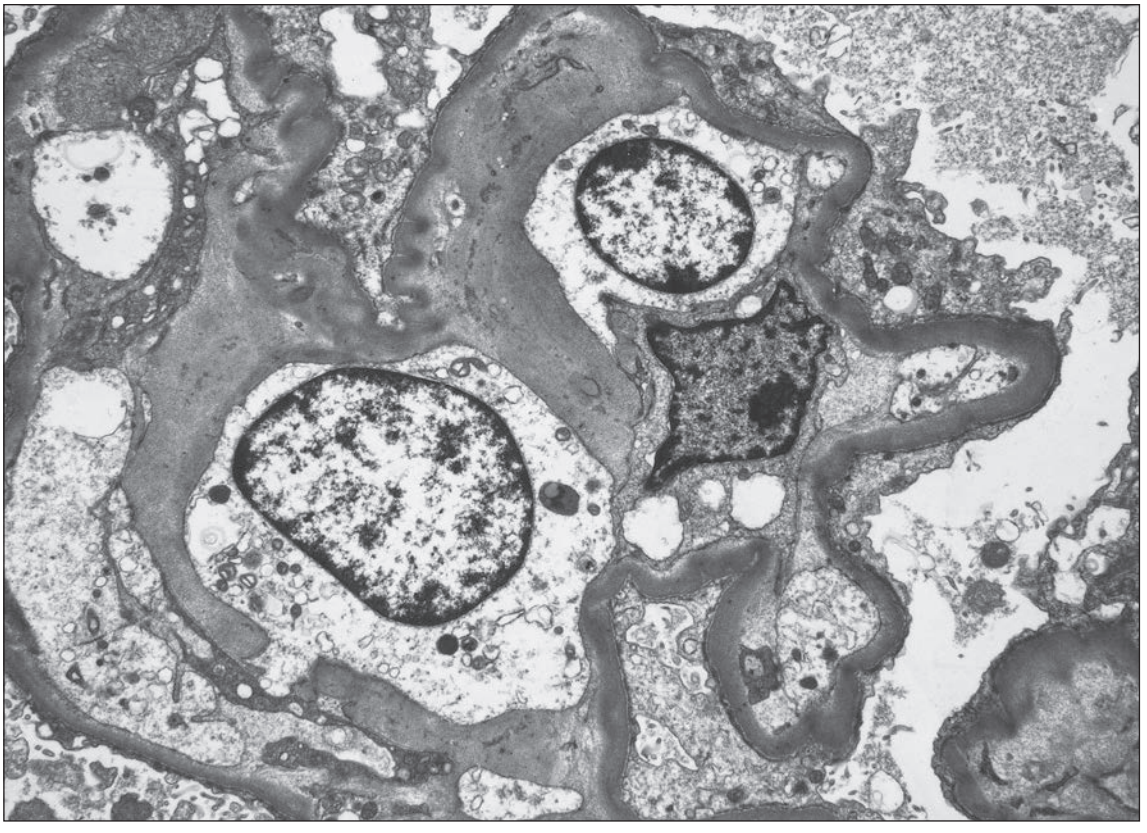

**FIGURE 18.22 Electron micrograph from a patient with TMA.** There is reduplication of the glomerular capillary basement membrane with cellular interposition. Severe thickening of the glomerular capillary basement membrane with an inner granular, moderately electron-dense layer containing some membrane-bound circular elements is also seen (×14,000).

in addition to arterial and venous thrombosis, sometimes leading to a "catastrophic" clinical presentation; virtually, any organ can be affected by such microangiopathic changes (330).

## Outcome and Prognostic Features

Until the 1970s, many children died during the acute anuric or oliguric stage in classic HUS. After the advent of hemodialysis, the overall mortality rate has declined to 12% in cases of HUS overall, and it is only about 4% with classic HUS (331). Although acute renal failure manifests in up to 70% of patients with classic HUS, most patients recover (48). At this time, acute renal failure has practically been eliminated as a cause of death in the acute phase of HUS.

Many of the historical data about prognosis and outcome of aHUS rely on series either predating the advent of plasma exchange therapy or those that applied therapeutic modalities that are outdated now. However, information obtained by these studies is still useful for comparison with newer studies and also for establishing the natural course of the disease without modern therapy. Most studies indicate that pediatric patients with aHUS have a worse prognosis and renal outcome than those with classic HUS (332,333). However, a study from the western United States showed that children with atypical disease had milder nephropathy and, with the exception of those with recurrent disease, did not experience a worse outcome (334). The prognosis is also much better in infants than in children. However, age in itself at the onset of HUS is not a prognostic feature (105). The difference in outcome between children and infants is most likely related to the high incidence of the atypical subset of HUS in children greater than 3 years old, a subset that is uncommon in infants and carries a worse prognosis.

A number of clinical-laboratory features such as the severity of gastrointestinal tract symptoms (335), the longer duration of dialysis (336), the duration of anuria (337,338), hypertension (337), initial peripheral leukocytosis (339), and neurologic involvement during the acute phase (340) have been proposed as predictors for poor long-term renal outcome in pediatric patients with HUS. However, many of these proposed prognostic factors have not been uniformly confirmed by various investigators. A large study from Italy that included 387 children with HUS with a median age of 1.9 years indicated concomitant absence of prodromal diarrhea and VTEC infection as the best indicators of poor renal prognosis (333).

Most patients with classic HUS survive the acute phase, but long-term follow-up suggests that it is not an entirely benign disease. Approximately 30% to 50% of such patients have subsequent evidence of chronic kidney disease and/or hypertension (341,342). When compared to age- and sex-matched controls with only *E. coli* O157:H7 gastroenteritis, children who recovered from classic HUS frequently have persistent microalbuminuria more than 3 years after the acute episode (343,344). A systematic review of the literature addressed the long-term prognosis of patients with classic HUS (105). Data on 3476 patients (aged 1 month to 18 years at recruitment) from 49 studies published between 1950 and 2001 with a mean follow-up of 4.4 years revealed 12% incidence of death or permanent ESRD

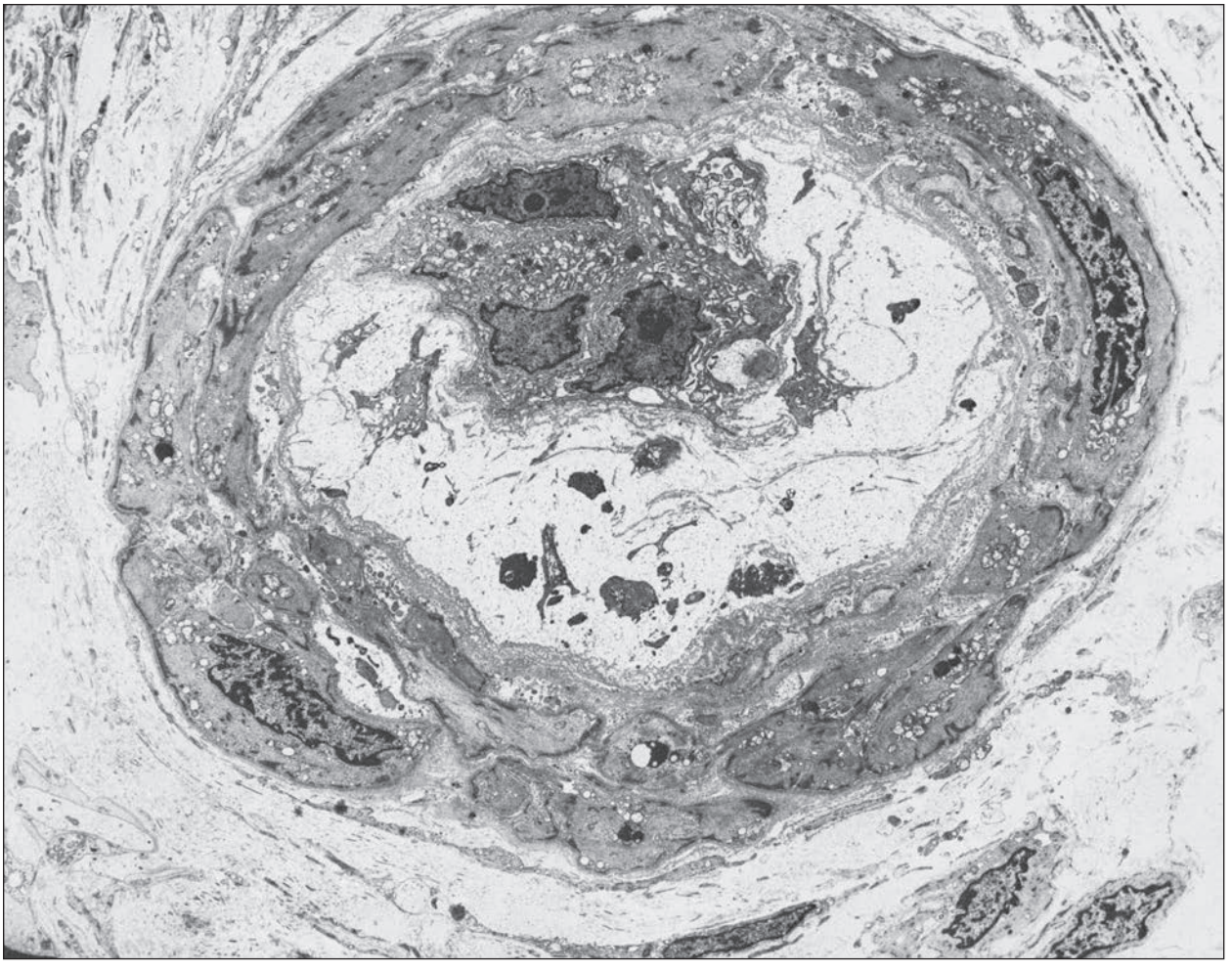

**FIGURE 18.23 Electron micrograph from a patient with TMA.** The arteriolar lumen is obliterated, and the subintima is widened by a lucent zone containing electron-dense strands and granules (×6000). (Courtesy of Dr. Tatiana Antonovych.)

(death: 9%, ESRD, 3%) and 25% incidence of long-term renal sequelae. However, the incidence of glomerular filtration rate lower than 80 mL/min per 1.73 $m^2$, hypertension, and proteinuria was extremely variable ranging from 0% to 64% in various studies. The higher severity of acute illness, particularly central nervous system symptoms and the need for initial dialysis, was strongly associated with worse long-term prognosis. It has been postulated that severe glomerular endothelial injury and consequent renal ischemia results in permanent renal capillary damage that predisposes to hypoxia-induced fibrosis (345,346).

In general, adults fare less well than children, likely influenced to a large extent by higher prevalence of aHUS in adults. Approximately 50% of patients with aHUS progress to end-stage kidney or have irreversible brain damage, and 25% may not survive the acute phase (35,309). In a French pediatric cohort, mortality during the acute phase of aHUS during the first episode was 8.6%, and 24% of those who survived developed ESRD (33). However, in an Italian cohort of aHUS with both adult and pediatric patients, the mortality at first episode was only 2% in adults and 12% in children, and 32% of survivors developed ESRD (34).

Recent studies have demonstrated that the prognosis of aHUS may be determined by specific causative gene mutation. Patients with *MCP* mutations have better prognosis than patients with *factor H* mutations (76). Relapses with complete recovery are characteristic of children who have aHUS due to mutations involving MCP (33,34). Although data are limited and there is risk of uncontrolled complement activation due to surgery, correction of the genetic defect by a combined liver-kidney transplantation in patients with *factor H* mutations may prevent recurrences and be associated with favorable long-term outcome (347,348).

Until recently, aHUS could not be clearly distinguished from TTP and results from various studies likely reflect prognosis of TMA due to both aHUS and TTP. A survey of cases of HUS/TTP in adults until 1973 revealed that 73% died of renal failure or had terminal renal failure requiring dialysis (349). In follow-up studies, the mortality rates in adult HUS/TTP were still relatively high, with ≤30% overall mortality (350–353). However, the outcome and prognosis of HUS in adults are less clearly defined than in children because adult HUS is less common and more heterogeneous than childhood HUS. A study from Canada presented data from 37 adult patients with HUS/TTP (352). Eleven patients had a prodrome of colitis, and in seven of these patients, VTEC infection was detected. In 10 patients, the HUS was related to underlying diseases, such as SLE or cancer. The remaining 16 patients had neither diarrheal illness nor an underlying disorder. The mortality rate was 30%; 14% developed chronic renal failure; and 24%

had recurrent episodes. None of the patients with colitis died, and no recurrences were noted in the "colitis" group. Those patients who developed HUS without colitis had a less favorable survival and experienced recurrences. The findings of this study prompted the conclusion that the disease or trigger (such as infection) that gives rise to HUS/TTP is the most important determinant of prognosis. These investigators also noted that most of these patients also had symptoms of TTP, thus making the separate prognostic evaluation of aHUS and TTP nearly impossible. Similarly, a wide variety of causes have been encountered for HUS/TTP in 55 adult patients seen between 1990 and 1998 in a study from France (353). In patients with the "primary forms" of HUS (i.e., HUS without underlying diseases or conditions such as HIV infection, malignancy, kidney, liver, or bone marrow transplantation, or nephropathies), the relatively low mortality rate (13.3%) and excellent renal outcome (73.3% of patients recovering normal renal function) were attributed to improved therapy (i.e., plasma infusions).

### Relation of Pathologic Picture to Clinical Findings and Prognosis

A comprehensive study of HUS in childhood and infancy was carried out by Habib et al. (318). Pathologic changes were correlated with the clinical picture and outcome. Three pathologic categories were distinguished: *cortical necrosis, predominantly glomerular involvement*, and *predominantly arterial involvement.* The prognosis was best in the group with predominantly glomerular involvement and by far the worst in the group with predominantly arterial involvement. The one with predominantly arterial involvement tended to occur in children, as opposed to infants. This is also the type that is common in adults.

Another study, by Thoenes and John (87), also showed that the histologic changes of TMA could be assorted into one of three categories. In the first group, the glomeruli showed the classic changes of TMA, accompanied by relatively mild arteriolar changes. This form was most frequent in young children, more susceptible to HUS rather than TTP. In the second group, severe obliterative changes predominated in the interlobular arteries. Arterioles showed fibrinoid changes, and the glomeruli revealed thickening and wrinkling of capillary walls and occasional fibrinoid necrosis. This form was most common in a heterogeneous group of adults with the clinical features of malignant hypertension, postpartum renal failure, acute renal failure, and acute or subacute glomerulonephritis. The third group, which showed mixed glomerular and arterial lesions, was usually seen in older children. A study by Morel-Maroger et al. (308) confirmed the importance of vascular lesions in a large series of TMA in adults. The presence of arterial occlusion was a harbinger of poor prognosis. In a follow-up clinicopathologic study of 34 pediatric patients with aHUS, renal arteriolar and/or arterial changes were identified in 15 patients (309); the presence of arterial disease correlated with a poor prognosis. This study emphasized the prognostic value of renal biopsy in aHUS. Another study from Iran (354) that semiquantitatively scored the histologic findings in the kidney biopsies of 29 children diagnosed with aHUS from 1992 to 2005 confirmed the importance of vascular damage in predicting their long-term outcome and development of chronic kidney disease.

The impact of the renal biopsy findings on the long-term outcome of classic (typical) childhood HUS was addressed in a study from the Necker-Enfants-Malades Hospital in Paris, France (355). Twenty-nine patients were followed for 15 to 28 years, and the long-term outcome showed a close correlation with the original biopsy findings. Ten of eleven patients with cortical necrosis developed ESRD ($n$ = 4), chronic renal failure ($n$ = 3), or significant residual renal functional abnormalities ($n$ = 3). The nine patients who had TMA involving less than 50% of the glomeruli were symptom free or had only mild renal sequelae.

However, a report from the Southwest Pediatric Nephrology Study Group (83) raised some doubt about the prognostic impact of renal biopsies from samples taken early after the onset of the disease. In this study of children, no arterial lesions were identified in renal tissues obtained during the first 16 days of hospitalization, as opposed to those biopsies from samples taken after 16 or more days of hospitalization. The explanation for the apparent absence of arterial lesions during the first 16 days was that arterial changes may evolve slowly over time and may not be recognizable at the light microscopic level in the early stages of the disease. Moreover, sampling error could be a significant factor in the interpretation of renal biopsy specimens from patients with HUS. Furthermore, only the most severely affected patients undergo biopsy, and greater than 90% of patients who do not undergo biopsy seem to be cases that spontaneously resolve.

In summary, the presence of renal arterial occlusive lesions determines the outcome in a given patient. As a rule, extensive glomerular changes in themselves may not necessarily signify a poor prognosis as long as the changes are confined to the glomeruli and are accompanied by only minor arteriolar changes. Exceptions to this rule exist, and extensive cortical necrosis may be seen in the presence of widespread glomerular lesions, but with sparing of the arteries. Information on the sequence of changes leading to chronic renal failure is scanty.

## ETIOLOGY AND PATHOGENESIS OF HEMOLYTIC-UREMIC SYNDROME, THROMBOTIC THROMBOCYTOPENIC PURPURA, AND OTHER THROMBOTIC MICROANGIOPATHIES

The basic pathologic abnormalities of TMA are endothelial damage, intimal thickening, coagulation both within the vessel wall and the vascular lumina, and the sequelae of vascular occlusion. Endothelial damage, characterized by swelling, cytolysis, and detachment of the endothelium from basement membranes, is seen in the glomeruli, arteries, and arterioles. Edema in the intima of small arteries and arterioles and the subendothelial zone of the glomerular capillaries, which suggests increased permeability of the endothelial cells, is one of the most typical morphologic findings. The presence of fibrin in blood vessel walls is probably related to increased permeability, although it may also represent an intravascular fibrin thrombus that has been incorporated into the vessel wall. Coagulation may be a direct consequence of endothelial damage. Platelets and fibrin are found in the glomeruli and small arteries and arterioles. Fibrin thrombi can also be seen in the glomerular capillaries and in the lumina of renal arterioles and small arteries. In addition, a particulate or flocculent electron-dense material is present in the widened subendothelial region of the glomerular capillary wall. The exact nature of the subendothelial material is not known, but it is clearly related to fibrinogen (or fibrin) or other coagulation proteins and probably some matrix proteins (e.g., fibronectin). In TTP, microthrombi are widely disseminated throughout the microvasculature typically

affecting several organs. However, microthrombi can also be seen in HUS, both classic and atypical, in organs other than the kidney, such as the colon, liver, pancreas, heart, and brain.

## Endothelial Damage

Endothelial damage is the crucial feature that precedes the development of additional vascular lesions. Injury to endothelium may result in switch of endothelial anticoagulant phenotype to procoagulant phenotype, decreased fibrinolytic activity, exposure of thrombogenic subendothelial surfaces, platelet activation, imbalance between prostacyclin ($PGI_2$) and thromboxane $A_2$ ($TXA_2$) production, release of unusually large vWF multimers, cellular activation with up-regulation of adhesion molecules, chemokines, cytokines, and transcription factors, inhibition of protein synthesis, apoptosis, and cell death. All of these factors may contribute to platelet aggregation, thrombus formation, and impaired removal of fibrin with subsequent severe vascular and organ damage. Although the pathogenetic pathways of the endothelial injury for some of the causative agents have been well delineated, for most of the proposed causative agents, the exact mechanism by which the endothelium is injured is still not fully understood. Some factors thought to play a major role in the endothelial injury in the pathogenesis are discussed.

## Classic HUS

### Shiga toxins

Shiga toxins (Stxs) play a critical role in the pathogenesis of classic HUS primarily by mediating endothelial cell injury (Fig. 18.24). The Stx family comprises two main groups, Stx1 and Stx2, each of which contains an increasing number of variants, such as Stx1c, Stx2c, Stx2d, Stx2e, Stx2f, and Stx2g (134). Structurally, both Stxs are composed of an enzymatically active A subunit of 32 kDa and a homopentameric 7.7-kDa B subunit that allows binding of the toxin to specific globotriaosylceramide (Gb3) cell surface receptors on susceptible host cells, including endothelial cells in the glomeruli, intestines,

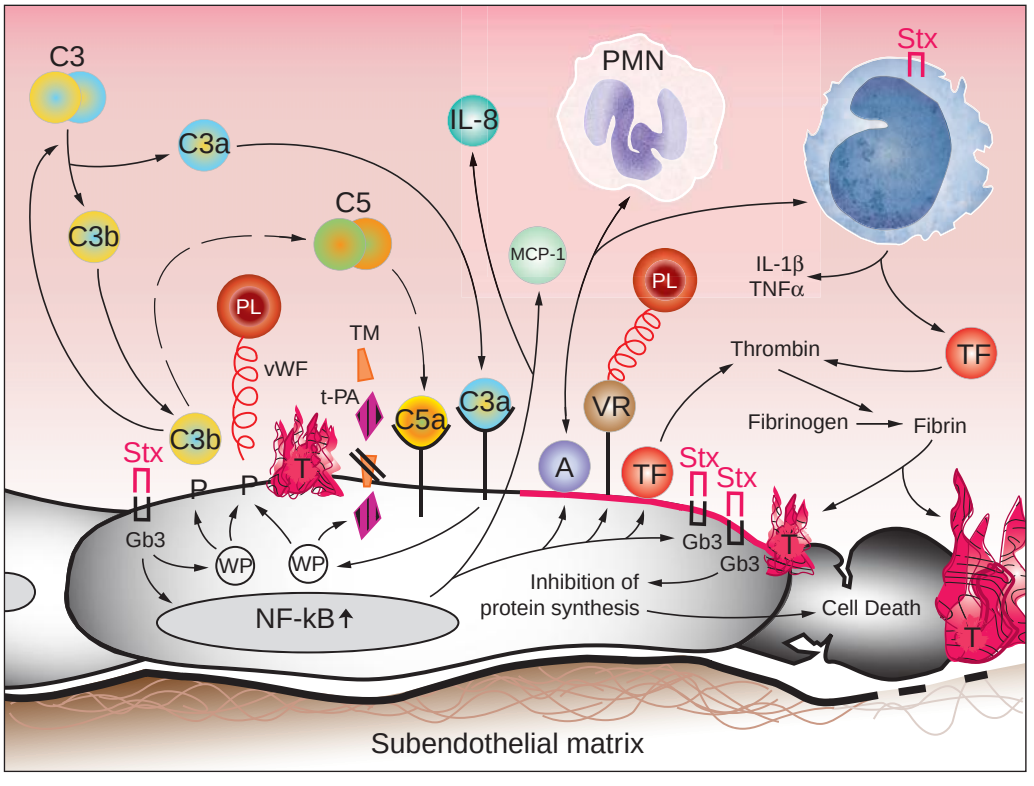


**FIGURE 18.24 The pathogenesis of microvascular thrombosis in classic HUS.** Upon binding of Shiga toxin (Stx) to its specific endothelial receptor Gb3, the endothelial cells acquire a proinflammatory and prothrombotic phenotype, leading to endothelial injury and microvascular thrombosis (T). At low doses, Stx inflicts endothelial injury by NF-κB–dependent up-regulation of endothelial MCP-1, IL-8, and various adhesion molecules (A), such as E-selectin, ICAM-1, and VCAM-1, that promote leukocyte recruitment and adhesion to the endothelial cells. Shiga toxin also induces surface mobilization of endothelial P-selectin (P) and release of the UL von Willebrand factor (vWF/ULvWF) multimers from the Weibel-Palade (WP) bodies. P-selectin is implicated in vWF-dependent platelet (PL) deposition and also serves as a high-affinity receptor for C3b promoting alternative complement activation with additional C3a receptor–potentiated release of ULvWF multimers, P-selectin, and tissue plasminogen activator (t-PA). Via t-PA–facilitated shedding of the endothelial thrombomodulin (TM) along with impaired cleavage of the ULvWF multimer-platelet strings and up-regulation of endothelial tissue factor (TF), microvascular thrombosis may develop. Additional factors promoting thrombosis include Stx-induced up-regulation of endothelial vitronectin receptor (VR) and release of cytokines and TF from activated monocytes. At high doses, Stx causes cessation of the protein synthesis with subsequent endothelial cell death, exposure of the subendothelial basement membrane, matrix and TF, and activation of platelets and the coagulation cascade, culminating in intravascular clot formation.

and brain, renal tubular epithelial cells, alveolar macrophages, and peripheral blood leukocytes (134,356). Binding to the receptor is followed by internalization of the toxin, trafficking via endosomes, and subsequent retrograde transport from the Golgi complex to the endoplasmic reticulum (357). Trafficking of the toxin from the endosomes to the Golgi can be blocked by manganese as shown recently (358).

From the endoplasmic reticulum, the A subunit is translocated to the cytosol where it inhibits protein synthesis through depurination of a specific adenosine in 28S ribosomal RNA (359,360). Cessation of protein synthesis initiates a cascade of reactions called the ribotoxic stress response, resulting in cell death (361). It is also well documented that Stx can trigger programmed cell death signaling pathways in various cell types, including human microvascular endothelial cells (362,363). The apoptotic mechanism has also been entertained as a potential contributor to the pathogenesis of HUS caused by STEC (364).

However, there is also evidence that at concentrations with only minor effects on protein synthesis, Stx has a dramatic effect on endothelial cell gene expression and RNA metabolism altering the endothelial cell phenotype (365). Stx-treated human endothelial cells show up-regulation of genes encoding for cytokines, chemokines, cell adhesion molecules, and transcription factors mediated by the nuclear factor–κB (NF-κB) and the tumor necrosis factor (TNF)/stress-related signaling pathways (366). Upon exposure to Stx, NF-κB–dependent up-regulation of monocyte chemoattractant protein-1 (MCP-1), IL-8, and fractalkine (FKN) promotes, along with various adhesion molecules, such as E-selectin, ICAM-1, and VCAM-1, leukocyte recruitment and adhesion to the endothelial cells (367–369). Exposure to Stx also renders the microvascular endothelial cells thrombogenic at high shear stress mediated by up-regulation of endothelial vitronectin receptor ($\alpha v\beta 3$) that leads to platelet deposition via vWF-dependent bridging mechanism (370). Thrombus formation in this setting is also dependent on Stx-induced up-regulation of endothelial P-selectin and PECAM-1, in part via interaction of these adhesion molecules with platelets. TNFα– and IL-1–induced up-regulation of Gb3, the endothelial receptor for Stx, can further potentiate endothelial sensitivity to Stx (371). Since TNFα, IL-1β, IL-6, and IL-8 can be released locally from monocytes and macrophages upon stimulation with Stx (372), the inflammatory cytokines may play a significant role in augmenting Stx-mediated endothelial injury.

In vitro data also show that Stx can induce endothelial secretion of strings of unusually large vWF multimers (373). This finding, coupled with the observation of impaired cleavage of the unusually large vWF-platelet strings by ADAMTS13, in the presence of Stx is strongly suggestive of potential pathogenetic significance in classic HUS (373). It has also been reported that the B subunits of both Stx1 and Stx2 are sufficient to stimulate the secretion of ultra large vWF and to support platelet adhesion on human endothelial cells (374) and that Stx1B and Stx2B use different signaling pathways to induce vWF secretion (375).

TF is an activator of the extrinsic pathway of coagulation that leads to the generation of thrombin, conversion of fibrinogen to fibrin, and the formation of insoluble fibrin clot. In vitro, Stx and TNF-α stimulate TF expression in human glomerular endothelial cells that can be further enhanced by exogenous angiotensin II (376). Induced endothelial expression of TF by Stx along with downstream generation of thrombin, a potent stimulator of platelet activation, can further augment the prothrombotic forces in the microcirculation, a feature relevant to the pathogenesis of HUS.

Recent evidence indicates that complement activation may play a crucial role in the development of thrombotic complications in classic HUS. In vitro and in vivo data by Morigi et al. (377) showed Stx-induced alternative complement activation via P-selectin as a key mechanism of C3a-dependent microvascular thrombosis in classic HUS. Complement activation in response to Stx generated an increased amount of C3a that caused further endothelial P-selectin expression, loss of endothelial thrombomodulin, and thrombus formation (377). The authors also demonstrated in a murine model of HUS induced by Stx2 and LPS that up-regulation of glomerular endothelial P-selectin was associated with C3 and fibrin deposits, platelet clumps, and reduced thrombomodulin expression. Activation of the alternative complement system has also been detected in the plasma samples of children with acute-onset classic HUS (378). A study by Stahl et al. (379) revealed increased levels of C3 on platelet-leukocyte complexes in a patient with classic HUS suggestive of the pathogenetic role of inflammatory and thrombogenic events in classic HUS.

New data suggest that the CXCR4/stromal cell–derived factor 1 (SDF-1) chemokine pathway may also play a significant role in the pathophysiology of classic HUS. In vivo and in vitro studies revealed up-regulation both the receptor (CXCR4) and its ligand (SDF1) upon stimulation with Stx (380). Under flow conditions, inhibition of the CXCR4/SDF-1 interaction prevented Stx-mediated adhesion of platelets to human microvascular endothelial cells in an in vitro system. Inhibition of the CXCR4/SDF-1 interaction also restored platelets to basal levels and significantly attenuated Stx-mediated mortality in a mouse model of HUS induced by Stx (380).

Stx1 and Stx2 bind to different epitopes of the Gb3 receptor with differences in the binding affinity and kinetics. Binding of Stx2 to the receptor is slower; however, dissociation from the receptor is also slower than for Stx1 allowing longer time for internalization (381). This might explain the significantly higher in vitro toxicity of Stx2 on human endothelial cells. It has also been demonstrated that up-regulation of the genes encoding proinflammatory molecules is more efficient when mediated by Stx2 than Stx1 (366,382,383). In general, there is a higher association of more severe disease caused by bacteria producing Stx2 than those producing Stx1 (119). STEC bacteria can possess one or more different Stx alleles, and the different Stx subtypes are associated with different clinical outcomes (128,384–386).

Stxs gain access to systemic circulation via translocation across polarized intestinal epithelial cells, a process facilitated by neutrophil transmigration (387,388). In vitro data indicate that the toxin may induce IL-8 expression in the intestinal epithelium (389); local cytokine production may trigger an inflammatory response that then contributes to the tissue damage and loss of colonic barrier function. Shiga toxin 2–induced up-regulation of endothelial IL-8 and MCP-1 production, also shown in vitro, may play a role in neutrophil adhesion and transmigration via endothelium (368). Although erythrocytes, platelets, and monocytes can all bind Stx, both in vitro and in vivo data seem to support polymorphonuclear leukocytes as the carriers of the toxin within the circulation (390,391). Transfer of Stx from the transporter (i.e., polymorphonuclear

leukocyte) to the endothelial cells is facilitated by the 100-fold higher affinity of the receptor on the endothelial cells (390). The extent of renal damage in children with STEC-associated HUS could depend on the concentration of Stx present on the polymorphonuclear leukocytes and presumably delivered by them to the kidney. Patients with high amounts of Stx on polymorphonuclear leukocytes showed preserved or slightly impaired renal function, whereas cases with low amounts of Stx usually presented evidence of acute renal failure (392). The authors of this paper hypothesized that high amounts of Stx could induce a reduced release of cytokines by the renal endothelium, with a consequent lower degree of inflammation. Conversely, low toxin amounts can trigger the cytokine cascade, provoking inflammation, thereby leading to more significant tissue damage. There is also in vitro evidence to show that under flow conditions, Stx2 up-regulates endothelial expression of MCP-1 and interleukin-8 (IL-8), both of which are important modulators of leukocyte adhesion and transmigration (368).

Endothelial cells, mainly those of renal microvasculature, are the primary target of Stx in STEC-associated HUS. Basal levels of Gb3 receptors were shown to be approximately 50 times higher in renal endothelial cells than in the umbilical endothelial cells (393); differential expression of Gb3 receptors may be responsible for preferential involvement of the kidney in HUS. In vivo studies also suggest that the organ distribution of Stx receptors determines the localization of microvascular lesions in rabbits injected with Stx1 (394). However, cells other than endothelial cells such as renal tubular epithelial cells expressing high levels of Gb3 may also be targeted by Stxs and may contribute to tissue injury (395).

Although Stx is clearly the most important pathogenetic factor in the classic form of HUS, factors other than Stx may also play a significant role in the development of the disease. In vitro synergism of cytotoxic potential has been described between lipopolysaccharide (LPS, endotoxin) and Stxs and also between TNFα and Stx (396,397). In a baboon model of Stx1-induced HUS, coadministration of low doses of Stx1 with endotoxin augmented the response of otherwise subtoxic amounts of Stx (398). Endotoxemia has been described in patients with shigellosis-associated HUS (141,399), and specific antibodies to the LPS of Stx-producing organisms have been detected in children with classic HUS (400). In addition, Heyderman et al. (401) described decreased levels of core antibodies to LPS in patients with severe classic HUS. These investigators proposed that, in severe classic HUS, enteric inflammation results in the dissemination of LPS into the systemic circulation, consumption of endotoxin core antibodies, activation of inflammatory cells, and disruption of endothelial function.

The potential role of anti-Stx antibodies in the pathogenesis and diagnosis of classic HUS have also been investigated. Anti-Stx antibodies can be detected in some healthy patients and at higher frequency in those with HUS (402). The incidence and the specificity of the antibodies (i.e., anti-Stx2 vs. anti-Stx1 antibodies) in healthy children show significant variation from country to country (402–404). However, much higher antibody titers in patients with HUS, especially those with anti-Stx2 specificity, may help differentiation of patients with acute HUS from those without HUS when pathogenic bacteria are not isolated from the stool (402,403). The role of anti-Stx antibodies has also been hypothesized in the development of neurologic complications as well as enhancing the toxic effects of Stx on the endothelial cells (79).

## Atypical HUS

### Complement Abnormalities

Complement dysregulation is the main cause of aHUS. Inherited and acquired abnormalities of the alternative complement system account for approximately 70% of cases, affect most patients with the familial and the sporadic forms of the disease, and also affect a few cases with the secondary forms (11,36,247). In approximately 30% of the cases with aHUS, the etiology remains unknown. The complement system is part of the innate immune system with a primary role of protecting against infections. The three activation pathways—classic, lectin, and the alternative—converge to produce C3 and C5 convertases that cleave C3 and C5, respectively, followed by the formation of membrane-attack complex (MAC). The alternative pathway in the plasma is continuously activated by hydrolysis of C3 (so-called "C3 tickover") with the formation of anaphylatoxin C3a and the opsonic fragment C3b, which is being deposited onto plasma-exposed surfaces, including endothelial cells (405). If unchecked, complement activation that follows may lead to complement-mediated injury of the host cells mediated by MAC (Fig. 18.25). To prevent of such self-inflicted injury, the complement system is tightly regulated by plasma membrane-bound factors, including CFH, complement factor I (CFI), MCP (CD46), and thrombomodulin (TM). CFI down-regulates the alternative and classical complement pathways by cleaving the alpha-chains of C3b and C4b to inactive forms (i.e., iC3b, C4c, and C4d) and dissociating the multicomponent C3 and C5 activity in the presence of cofactors, CFH, MCP, and TM. CFH binds to C3b in the fluid phase and also on membranes, and therefore it can regulate both fluid phase and membrane-bound alternative pathway amplification of C3b. Thrombomodulin, a transmembrane protein with anticoagulant and anti-inflammatory properties, regulates complement activation on cell surfaces (406). Genetic defects of each of these four complement regulatory proteins result in amplified production of C3 convertase, which leads to excess generation of C5 convertase and formation of C5a and MAC on the endothelial cell surface. Gain-of-function mutations in genes encoding the alternative pathway C3 convertase components, C3 and CFB, also result in chronic alternative pathway activation. In rare cases, antibodies against CFH are responsible for impaired regulatory function of CFH (407). In addition, common genetic variants in *CFH, CD46*, and *CFHRs* genes are known risk factors for the development of aHUS (408). In all these forms, endothelial cell injury with acquired prothrombotic phenotype and exposure of the subendothelial surface due to retraction or cell death trigger platelet activation, intravascular coagulation, and thrombus formation (409). Inflammation, also secondary to complement activation, as well as endothelial cell apoptosis, can further exacerbate the injury. The disease will only manifest in patients who have complement abnormalities coupled with a triggering event usually in the background of at-risk haplotypes (408). Hence, the penetrance is only 60% in the familial forms of the disease (76).

The specific forms of the alternative complement pathway abnormalities associated with aHUS are summarized in Table 18.5 along with some of the clinical characteristics and are further detailed below.

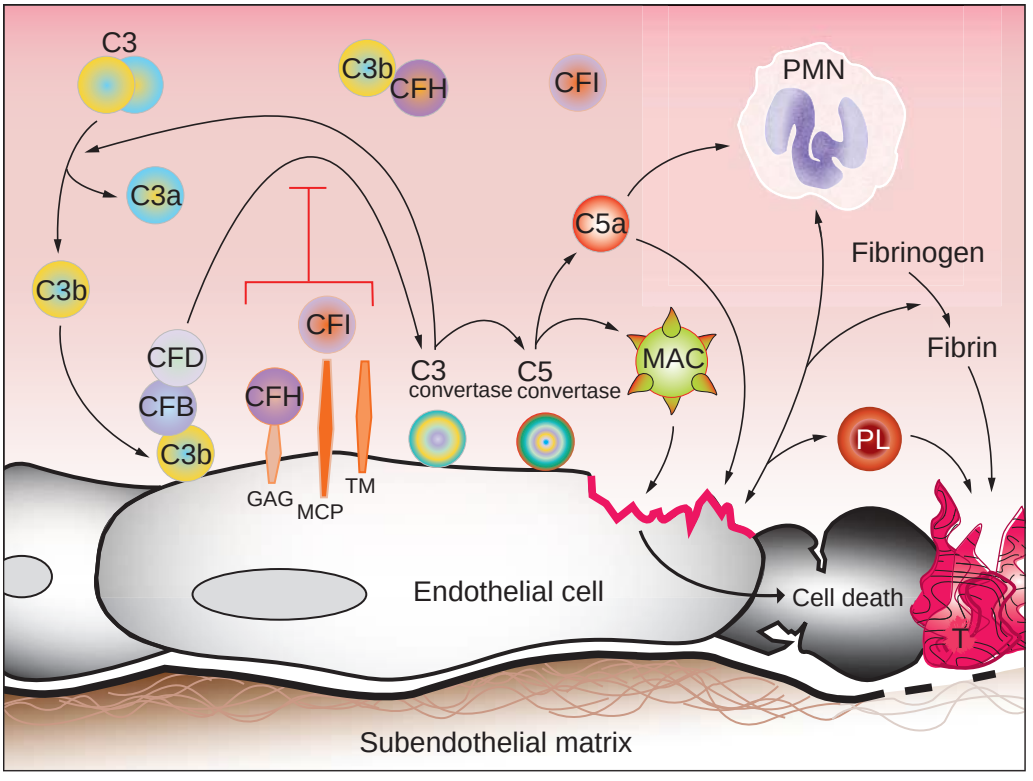


**FIGURE 18.25 The pathogenesis of atypical HUS due to dysregulation of the complement alternative pathway.** In this pathway, C3b binds to factor B (CFB) and is cleaved by factor D (CFD) to form the alternative C3 convertase. Under normal circumstances, host cells are protected from local amplification of endothelial cell surface–bound C3b, produced by continuous alternative pathway activation in the plasma (a process known as "tickover"), by complement regulatory proteins complement factor I (CFI) and complement factor H (CFH), in the presence of cofactors, membrane cofactor protein (MCP), and thrombomodulin (TM). Abnormalities affecting any of these 4 components and also C3 and complement factor B (CFB) result in amplified production of C3 convertase, which leads to the excess formation of C3a, C3b, C5a, and the membrane attack complex (MAC). Upon concerted effects from C3a, C5a, and MAC, the endothelial cells acquire a proinflammatory and procoagulant phenotype coupled with platelet activation, recruitment of inflammatory cells, onset of coagulation cascade, and clot formation. The proinflammatory and procoagulant phenotype is characterized by induced expression of endothelial leukocyte adhesion molecules, stimulation of endothelial prothrombinase and TF activity, cytokine production, and secretion of vWF multimers. Retraction of the endothelial cells by C5a also exposes the subendothelial basement membrane with thrombogenic substances including vWF and TF that trigger coagulation. Lytic doses of MAC have similar effects.

**TABLE 18.5 Clinical characteristics of patients with various subgroups of atypical hemolytic-uremic syndrome with complement dysregulation**

| Gene or subgroup | Frequency in aHUS | Minimal age at onset | | Risk of death or ESRD at first episode or within <1 yr | Risk of relapses | Risk of recurrence after renal transplantation | Percentage of pts with low C3 |
|---|---|---|---|---|---|---|---|
| | | Children | Adults | | | | |
| CFH | 20%–30% | Birth | Any age | 50%–70% | 50% | 75%–90% | 30–50 |
| CFI | 4%–10% | Birth | Any age | 50% | 10%–30% | 45%–80% | 20–30 |
| MCP | 5%–15% | >1 yr | Any age | 0%–6% | 70%–90% | <20% | 0–27 |
| C3 | 2%–10% | 7 mo | Any age | 60% | 50% | 40%–70% | 70–80 |
| CFB | 1%–4% | 1 mo | Any age | 50% | 3/3 not in ESRD | 100% | 100 |
| TM | 3%–5% | 6 mo | Rare | 50% | 30% | 1 patient | 50 |
| Anti-CFH Ab | 6% | Mostly | 7–11 y | 30%–40% | 40%–60% | Yes if high Ab titer | 40–60 |

CFH, factor H; CFI, factor I; MCP, membrane cofactor protein; CFB, factor B; TM, thrombomodulin; Ab, antibodies; ESRD, end-stage renal disease; pts, patients.

Adapted from Loirat C, Fremeaux-Bacchi V. Atypical hemolytic uremic syndrome. *Orphanet J Rare Dis* 2011;6:60.

### Complement Factor H

Among various mutations causing aHUS, those affecting CFH are the most common with more than 100 mutations identified so far (410). The mutation frequency of CFH is 40% to 45% in patients with the familial form of the disease and 10% to 20% in those with the sporadic forms (36). Most of the mutations are heterozygous clustering in the C-terminal of the molecule with diminished cofactor activity due to low binding of mutant forms to glycosaminoglycans on the endothelial cells or to surface-bound C3b (411,412). Reduced ability of C-terminal CFH mutants to bind C3b on platelets also leads to platelet activation and release of TF-containing microparticles promoting thrombosis (413). CFH levels are usually normal; however, the rare homozygous CFH mutations are associated with quantitative CFH deficiency and very low C3 levels (42). Plasma C3 level is decreased in 30% to 50% of patients with heterozygous mutations (11).

### Anticomplement Factor H Antibodies

Autoantibodies against CFH account for approximately 6% to 10% of cases with aHUS (11,36). Patients with the sporadic form of the disease are affected. The autoantibodies are directed against the C terminal of the molecule inhibiting CFH binding to C3b (414). The effects are similar to those with C-terminal FH mutations. Interestingly, about 90% of patients with anti-CFH autoantibodies have complete deficiency of factor H-related proteins (CFHR) 1 and 3 secondary to deletion of the *CFHR1* and *CFHR3* genes (415,416). Genetic abnormalities of *CFHR1, CFHR3, CFHR5*, and *CFHR1-CFHR4A* have also been reported (417,418).

### Membrane Cofactor Protein

Mutations of the gene encoding MCP have been described in 5% to 15% of patients with aHUS, and they are more frequent in children than in adults (11,419). Most of the mutations are heterozygous and approximately 25% are homozygous or compound heterozygous. The mutant forms may show decreased C3b binding capability, decreased cofactor activity, or decreased expression on blood leukocytes (76,420).

### Complement Factor I

Mutations of CFI account for 4% to 10% of patients with aHUS (11). Approximately 40 mutations have been reported, all heterozygous. The mutations result in low CFI levels in about 50% of patients (421). Others disrupt cofactor activity with altered degradation of C3b/C4b in the fluid phase and on surfaces (421). Mutations involving more than one gene in various combinations, such as *CFH-TM, CFH-CFI, CFH-CFI-MCP, CFI-MCP,* and *CFH-MCP,* have also been described (34).

### Complement Factor B

The rare heterozygous gain-of-function mutations of CFB account for approximately 1% to 4% of patients with aHUS (11,422). The mutated forms bind excessively to C3b, induce an increased stability and activity of the C3 convertase, with enhanced formation and deposition of C5b-9 complexes at endothelial cell surfaces (423). Permanent activation of the alternative pathway results in very low C3 levels in these patients. Plasma CFB levels may be normal or low.

### C3

Heterozygous mutations of C3 account for about 4% to 10% of patients with aHUS (11). Most C3 mutations reduce C3b binding to CFH and MCP, leading to impaired degradation of mutant C3b and increased formation of C3 convertase (424). Plasma C3 levels are low in the majority of patients.

### Thrombomodulin

In up to 3% to 5% of patients with aHUS, heterozygous mutations of the thrombomodulin (TM) gene are identified (11). Thrombomodulin, a key component in the protein C anticoagulant and anti-inflammatory pathway, facilitates complement inactivation by CFI in the presence of CFH (406). The mutant forms are less efficient in degrading C3b and in generating activated thrombin-activatable fibrinolysis inhibitor (TAFIa), a plasma carboxypeptidase B that cleaves C3a and C5a (406).

### Other Considerations

Reduced penetrance and variable inheritance are also characteristic features of aHUS with gene mutations. In two large independent cohorts with 152 patients, the penetrance of disease phenotype was reported to be approximately 50% (424b). A possible explanation for the incomplete penetrance is that polymorphic changes in complement regulatory proteins may act as modifiers. Specific single nucleotide polymorphism (SNP) variants of both CFH and MCP genes were shown to be associated with aHUS with concurrent mutations of the same genes (294,424b). Caprioli et al. (294) showed that the association between aHUS and specific SNP variants of the CFH gene was also present in patients without CFH mutations. In one of the two cohorts of patients included in the study of Fremeaux-Bacchi et al. (424b), the association between aHUS and specific SNP variants of the CFH gene was only present in those patients without known mutations in CFH, MCP, and IF genes. However, in the other cohort of patients from the same study, an association between aHUS and specific SNP variants of the CFH gene was only seen in those patients known to have a mutation.

Some patients may have complex genetic abnormalities. In two siblings described by Noris et al. (424c) who presented with recurrent familial TMA (one with exclusive neurologic symptoms and the other with severe renal involvement), both patients had severe ADAMTS13 deficiency as a result of two heterozygous mutations. In addition, a heterozygous mutation of factor H was found in the patient who developed chronic renal failure but not in her sister who presented with exclusive neurologic symptoms.

In summary, decreased levels or functional deficiencies of some of the complement regulatory proteins of the alternative pathway are associated with HUS in approximately 70% of patients with the atypical form of the disease. Currently, these heterogeneous abnormalities, both genetically determined and acquired, resulting primarily from the dysregulation of the alternative complement pathway, are considered to be predisposing factors for aHUS. Precise molecular mechanisms leading to HUS in these patients have not been delineated. Steady complement activation on the surface of the endothelial cells may result in endothelial cell activation and/or injury predisposing them to further injury leading to HUS in some patients. However, there are individuals with the predisposing genetic mutation(s) who remain asymptomatic pointing to additional extrinsic and/or intrinsic factors in the development of the disease. The familial forms with complement abnormalities reveal genetic heterogeneity, and a significant proportion of the sporadic forms also have genetic abnormality. However, approximately 30% of patients with the atypical form of HUS,

including those with decreased complement levels, still remain without identified etiologic factors.

### *Streptococcus pneumoniae*–Associated HUS

HUS may be associated with infections with neuraminidase-producing organisms (15,16,64,65,425–433). Neuraminidase, which is produced by certain bacteria such as *S. pneumoniae* and possibly some viruses, has the potential to damage endothelial cells, platelets, and also red blood cells. The pathogenesis has been attributed to the action of neuraminidase to expose the usually hidden T-crypt antigen (Thomsen-Friedenreich antigen) by removing part of the protective glycocalyx (*N*-acetylneuraminic acid) from membrane surfaces (89). Because most people have preformed circulating IgM antibodies to this antigen, the antigen-antibody reaction followed by complement activation through the classical pathway damages endothelial, red-cell, and platelet surfaces (89,434) and leads to intravascular thrombosis, hemolysis, and thrombocytopenia. Hemolysis caused by the direct action of neuraminidase on the red blood cells provides an explanation for those cases of HUS in which anemia occurs with scanty or no evidence of a TMA (9,20). The recent rise in the incidence of pneumococcal HUS has been attributed to the changing epidemiology of invasive pneumococcal infections since the introduction of heptavalent pneumococcal conjugate vaccine (PCV-7) (15,64).

## Thrombotic Thrombocytopenic Purpura

### von Willebrand Factor and ADAMTS13 Abnormalities

Endothelial cells are the primary source of circulating vWF multimers. However, vWF multimers that are produced by the endothelial cells are larger than those found in normal plasma. In normal circumstances, vWF-cleaving metalloprotease (ADAMTS13) degrades the secreted multimers by cleaving vWF on the surface of the endothelial cells at the $Tyr^{1605}$-$Met^{1606}$ peptide bond in the A2 domains of monomeric subunits (435,436). In addition, ADAMTS13 also cleaves vWF in the flowing blood (438) and at the sites of tissue injury (438b). Similar to that of vWF, the primary source of the ADAMTS13 is the endothelium (438c). Genetic or acquired deficiencies of ADAMTS13 activity result in deficient cleavage and persistence of unusually large (UL) vWF multimers (i.e., multimeric forms of vWF, larger than that in normal plasma) in circulation. High shear stress rates favor the interaction of large vWF multimers with platelet glycoprotein Ib-IX-V receptors that, in turn, activates platelets and promotes glycoprotein IIb-IIIa receptor–dependent platelet aggregation and formation of platelet thrombi in the microcirculation (435). Genetic deficiencies are linked to homozygous or compound heterozygous mutations of the ADAMTS13 gene, while the more common acquired deficiencies are due to circulating inhibitory immunoglobulin G (IgG) autoantibodies against ADAMTS13 (71,163,205,441–443). The autoantibodies bind the spacer domain of ADAMTS13, a region critical for recognition and proteolysis of vWF (163).

The ULvWF multimers are secreted from the Weibel-Palade bodies of the endothelial cells upon stimulation and remain, at least transiently, anchored to the endothelial cell membrane, possibly via P-selectin (444). Without cleavage by ADAMTS13, the strings of ULvWF multimers that are highly adherent to platelet glycoprotein Ibα-IX-V surface receptors (445) may form the niduses of platelet-rich thrombi, typical of TTP (446). In vitro studies indicate that some of the inflammatory cytokines (IL-8 and TNFα) may stimulate the release of ULvWF from the endothelial cells and that IL-6 inhibits the cleavage of ULvWF (447). This observation links inflammation to the accumulation of hyperreactive ULvWF, both in the plasma and on the surface of endothelial cells that may contribute to platelet aggregation and adhesion on the vascular endothelium. Although triggering events are rarely identified in patients with idiopathic TTP, accentuated secretion of vWF multimers by inflammatory cytokines may perhaps provoke the disease in some patients with low plasma ADAMTS13 activity (169).

However, in some patients who have ULvWF multimers in the circulation, the ADAMTS13 levels are normal (167). This raises the possibility that abnormalities of vWF-cleaving proteases other than ADAMTS13 or of disulfide isomerases may also be involved in the pathogenesis of some cases of TTP (448). Other patients may have persistent severe ADAMTS13 deficiency, either congenital or acquired with a demonstrable autoantibody inhibitor, but have no clinical signs of TTP.

The relationship between clinical disease phenotype and deficient ADAMTS13 activity has been addressed in a number of studies in patients with TTP and HUS (448–452). Although some studies reported nearly perfect correlation between TTP and severe deficiency in ADAMTS13 activity (449,453), other studies showed that a significant proportion of patients with the clinical diagnosis of HUS (typical, atypical, and familial) can also have severe ADAMTS13 deficiency (432,452,454,455). Other studies show that up to 50% of patients with idiopathic TTP present without detectable severe ADAMTS13 deficiency (441,453). Among those with the diagnosis of idiopathic TTP and severe ADAMTS13 deficiency, the incidence of autoantibodies was 62% in one study (453). Furthermore, severe ADAMTS13 deficiency has been well documented in patients with systemic infections and malignancies with and without concurrent ADAMTS13 inhibitors (172,302,456). Therefore, ADAMTS13 deficiency is not a reliable marker to distinguish TTP from HUS and is not specific for the diagnosis of TTP (74). Still, severe ADAMTS13 deficiency defines a subset of patients among TMA patients with distinct laboratory and clinical profiles (457). Patients with severe ADAMTS13 deficiency and TMA showed more relapses and significantly lower platelet count, medium hematocrit levels, and serum creatinine versus those patients who had TMA but did not have severe ADAMTS13 deficiency (457). In contrast, neither the presenting features nor the clinical outcomes were different in another study in patients with idiopathic TTP/HUS who had severe ADAMTS13 deficiency from those who did not (164). Many patients in this study in both categories responded to plasma exchange treatment, a clear indication that severe ADAMTS13 deficiency cannot be used as a basis for withholding plasma exchange treatment (164).

Some data indicate that ADAMTS13 levels and the presence of anti-ADAMTS13 antibodies during remission(s) may have prognostic ramifications for future disease recurrences. Patients with severely reduced levels of ADAMTS13 and/or with anti-ADAMTS13 antibodies during remission had an approximately threefold greater likelihood of developing another episode of TTP than patients with higher protease activity and no antibody (448). Recent data also indicate that higher anti-ADAMTS13 antibody titers may have predictive value for acute episode severity in patients with TTP. Higher IgA, IgG1, and IgG3 antibody titers during the acute phase were reported to correlate with disease severity in patients with TTP (458).

## Complement Abnormalities

There are also some data supporting the potential role of complement activation and polymorphonuclear leukocytes in the pathogenesis of TTP (459,460). In vitro, human microvascular endothelial cells showed C3 and MAC deposition and surface expression of P-selectin upon treatment with serum from patients with TTP (459). Neutrophils isolated from patients during the acute phase of the disease released excessive amounts of reactive-oxygen species (ROS), N-derived oxidants, and proteinases and induced damage and thromboresistance loss in human microvascular endothelial cells ex vivo (459). Réti et al. (460) reported features of both classical and alternative complement activation in patients with TTP.

## Systemic Infections

Severe systemic infections can mimic the clinical features of TTP or HUS. Out of the 451 patients in the Oklahoma TTP-HUS Registry, 31 (7%) had severe infections, 16 of which (52%) presented with the classic pentad of TTP, and 4 of them had severe ADAMTS13 deficiency with demonstrable inhibitor activity in 2 of them (172). A review of the literature conducted in conjunction with the analysis of the Oklahoma TTP-HUS Registry revealed a wide array of systemic infections in association with the clinical presentation of TTP. Although some of these infections, such as brucellosis, streptococcal infection with acute glomerulonephritis, angioinvasive fungal infections, CMV, HIV, ehrlichiosis, and Rocky Mountain spotted fever, may cause microvascular injury, in the majority of the cases, the etiology of the MAHA is uncertain. The possibility that some of these cases may have severe systemic infections with concurrent TTP or HUS, triggered by infection, has also been entertained (173). Well-documented association of certain infections and also inflammatory conditions with TTP supports the role of severe infections as potential triggers for TTP (169,174). Since TTP can never be excluded with certainty in patients who are acutely ill and present with MAHA and thrombocytopenia, these cases represent a difficult differential diagnostic and therapeutic challenge.

## Platelet Activation and Aggregation

Platelet activation and aggregation is considered to be a major feature in the pathogenesis of both HUS and TTP. Thrombocytopenia is caused by consumption of activated platelets in microthrombi and mechanical destruction in peripheral, damaged microvessels. Activation of the platelets can be initiated by multiple mechanisms, including endothelial damage, exposure of the subendothelium, shear stress, binding of agonists such as thrombin or thromboxane $A_2$, platelet alpha granule–derived chemokines, complement activation, Stx and LPS, bacterial virulence factors, monocyte and endothelium-derived chemokines, and ULvWF multimers (461). Platelet activation in TMA can be initiated by endothelial damage and thrombin generation, which is followed by platelet aggregation at the site of injury. Shiga toxin–induced endothelial cell activation can trigger platelet activation via induction of vitronectin receptor ($\alpha v\beta 3$) with the participation of vWF (370). Recent data also indicate that platelet activation occurs upon direct exposure to Stx and LPS in classic HUS, due to abnormal complement activation on the surface of the platelets in aHUS, and via ULvWF multimers anchored to P-selectin on the surface of the endothelial cells in TTP (413,462,463). In vitro data suggest a potential role of EHEC LPS in platelet activation via toll-like receptor 4 (TLR4) and CD62 in classic HUS (462). The same study also showed O157 LPS on circulating platelets in patients with classic HUS. A role of CFH in modulating platelet structure and function has also been demonstrated in aHUS (413,463). A reduced ability of C-terminal CFH mutants to bind to platelets enables complement activation on the surface of the platelets with subsequent platelet activation, which may contribute to the development of thrombocytopenia in aHUS. Platelet activation results in the release of various chemokines and cytokines, leading to endothelial cell activation, recruitment of inflammatory cells, and induction of TF expression by both platelets and monocytes (464).

## Coagulation Disturbances

Although intravascular coagulation is an important feature of HUS and TTP, no apparent clinical or laboratory signs of disseminated intravascular coagulation are usually present. The lack of disseminated intravascular coagulation in HUS and TTP may be due to an organ-limited activation of coagulation (i.e., kidney in HUS), or low-grade coagulation. Thrombotic changes in HUS and TTP can be explained on the basis of imbalance between fibrin formation and its removal. A defect in fibrin removal has been shown in human HUS by the demonstration of a plasma inhibitor of glomerular fibrinolysis. The circulating inhibitor of fibrinolysis in HUS has been identified as plasminogen activator inhibitor type 1 (PAI-1); normalization of plasma PAI-1 levels in children with HUS correlated with improvement in renal function (465). Elevated plasma levels of PAI-1 were later confirmed by another study of patients with classic HUS (466). Additional laboratory features of altered coagulation that had preceded the clinical onset of HUS included elevations in plasma concentrations of prothrombin fragment 1 + 2, tissue plasminogen activator (t-PA) antigen, t-PA-PAI-1 complex, and d-dimer levels. These findings were suggestive of thrombin generation and inhibition of fibrinolysis that precede and may also be the cause of renal injury in classic HUS. Up-regulation of PAI-1 was also shown in the glomeruli and arterial walls on renal biopsies from patients with TMA (467).

The potential role of TF in abnormal coagulation in classic HUS has been addressed by some studies. Elevated serum levels of TF and tissue factor pathway inhibitor (TFPI) were reported in patients during the acute phase of classic HUS (468). Thrombin activity in the circulating blood was inferred from the increase in thrombin antithrombin-III complex, and there was a correlation between plasma TF levels and plasma soluble thrombomodulin levels, a marker of endothelial cell injury.

A more recent study reported an increased number of microparticles with surface-bound TF, mainly originating from platelets and monocytes, in the plasma of patients with classic HUS (469). These patients also showed functional TF activity in their serum. In vitro, TF-containing microparticles were released from monocytes and platelets upon stimulation with Stx and LPS, an effect that was augmented by simultaneous exposure of Stx and LPS and also high shear stress (469).

## Inflammation

There is strong evidence indicating a significant role of neutrophils and monocytes in the pathogenesis of classic HUS. A high polymorphonuclear leukocyte count at presentation correlates with poor prognosis (339,470), and glomerular accumulation of mononuclear and polymorphonuclear leukocytes has also been documented in the kidneys of patients with classic HUS (311). Significantly elevated circulating levels of neutrophil activator IL-8 were shown to correlate with both

serum $\alpha_1$-antitrypsin–complexed elastase (a marker of neutrophil activation) and, in classic HUS, the circulating polymorphonuclear count (471).

Several lines of in vitro experimental evidence also support the pathogenetic role of neutrophils and monocytes in classic HUS. Upon stimulation with Stx, monocytes produce cytokines TNFα, IL-1β, IL-6, and IL-8 via transcriptional activation of NF-κB and AP-1 (372,472). Locally-produced cytokines can augment microvascular injury by up-regulation of the endothelial Stx receptor Gb3 and also via amplification of the inflammatory response (371). Direct interaction of the inflammatory cells with activated endothelial cells can further contribute to the endothelial cell injury. Activated neutrophils can generate superoxide anions ($O_2^-$) that, combined with endothelial-derived nitric oxide (NO), form the highly cytotoxic hydroxyl radical. Evidence indicates increased levels of oxidative stress during the acute stage of classic HUS in association with signs of elevated lipid peroxidation (473). Polymorphonuclear leukocytes stimulated by Stx induce apoptosis in cocultured endothelial cells (474).

## Antiphospholipid Antibodies

Antiphospholipid antibodies (i.e., lupus anticoagulants, anticardiolipin, and anti-β2-glycoprotein-1 antibodies) may prolong phospholipid-dependent coagulation times, but paradoxically, they are associated with an increased risk of venous and arterial thrombosis (475). Antiphospholipid syndrome (APS) is currently defined by the presence of at least one of two clinical criteria (vascular thrombosis or pregnancy morbidity) and at least one of three laboratory criteria (lupus anticoagulants, anticardiolipin, and/or anti-β2-glycoprotein-1 antibodies) (476). Historically, APS has been divided into three main groups; the primary form occurs without underlying diseases, while the secondary forms develop in the background of other diseases, among which SLE is the most common. Catastrophic APS is characterized by simultaneous vascular occlusions typically affecting small vessels of multiple organs (477,478). Antiphospholipid antibodies have also been described in association with microvascular thrombosis in various clinical conditions, such as primary APS, SLE, lupus-like syndrome, postpartum renal failure, preeclampsia-eclampsia, HELLP syndrome, catastrophic APS, HUS, TTP, malignant hypertension, and SSc (479). The most common renal manifestations of APS are those of microvascular thrombotic lesions, that is, TMA; however, arterial and arteriolar intimal fibroplasia with or without luminal occlusion, organized arterial thrombi, cortical infarction, focal cortical atrophy, renal vein thrombosis, membranous and proliferative glomerulonephritides, C3 nephropathy, pauci-immune crescentic glomerulonephritis, minimal change disease, and focal segmental glomerulosclerosis have also been described in association with APS (475,480–483). The term microangiopathic APS has been proposed for a subset of patients with APS who present with microvascular thrombotic complications (484).

The clinical presentation in patients with renal involvement varies based upon the nature of the underlying pathology; patients with acute TMA may present with rapidly progressive renal failure, proteinuria, sometimes in the nephrotic range, and severe hypertension, often in the malignant range (480), MAHA and thrombocytopenia can also be seen. In patients with chronic TMA, the clinical presentation is more subtle; systemic hypertension is common but variably associated with renal insufficiency, proteinuria, or hematuria (483,485). It has also been well documented that APS can lead to slow loss of renal function without history of apparent acute events. Chronic arterial and arteriolar changes with intimal fibroplasia, arterial and arteriolar fibrous and fibrocellular occlusions, features of chronic TMA, and nonspecific focal cortical atrophy are common findings in the kidney biopsies of these patients as shown in a study of primary APS (483). Renal TMA in patients with the secondary forms of APS develops in the background of various glomerular, vascular, and tubulointerstitial pathologies. Morphologic distinction of some of the APS-related chronic vascular changes from those secondary to hypertension could be challenging. Distinction might be aided by the apparent discrepancy between the morphologic severity of such lesions and the severity of the hypertension.

A systematic review of the literature identified 46 patients between 1983 and 2002 with antiphospholipid antibodies and thrombotic MAHA (486). More than half of the patients had primary APS (61%), one third had SLE (33%), while the remainder had either lupus-like syndrome (4%) or SSc (2%). The most common clinical manifestations were those of HUS (26%), catastrophic APS (23%), postpartum and pregnancy-related acute renal failure (13%), malignant hypertension (13%), and TTP (13%). Morphologic findings that were available from 32 patients indicated fibrin thrombi in the glomerular capillaries, arterioles, and interlobular arteries in 75%, 46%, and 33% of cases, respectively.

A detailed morphologic study by Nochy et al. (483) described a spectrum of small vessel vasoocclusive lesions, both acute and chronic, in patients with primary APS. These changes that include acute and chronic TMA, arterial and arteriolar intimal fibroplasia, vascular luminal occlusion, and focal cortical atrophy were also confirmed by other studies (485,487) and are now referred to as APS nephropathy.

However, the reports are somewhat conflicting about the relationship between antiphospholipid antibodies and the renal changes in SLE. Farrugia et al. (488) reported that the renal morphologic changes in most patients who had lupus anticoagulant-positive SLE and renal dysfunction were indistinguishable from those observed in patients with lupus anticoagulant-negative SLE and renal involvement. However, some of the lupus anticoagulant-positive patients had renal TMA, which was accompanied by a worse prognosis. In contrast, Bhandari et al. (489) reported an association between the presence of anticardiolipin antibodies and serum as well as pathologic markers of disease activity in a study of 51 patients with SLE and biopsy-proven nephritis. The correlation between the presence of intraglomerular thrombi and anticardiolipin antibodies was also significant. Moreover, the presence of thrombi was associated with a worse outcome.

A number of more recent studies support the notion that antiphospholipid antibodies in patients with SLE have both pathologic and clinical significance. In a study by Daugas et al. (490) of the 114 patients with SLE, APS nephropathy was identified in 32% of patients who had renal biopsies. Antiphospholipid syndrome nephropathy was statistically associated with lupus anticoagulants, and it was also an independent risk factor for more severely altered renal function. Another well-documented study of 151 patients with SLE reported a strong correlation between APS nephropathy and the presence of lupus anticoagulants (491). A more recent study from the same group also revealed that the frequency of chronic lesions of APS nephropathy was similar among those with SLE, primary APS, and catastrophic APS (485). A strong

association was found between lupus anticoagulant positivity and the development of chronic renal insufficiency in the long term in patients with SLE (492).

Catastrophic APS usually affects multiple organs simultaneously, with renal involvement being the most common (478). The lesions are typically those of severe acute TMA with an approximately 50% mortality rate. The onset of catastrophic APS is often triggered by various precipitating factors, including infections, surgical procedures, withdrawal of or inadequate anticoagulation, neoplasm, lupus flares, and drugs such as oral contraceptives (478).

Experimental data strongly suggest that antiphospholipid antibodies play a pathogenetic role in the thrombotic complications of APS via multiple pathways, including activation of endothelial cells, monocytes and platelets, inhibition of activated protein C and antithrombin activity, and complement activation (493). There is also evidence indicating that some of the antiphospholipid antibodies can hinder inactivation of procoagulant factors and resolution of clots by binding to proteases involved in hemostasis and fibrinolysis, such as plasmin, tissue plasminogen activator, and activated factor X (493,494). Antiphospholipid antibodies can activate the endothelial cells and monocytes leading to induced expression of adhesion molecules by the endothelial cells and up-regulation of TF expression by both the endothelial cells and monocytes (493,495). Antiphospholipid antibody–induced activation of the platelets may initiate platelet adhesion and thrombosis (496). NF-κB and p38 mitogen–activated protein kinase (p38 MAPK) are important mediators of these processes (493,495,497). Activation of the endothelial cells, platelets, and monocytes sets the stage for thrombotic complications that can be triggered by complement activation (498) perhaps in the presence of a second hit (493,499).

### Animal Models

Animal models that fully recapitulate the entire spectrum of Shiga toxin–mediated HUS as seen in humans following oral ingestion of bacteria are lacking. In a baboon model developed by Taylor et al. (500), the animals show thrombocytopenia, MAHA, and glomerular TMA but only with intravenous infusion of Stx 1. It has also been shown that 100 ng/kg of Stx 1 administered as a single bolus dose results in severe HUS, while administering the same total amount of toxin as four 25 ng/kg doses every 12 hours does not (501), unless LPS is administered concurrently (398). However, intravenous administration of four 25 ng/kg doses of Stx2 did cause HUS (147). These findings in baboons corroborate epidemiologic observations (119) and cell culture findings (366,383) regarding higher toxicity of Stx2.

There are also subprimate models of Stx-mediated HUS, including those in ferrets (502), canines (503), pigs (504), and mice (377,505). Ferrets were shown to develop glomerular lesions mimicking those seen in HUS following oral infection with *E. coli* O157:H7 (502). Also, oral infection of gnotobiotic pigs with *E. coli* 157:H7 or O26:H11 results in renal TMA with arteriolar and glomerular involvement similar to that seen in humans (504). However, thrombocytopenia, MAHA, and renal failure, typical of human disease, are lacking in this model. Intraperitoneal coinjection of purified Stx2 with LPS in C57BL/6 mice results in thrombocytopenia, hemolytic anemia, and renal failure with glomerular microthrombi closely mimicking human HUS (506).

Administration of an extrinsic toxin (Stx) to ADAMTS13-deficient mice with a genetic background of CASA/Rk (a mouse strain with elevated plasma vWF) triggers TTP (507). However, in ADAMTS13-deficient mice that were also either haploinsufficient ($vWF^{+/-}$) or completely deficient ($vWF^{-/-}$) in vWF, the lack of vWF resulted in complete protection from Stx-induced thrombocytopenia (508).

### Summary of Pathogenesis

The pathogenesis of HUS and TTP is complex, but it appears best explained by regarding endothelial damage as the initial event. Predisposing factors for the development of the disease include hereditary and acquired deficiencies of various complement regulatory proteins and the vWF-cleaving protease. Endothelial damage may be initiated by various factors including Stx, neuraminidase, lytic antiendothelial cell antibodies, apoptosis-inducing factor, cyclosporine, and mitomycin. Endotoxin, proinflammatory cytokines, and polymorphonuclear leukocytes may also contribute to endothelial damage. Changes in the endothelial cell anticoagulant and procoagulant properties, decrease in $PGI_2$ formation, appearance of UL vWF multimers, and exposure of thrombogenic subendothelial surfaces lead to platelet activation and aggregation. In turn, these factors are responsible for the formation of thrombi and cellular proliferation in the glomeruli and arterioles. Red blood cell hemolysis is caused by mechanical disruption in traversing fibrin meshwork or by the direct action of neuraminidase and other circulating antibodies. Thrombocytopenia is produced in several ways, which include consumption in thrombi, circulating platelet-aggregating factors, Stx, neuraminidase, and vWF.

## TREATMENT OF TMA

Up until very recently, the mainstay for the treatment of all adult patients who fulfilled the clinical diagnostic criteria of TMA has been plasma exchange (9). Especially in those patients with the possibility of autoantibodies blocking ADAMTS13 activity adjuvant treatment with corticosteroids has also been applied. Some patients, especially those with an exacerbation after a response or a relapse after a remission, may also benefit from rituximab treatment (9). Inhibition of the terminal complement complex formation by the monoclonal C5 antibody eculizumab is the newest treatment option for patients with some forms of TMA. Eculizumab has been successfully applied to patients with aHUS, classic HUS, and also in renal transplants for recurrence of TMA (509–511). Combined liver-kidney transplants have been performed to treat patients who developed ESRD due to aHUS with CFH or CFB mutations, with limited success (11).

## SYSTEMIC SCLEROSIS (SYSTEMIC SCLERODERMA)

### Classification

*Systemic scleroderma, progressive systemic sclerosis*, and *systemic sclerosis* are three terms used interchangeably to describe this rare systemic connective tissue disease of unknown origin. Although no single classification of SSc is universally adopted, the two disease subset model with diffuse and limited forms is

the most common in use (512). In the first, diffuse form (synonym: diffuse cutaneous form), symmetrical skin involvement affects both the distal and proximal parts of the extremities and often the trunk and face. Rapid progression of skin changes and early appearance of visceral involvement are characteristic. In the second, limited form (synonym: limited cutaneous form), a more confined symmetrical involvement of the skin, affects the distal part of the extremities (often restricted to the fingers) and the face. The progression of the sclerotic process is relatively slow and visceral manifestations take a much longer time to become manifest in this form. The terms *CREST syndrome* (calcinosis, Raynaud phenomenon, esophageal hypomotility, sclerodactyly, and telangiectasia), *acrosclerosis*, and *acroscleroderma* were also used for this more limited, relatively indolent, form of this disease (513). A three-cutaneous-subset classification system distinguishes a limited form, in addition to the limited cutaneous, and diffuse cutaneous forms of the disease (513,514). Patients with the limited form of SSc have Raynaud phenomenon and SSc-specific nailfold capillary changes and/or SSc-specific autoantibodies. A rare form without skin involvement ("systemic sclerosis sine scleroderma") also exists (515). "Overlap" syndromes, which include sclerodermatomyositis and mixed connective tissue disease, are also included under the general heading of SSc (516). The survival of patients with the limited form is much longer, and death is often caused by diseases other than SSc. The group with the diffuse form runs the risk of severe renal damage.

*Scleroderma renal crisis* is the term used to describe the most severe form of renal involvement in SSc (517,518). This disorder is characterized by rapidly developing acute renal failure that is often accompanied by elevation of the blood pressure, sometimes to "malignant" levels. However, in approximately 10% of cases with scleroderma renal crisis, the disease develops in patients without elevated blood pressure (519). MAHA has been reported in 35% of patients with scleroderma renal crisis in a recent study (520), and thrombocytopenia can also be present (521). Proteinuria may be seen as part of scleroderma renal crisis, but it may occur independently of the crisis in certain patients. Apart from proteinuria, hypertension and azotemia also occur outside scleroderma renal crisis, and such patients have a more indolent course.

## Epidemiology and Clinical Presentation

SSc is a condition that occurs predominantly in women, with an approximate female-to-male ratio of 6:1 to 10:1 (522,523). It may occur at all ages, but most patients are between 45 and 64 years of age at clinical onset (522). The disease is rare in children (524). The annual incidence and prevalence of the disease vary largely among different surveys. A recent systematic literature review reported the prevalence ranging from 7/million to 489/million and the incidence from 0.6/million/year to 122/million/year (525). The prevalence is higher in the United States (276/million) and Australia (233/million) than in Europe (France: 158/million and England: 88/million). In a recent study from Norway, the prevalence of SSc was 99/million, with estimated prevalences of 13/million, 69/million, and 18/million for patients with limited SSc, limited cutaneous SSc and diffuse cutaneous SSc, respectively (526). However, it has been pointed out that interpretation of some of the epidemiologic studies might be hampered by the inconsistent use of classification criteria and subset definitions (527). Patients with the diffuse form of SSc have thickening and tightening of the skin as the earliest signs of the disease. The skin involvement starts in the fingers and then spreads to the forearms, upper arms, thighs, abdomen, and upper chest. The involved skin becomes increasingly shiny, taut, and adherent to the underlying subcutaneous tissue, with impairment of mobility of muscles, tendons, and joints. In contrast, in the limited form, skin involvement is restricted to the fingers, hands, and face. Raynaud phenomenon is the most common manifestation of SSc and usually precedes changes in the skin (528). Esophageal involvement occurs with similar frequency in both diffuse and limited forms, but heart, lung, and renal involvement is considerably more common in the diffuse form (528).

Laboratory findings include anemia, hypergammaglobulinemia, positive rheumatoid factor in one fourth, and antinuclear antibodies (ANA) in 80% to 98% of patients (529). Little, if any, correlation exists between the presence and titer of antinuclear antibodies and the clinical severity of the disease. There is no compelling evidence either that the antibodies play a direct role in the pathogenesis of the disease.

The major autoantibody response consists of *anticentromere, anti-topoisomerase I (also known as anti-Scl 70), anti-RNA polymerase III, anti-U1RNP (U1RNP), anti-Th/To (Th/To), anti-Pm/Scl (Pm/Scl), anti-Ku (Ku)*, and *anti-fibrillarin (U3RNP)* antibodies (530,531). These antibodies are reported to be mutually exclusive, and there is strong evidence of specific autoantibody clinical phenotype and autoantibody survival associations. Anticentromeric, anti-Th/To, and anti-Ku antibodies are more common in patients with the limited form of SSc, while anti-topoisomerase I and anti-RNA polymerase III antibodies are more common in those with the diffuse form of the disease (531,532). The presence of anti-topoisomerase I, anti-RNA polymerase III, and anti-U1RNP was associated with significantly reduced survival as compared with patients with anticentromeric antibodies in a recent study from Australia (531).

## Renal Involvement

A European multicenter study reported renal involvement (as characterized by proteinuria, or hematuria, or increased creatinine) in 52 of 290 patients (18%) with SSc at the time of first observation (533). Historical data from the United States and Italy indicate a 19% and 12% incidence of scleroderma renal crisis in patients with the diffuse form of the disease, respectively (534,535). More recent data show that the incidence of scleroderma renal crisis has decreased to less than 5% in patients with SSc and to less than 2% in those with the limited cutaneous form of the disease (532), possibly due to early aggressive treatment with ACE inhibitors. In up to 25% of patients, the diagnosis of scleroderma is made at the time of SRC presentation (521,536).

Steen et al. (537) compared 36 patients with the diffuse form of SSc and scleroderma renal crisis with 212 who had the diffuse form of disease without renal involvement. The first group had a shorter mean duration of disease—2.4 years versus 4.2 years—and had digital pitting scars less frequently. No other significant differences in clinical and laboratory findings were noted. Out of 60 patients with scleroderma renal crisis in this cohort, 47 patients had the diffuse form of the disease. Therefore, patients with the diffuse form of SSc

apparently are most prone to develop scleroderma renal crisis. In addition, scleroderma renal crisis more likely develops in those patients with the diffuse form of SSc who have a relatively short history of the disease. Renal damage is rare in the limited form of SSc.

In patients with scleroderma renal crisis, headaches, blurring of vision, and dyspnea are characteristic findings and are sometimes accompanied by convulsions. These features are related to the malignant hypertension that often accompanies scleroderma renal crisis (538). Some patients, however, do not develop hypertension, whereas others may have only modest elevations in blood pressure (538,539). Oliguria is a frequent early finding in scleroderma renal crisis. Levels of serum creatinine or BUN are consistently elevated and become relentlessly higher as the renal crisis progresses. Proteinuria and microscopic hematuria may be present, and MAHA is a feature in some cases (538). Renin levels are elevated in the blood (538), usually in patients with malignant hypertension.

Other patients with SSc may have various combinations of proteinuria, azotemia, and modest degrees of hypertension, and some die of nonrenal causes. Increased morbidity among those patients with proteinuria has been reported (540).

Among patients with scleroderma renal crisis, the frequency of anti-RNA polymerase III antibodies is 9.4% to 59% (518), and there is also a strong association of these antibodies with scleroderma renal crisis (541–543). In the Australian Scleroderma Cohort Study, the risk for developing scleroderma renal crisis was 25% among patients who were anti-RNA polymerase III positive compared with a 2% risk in the absence of this antibody (541).

Among various connective tissue diseases, SSc shows the poorest prognosis. The mean 10-year survival rate in patients with SSc in studies published between 1991 and 2002 was 72.0% ± 7.60% (534). More recent studies reported improved 10-year survival rates varying between 75% and 88% (544–547). In general, the presence of major organ involvement (lung, kidney, heart) is associated with lower survival rates, and lung and heart involvement are the leading causes of death among patients with SSc (548). In a large demographic study from Italy, the worse 10-year survival rate (34.8%) was observed in patients with scleroderma renal crisis and for a group of patients with simultaneous lung, heart, and kidney involvement (12.6%) (534). Among the patients who died during the mean follow-up of 7.1 years, the most common causes of death were cardiac (36%) and lung involvement (25%), followed by cancer (15%) and renal involvement (12%) (534). The 9-year cumulative survival rate of patients with the diffuse form of SSc with severe renal involvement was only 40% in a large study from the United States (535). However, when patients diagnosed with renal crisis before the availability of ACE inhibitors were excluded from the analysis, the survival rate was 68% (535). The leading causes of death 5 to 10 years after disease onset were comparable to those seen in the Italian study (534); however, when death occurred during the first 5 years from disease onset, renal involvement was the most common cause of death (30%) (535). In a recent study from Sweden, the 10-year survival rate was 76% for patients without scleroderma renal crisis but only 40% for those with scleroderma renal crisis (543). Another study reported a 35% scleroderma renal crisis–related mortality rate in a cohort of 606 patients, 20 of whom developed scleroderma renal crisis (520). However, the scleroderma renal crisis–related mortality was only 10% among those who were treated with plasma exchange in addition to ACE inhibitors.

The increased survival and decreased mortality due to scleroderma renal crisis is likely related to better treatment(s) available. In a prospective observational cohort study by Steen (538), the 5- to 10-year outcomes of 145 patients with scleroderma renal crisis treated with ACE inhibitors were investigated. Sixty-one percent of patients with scleroderma renal crisis had a good outcome that was similar to that of patients who did not have renal crisis. More than half of the patients who initially required replacement dialysis could discontinue dialysis 3 to 18 months later.

Certain patients with SSc and combinations of proteinuria, hypertension, and azotemia pursue an indolent course, although they die earlier than those without any evidence of renal disease. Many die of nonrenal causes.

## Pathologic Findings

Pathologic changes in the kidney in scleroderma renal crisis represent the acute renal changes of SSc, and this form is considered first. The changes are similar in those patients with and without hypertension.

### Gross Appearance

The kidneys are usually of normal or slightly increased size, although some may be slightly or moderately reduced and scarred because of preexisting arterial narrowing. The subcapsular surface often shows petechial hemorrhages or paler areas (Fig. 18.26). On cut surface, the paler areas are seen to be minute, wedge-shaped infarcts, much smaller than the usual infarcts seen in the kidney. Certain kidneys show a mottled, yellowish-red cortex with extensive necrosis. In general, the changes are similar to those found in HUS and, in certain cases, to the malignant phase of essential hypertension.

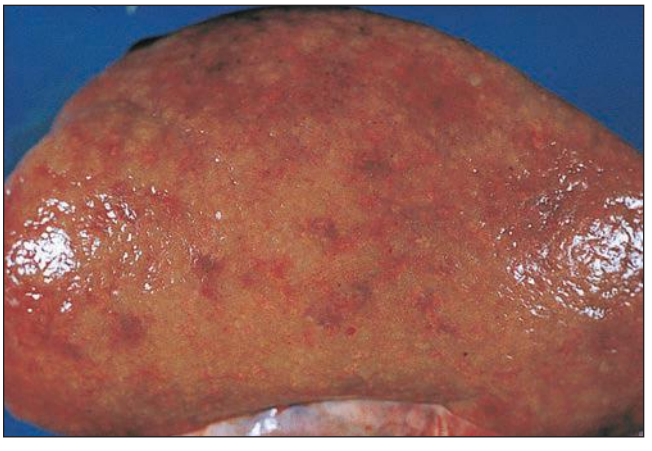

**FIGURE 18.26 Systemic sclerosis (scleroderma renal crisis).** Autopsy kidney. The surface is finely granular, indicating preexisting renal disease secondary to hypertension. There are petechial hemorrhages beneath the capsule. (Courtesy of Dr. Jan Pitha.)

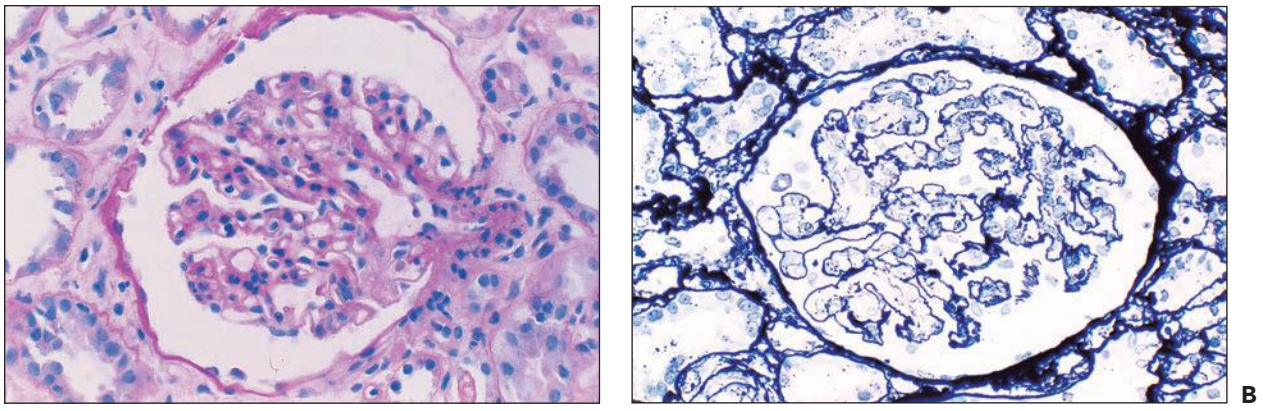


**FIGURE 18.27 Systemic sclerosis (scleroderma renal crisis).** The glomeruli may be unremarkable **(A)** (periodic acid-Schiff reaction) or may show ischemic features **(B)** with thickening and wrinkling of the glomerular capillary basement membranes. The lack of staining with methenamine silver **(B)** in some of the mesangial areas indicates mesangiolysis.

## Light Microscopy

### Glomeruli

The glomerular changes may vary considerably (Fig. 18.27). Some glomeruli may show little change, whereas others may be congested and infarcted. In some cases, one sees a thickening of the capillary walls with a double-contour appearance with silver or PAS methods, similar to that seen in HUS. The glomeruli in scleroderma renal crisis may have a fibrillar appearance identical to the changes seen in HUS. Accumulation of glomerular intracapillary eosinophilic material with the staining characteristics of fibrin occurs; these fibrin thrombi are localized to the capillary lumina. In other instances, the eosinophilic areas are larger and are referred to as *fibrinoid necrosis*. The areas of fibrinoid necrosis may contain nuclear debris and ruptured glomerular capillary basement membranes. Fragmented red blood cells can be identified in glomeruli, mostly when intravascular fibrin is present in the glomeruli and small blood vessels. These red blood cells reflect the MAHA that may be present. Occasionally, mild intracapillary or mesangial hypercellularity can be seen. Mesangiolysis may also be present. Some glomeruli are sclerosed, either globally or partially, and others show ischemic changes consisting of wrinkling and thickening of the capillary walls with shrinkage and simplification of the glomerular tuft. Crescents are encountered rarely, as is the case in the closely related lesions of HUS and the malignant phase of essential hypertension. The juxtaglomerular apparatus is prominent, especially in those cases with severe occlusive arterial and arteriolar lesions. Focal C4d glomerular positivity reported in some of the biopsies in patients with scleroderma renal crisis correlated with tubulointerstitial capillary C4d positivity (549).

### Arteries and Arterioles

Arteries of interlobular size, smaller arcuate arteries, and afferent arterioles undergo severe changes, but larger arteries may be normal or show only nonspecific fibrous intimal thickening commensurate with the age of the patient and arteriosclerotic changes elsewhere.

The characteristic changes in interlobular arteries and smaller arcuate arteries consist of mucoid intimal thickening with concentrically arranged myointimal cellular proliferation ("onion skin" lesion). The intimal thickening typically produces considerable reduction of the arterial lumen (Figs. 18.28 and 18.29). The mucinous intimal change, which is similar to that seen in severe hypertension, consists of acid mucopolysaccharides of the hyaluronic acid type. It stains with Alcian blue and metachromatically with toluidine blue, but it is only slightly positive with the PAS reaction. Trichrome stain gives either clear reaction or only a weakly blue staining in the thickened mucoid intima indicating no or little mature collagen deposition. Often, eosinophilic material is present in the thickened intima of the smaller interlobular arteries (Fig. 18.30). This finding is consistent with fibrin with phosphotungstic acid-hematoxylin and Fraser-Lendrum stainings and by immunohistochemical techniques. Fibrin may be found immediately below the endothelial cells or deep in the thickened intima (Figs. 18.31 and 18.32). It represents infiltration of fibrin across the endothelial barrier, incorporation of an intraluminal thrombus into the vessel wall, or coagulation within the vessel wall. In addition, red blood cells or fragments of red blood cells (schistocytes) may be seen in the vascular lumina and permeating the vessel wall. The endothelial

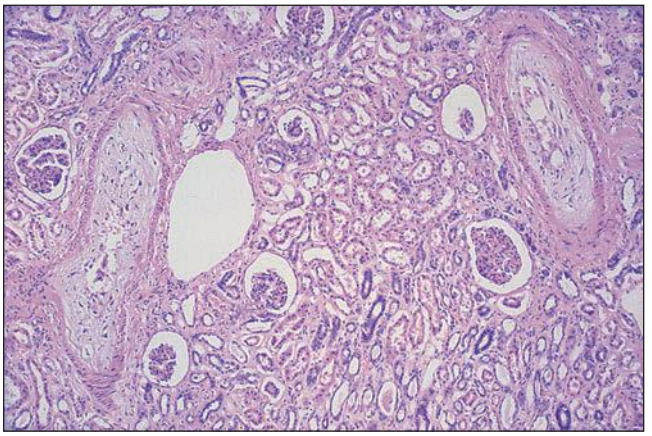

**FIGURE 18.28 Systemic sclerosis (scleroderma renal crisis).** Mucoid intimal hyperplasia of the interlobular arteries with prominent luminal narrowing. The glomeruli are ischemic. (H&E)

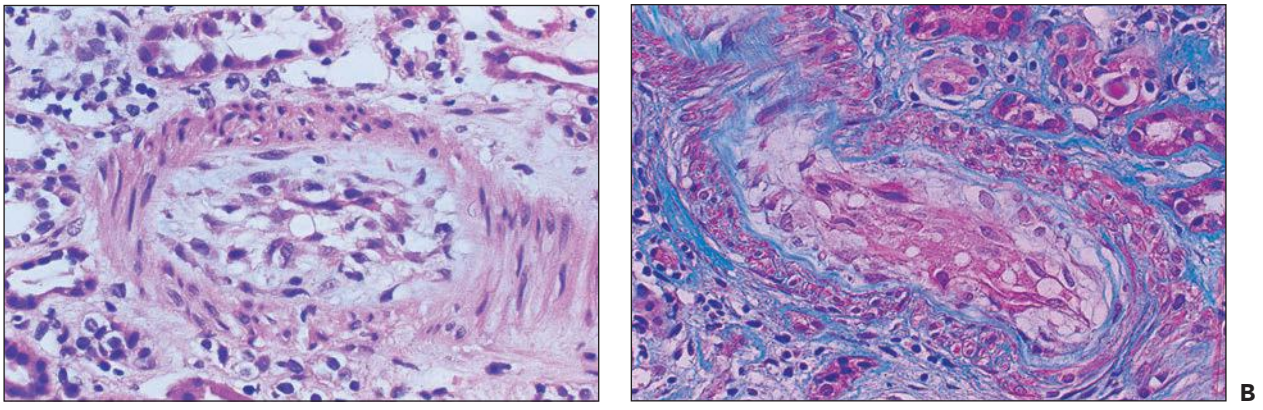

**FIGURE 18.29 Systemic sclerosis (scleroderma renal crisis). A:** A small interlobular artery shows luminal narrowing due to pale mucoid intimal thickening and myointimal cellular proliferation. (H&E) **B:** On the trichrome-stained section, the intima is pale, indicating little if any collagen deposition. (Masson trichrome.)

cells may be swollen or focally denuded. Eosinophilic granular thrombi may be present in the lumina of small arteries and arterioles; they were present in all the cases in which MAHA was described (550). The internal elastic lamina is usually intact, but it may be slightly frayed with no major breaks or gaps. The media and the adventitia of the affected arteries are usually unremarkable; however, the media may be thinned by stretching around the extended intima, and the adventitia has been noted by some authors as being slightly fibrotic (439).

Fibrinoid necrosis of the afferent arterioles consists of fibrin in the arterial walls, as seen with small arteries, but the extent of the change is much greater, and the entire wall may be involved. The arteriolar wall may have a smudgy appearance because of swelling of endothelial cells and medial myocytes. Inflammatory cells are usually not seen in fibrinoid necrosis, but such cells may occasionally be present. Sometimes, the arteriolar fibrinoid necrosis is accompanied by luminal thrombus, and on occasion, the process continues into the hilar region of the glomerulus. Arteriolar thrombi were detected in greater frequency than glomerular thrombi in patients with scleroderma renal crisis (549). Diffuse or focal C4d positivity in the renal arteries and arterioles has also been shown in patients with scleroderma renal crisis (549).

Using morphometric techniques on autopsy kidneys, Trostle et al. (551) found greater intimal thickening of arterioles and interlobular arteries in patients with diffuse SSc and renal crisis when compared with sex- and age-matched controls. In the group of patients with diffuse SSc, those with renal crisis had greater intimal thickening of the vessels than those without renal crisis.

### Tubules

The tubular changes in SSc are secondary to vascular changes. Atrophy of the proximal convoluted segment is frequent. Tubules are necrotic in the infarcted areas, and sometimes enclaves of necrotic tubules may be found without necrosis of the nearby glomeruli.

### Interstitium

In many cases, no changes are noted in the interstitium, presumably because of the great rapidity of the process. However,

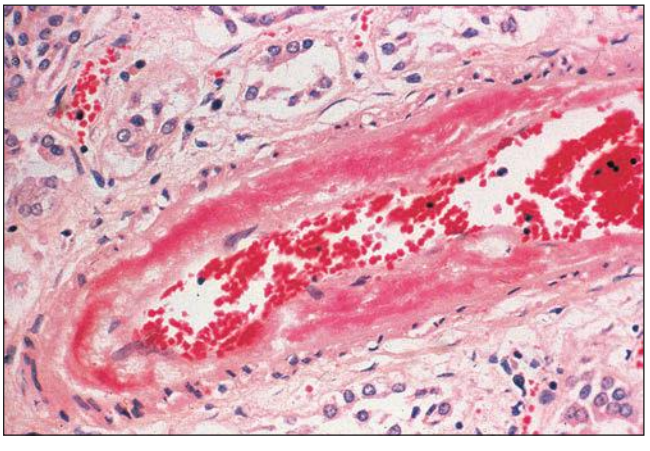

**FIGURE 18.30 Systemic sclerosis (scleroderma renal crisis).** Extensive (circumferential) fibrin insudation (fibrinoid necrosis) is seen in a small interlobular-size artery. (H&E)

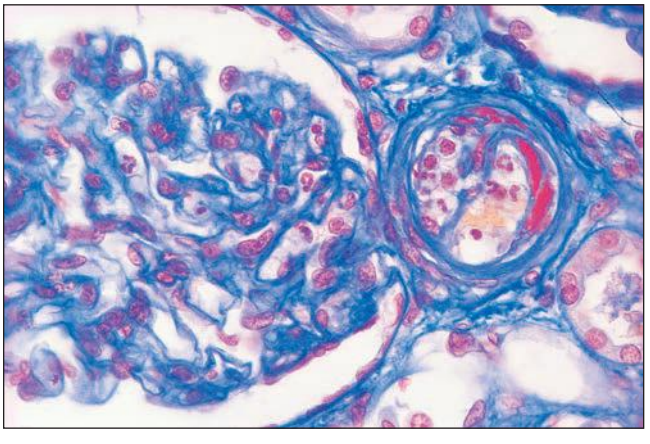

**FIGURE 18.31 Systemic sclerosis (scleroderma renal crisis).** The arteriole reveals fibrin insudation within the wall. The glomerulus is ischemic. (Lendrum stain.)

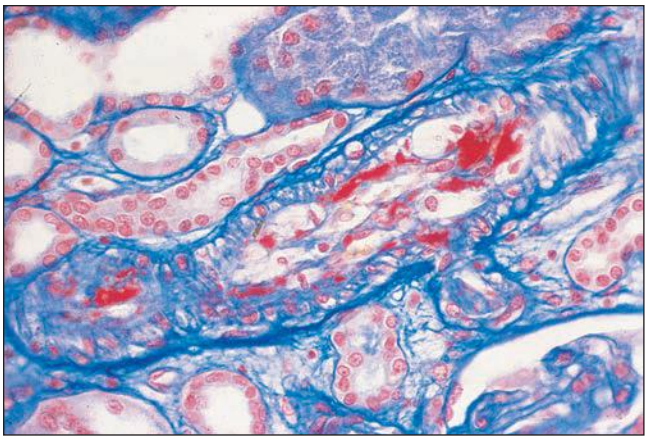

**FIGURE 18.32 Systemic sclerosis (scleroderma renal crisis).** Detachment of the endothelium from the underlying basement membrane is accompanied by subendothelial fibrin deposition in a small interlobular-size artery. (Lendrum stain.)

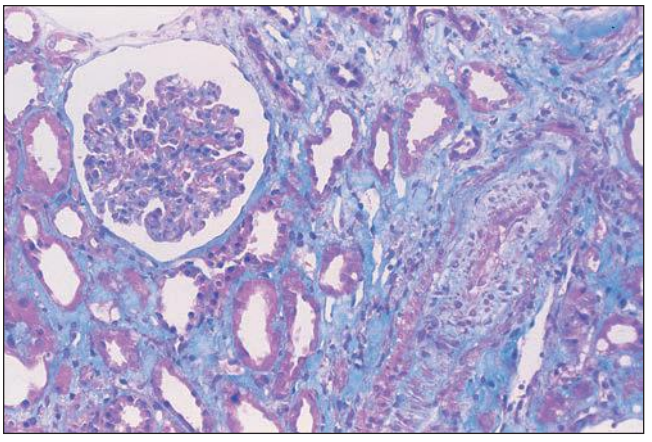

**FIGURE 18.33 Systemic sclerosis.** There is significant chronicity with extensive tubulointerstitial fibrosis, ischemic glomeruli, and intimal fibroplasia of an interlobular size artery. The patient presented with scleroderma renal crisis; however, she had a long-standing history of systemic sclerosis. (Masson trichrome.)

in patients with preexisting renal disease, extensive interstitial fibrosis may be seen (Fig. 18.33). Foci of chronic inflammatory cells may be present in association with scars. In some cases, plasma cells may be conspicuous. Often, the small renal infarcts show dense infiltration with neutrophils. This finding does not imply infection but simply represents the normal cellular reaction at the periphery of an infarct; because the infarcts are small, the neutrophilic infiltration appears extensive. Batal et al. (549) reported finely granular peritubular capillary C4d positivity in biopsies of patients with scleroderma renal crisis. The extent of the positivity correlated with worse clinical outcome and also with peritubular capillary leukocyte margination and mild interstitial inflammation.

## Immunofluorescence Microscopy

Fibrin or fibrinogen can be observed along the glomerular capillary walls and sometimes in the mesangium. Glomerular immunostaining with IgM and with C3 is more frequently seen than staining with IgG, IgA, C1q, and C4.

Immunostaining of the interlobular arteries and arterioles is often focal; fibrinogen staining is usually encountered in both arterioles—when fibrinoid changes are present—and the thickened intima of interlobular arteries (Fig. 18.34A). Immunoglobulins are often found in the same sites, and IgM invariably stains more intensely and is found more commonly than IgG and IgA (Fig. 18.34B). Complement factors C3 and C1q are found with the same frequency as IgM, and C4 also has been commonly reported.

## Electron Microscopy

Few studies have noted the electron microscopic appearance of the kidney in SSc. Glomerular changes similar to those described earlier for HUS may be seen (Fig. 18.35). The broad electron-lucent widening between the endothelium and the lamina densa (i.e., lamina rara interna with electron-lucent "fluff") is the most characteristic feature and sometimes contains scattered electron-dense granular or fibrillar material. This widening of the lamina rara interna corresponds to the thickened glomerular capillary walls seen by light microscopy. Thickening and occasional "reduplication" of the glomerular basement membranes and hyperplasia of mesangial cells have been described. In one case, scattered hyaline deposits with a periodicity of 9 to 12 nm were found in a subendothelial position in the glomerular capillaries. Glomerular intracapillary thrombi, if present, are composed of fibrin and platelets frequently admixed with fragmented red blood cells.

The study by Pardo et al. (552) deals only with renal vascular lesions in the milder forms. Granular deposits were found in a subendothelial position in the arterioles and were indistinguishable from those seen in patients with hypertension. The main conclusion drawn was that the vascular lesion in SSc was not unique at this stage of its evolution. In the severe, acute form, the thickened intima of interlobular arteries is composed of more or less parallel bands of material resembling basement membrane lying in a translucent, finely granular bed of ground substance (553–555). Fibrin tactoids and fragmented red blood cells can occasionally be seen embedded in the ground substance. Elongated myointimal or smooth muscle cells are present, but no discrete "immune-type" electron-dense deposits corresponding to the positive immunofluorescence findings have been noted. However, differentiation of "immune-type" deposits from areas of hyaline insudation may be difficult.

## Chronic Forms

The kidneys of these patients lack distinctive pathologic features and, when seen at autopsy, reveal fibrous intimal arterial thickening with areas of interstitial fibrosis or tubular atrophy. Although vascular changes such as arterial intimal fibroplasia or arteriolar thickening are nonspecific, in some instances, the arterial narrowing may be directly related to SSc. In an autopsy case-control study of 35 patients with one of two forms of SSc (CREST syndrome [$n$ = 9] or diffuse form [$n$ = 26]), Trostle et al. (551) showed that even without scleroderma renal crisis, involvement of the renal arteries and arterioles is present. Seventeen patients with the diffuse form of SSc who had no history of acute renal failure had significantly more severe intimal thickening of small- and medium-sized intrarenal arteries than

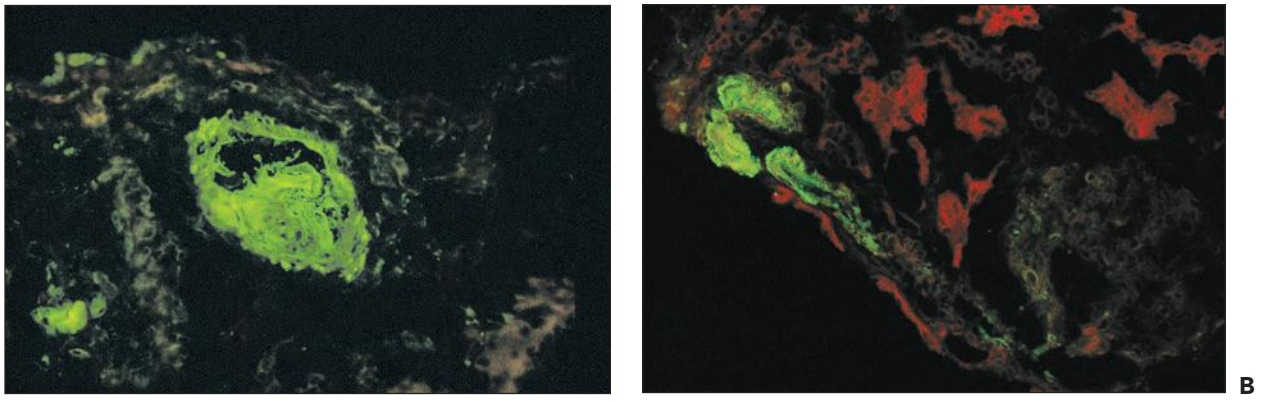


**FIGURE 18.34 Systemic sclerosis (scleroderma renal crisis).** Photomicrograph shows strong fibrinogen positivity in an interlobular size artery **(A)**. IgM positivity is present in the arteriolar wall and also along some of the glomerular capillary walls **(B)**. (Direct immunofluorescence.)

the age- and sex-matched controls. In patients with CREST syndrome, a significant intimal thickening of the small arteries was noted. However, the most severe intimal thickening was observed in patients with scleroderma renal crisis. In patients with longstanding SSc (and also in those patients who survive scleroderma renal crisis), the interlobular and arcuate arteries may show intimal fibrosis with roughly concentric reduplication of the elastic internal lamina, leading to severe narrowing of the lumen. This pattern of chronic vascular change with reduplication of the elastic internal lamina may also be seen in patients with longstanding essential hypertension and in those with the late stage of severe hypertension; this vascular change

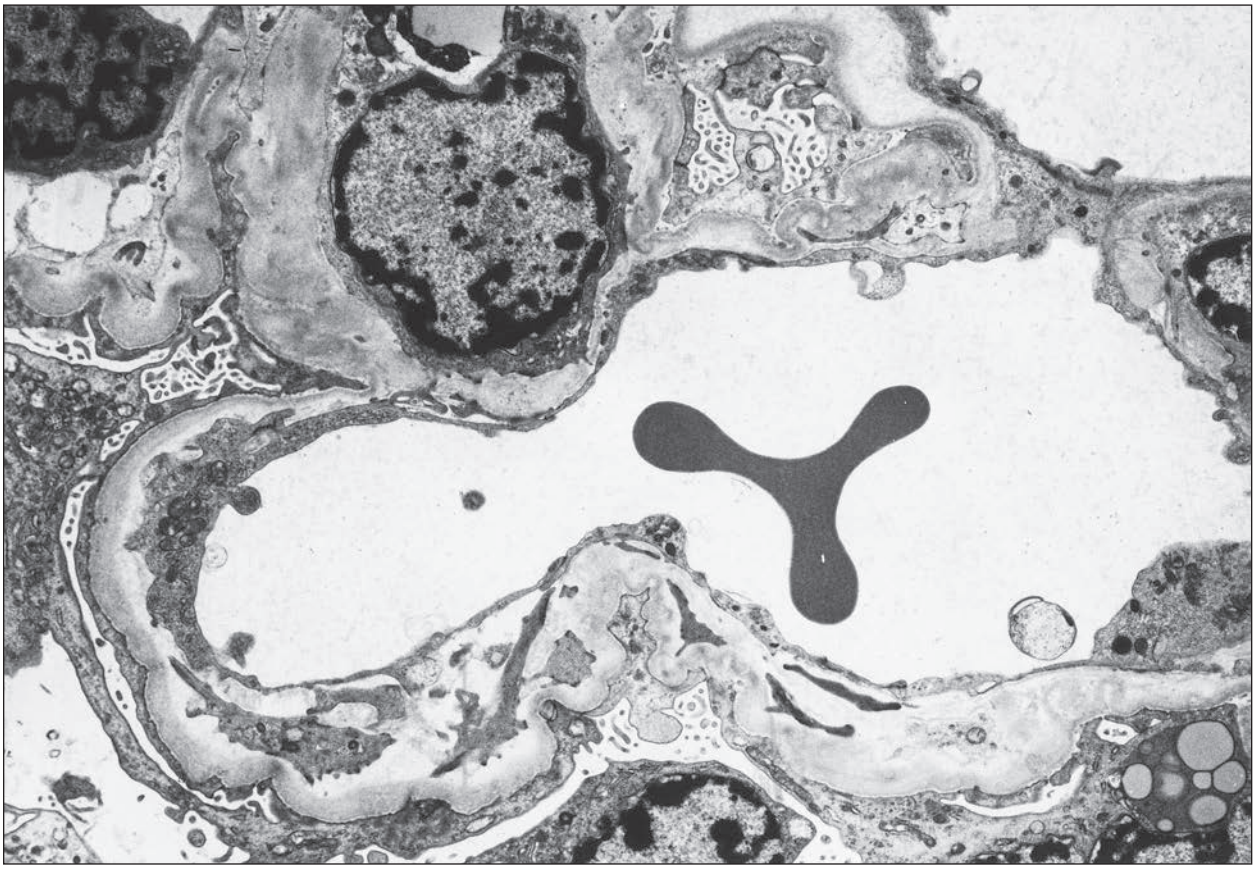

**FIGURE 18.35 Electron micrograph of a glomerulus from a patient with systemic sclerosis.** A wide electron-lucent zone on the endothelial side of the capillary basement membrane is seen with mesangial cell interposition (×7000). (Courtesy of Dr. Ginette Lajoie.)

has been frequently referred to as onion skin pattern. However, the term *onion skin* lesion is also used for the earlier stages of arterial changes in TMA characterized by concentric intimal proliferation of myointimal cells embedded in the loose mucoid material.

## Hypertension and Relationship to Vascular Changes

Hypertension is a common feature in SSc. Oliver and Cannon (556) found hypertension in 44 (52%) of their 84 patients with SSc. Of the 44 hypertensive patients, 11 presented with the abrupt onset of severe hypertension associated with rapid deterioration of renal function; they had a poor prognosis—9 died within 2 months and the other 2 underwent bilateral nephrectomy and long-term dialysis. The remaining 33 patients had mild to moderate hypertension, and only 5 died during the follow-up period, the mean of which was 4.1 months. There was a longer interval between clinical onset of scleroderma and the development of hypertension in these 33 patients. Presumably, the pathologic features of this group would correspond to what was described in the previous section as the "chronic form," but knowledge of the natural history and pathology of this disorder is limited.

The temporal relationship between hypertension and morphologic vascular changes (i.e., which comes first) has been the subject of debate since Volhard and Fahr (557). In SSc, the relationship of hypertension to vascular disease is certainly an intriguing question. Considerable evidence indicates that the vascular lesions in patients with scleroderma renal crisis may be the cause of the hypertension rather than the consequence. In a study published by Kovalchik et al. (558), renal biopsies were performed on nine patients with SSc whose blood pressures and levels of serum creatinine were not elevated. Four patients had severe vascular changes, two had mild changes, and three had normal vessels. Plasma renin activity was raised in three of the four with severe vascular changes, and further elevation of activity took place in response to cold pressor testing in these four patients, but not in the others. This study suggests that in SSc, the vascular changes may be primary and the cause of the hypertension. Other cases of SSc have been described with severe vascular changes in the kidney, usually accompanied by renal failure in the presence of a normal blood pressure (521,550,559). Such cases without hypertension have been associated with worse clinical outcomes than those with hypertensive scleroderma renal crisis (519,536). One study found no correlation between the vascular changes and the blood pressure in patients with scleroderma renal crisis (521).

Hypertension may be explained on the basis of severe arteriolar narrowing, which may stimulate the renin-angiotensin system to produce excessive amounts of renin and initiate hypertension. Those patients with severe hypertension almost invariably have high plasma renin concentrations or high levels of plasma renin activity (560).

Although endothelin is thought to play a significant role in the pathogenesis of SSc, plasma levels of endothelin are not significantly elevated in patients with SSc (561). However, overexpression of endothelin-1 has been documented by immunohistochemistry in the glomeruli and arterioles of patients with scleroderma renal crisis (562).

## Etiology and Pathogenesis

Endothelial or vascular abnormalities, dysregulated cellular and humoral immune responses, and increased collagen synthesis are considered to be the major contributors in the pathogenesis of SSc (563–567) (Fig. 18.36). These pathogenic components are thought to be closely interrelated through cytokines, growth factors, autoantibodies, and additional mediators derived from various sources such as inflammatory cells, endothelial cells, epithelial cells, platelets, and fibroblasts. However, these alterations appear to be predominantly downstream events, and the initiating factors of the disease are not fully elucidated. There is evidence that the endothelial cell injury including apoptosis is an early event and possibly the primary trigger. The significance of genetic susceptibility is highlighted by the recent identification of SSc-associated SNPs in genes encoding cytokines, chemokines, and extracellular matrix

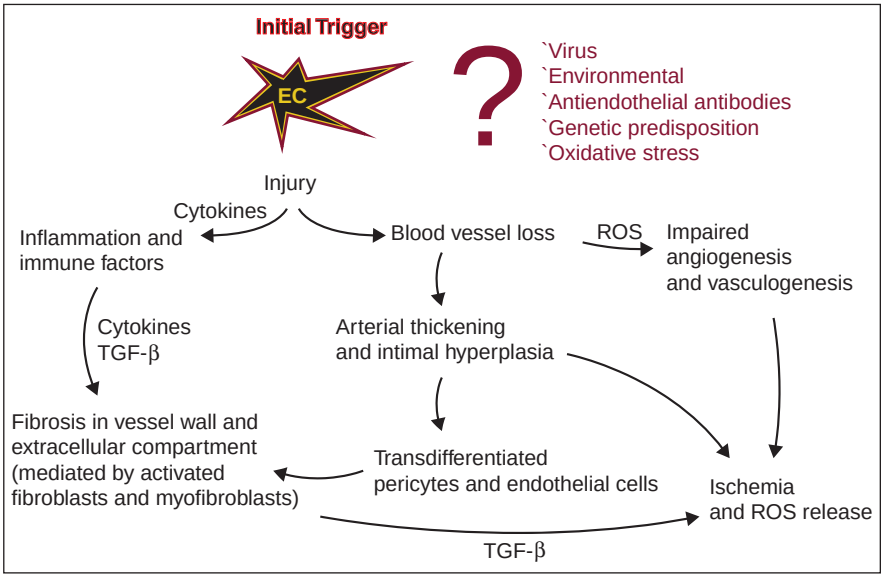


**FIGURE 18.36 The pathogenesis of systemic sclerosis.** Endothelial cell injury appears to be an early initiator of the cascade of events that eventually result in the clinical phenotype of systemic sclerosis. Increased vascular permeability, inflammation, altered collagen deposition, various cytokines, oxidative stress, and genetic and environmental factors all play a role in this process.

proteins (222,225). A strong linkage of certain HLA class II allelic variants to the clinical phenotype has also been described (96,568,569). In addition to genetic predisposition, several environmental or acquired factors have been implicated in the pathogenesis of SSc. These include various viruses (herpesviruses, CMVs, parvoviruses, and retroviruses), chemical and physical agents. The list of environmental exposures linked to SSc includes silica and metal dust, organic solvents, vinyl chloride, pesticides, and industrial fumes, among others. Although not rigorously tested, microchimerism is a novel concept in the pathogenesis of SSc. According to this hypothesis, fetal and maternal cells that cross the placenta during pregnancy may persist in the circulation and tissues of mother and child. These cells may become activated later with resultant graft versus host disease (GVHD) manifested as SSc. The similarities observed between SSc and chronic graft versus host disease and identification of allogeneic cells in the peripheral blood and skin of affected patients support this hypothesis (570,571).

## Genetic and Epigenetic Factors

Both candidate gene and more recent genome-wide association studies (96,118,569) have identified several genetic backgrounds associated with SSc. The disease is more prevalent in the US than in European populations (295) with greater propensity to affect and cause more severe disease in Hispanics, African Americans, and Native Americans (296,296b). The presence of systemic sclerosis in a first-degree relative, especially a sibling, is associated with a several fold (≥15 fold) higher risk of developing systemic sclerosis than the general population (572). The association between HLA class II genotype and clinical phenotype including autoantibody profile such as anticentromere antibody or anti–topoisomerase I has been reported in several studies (96,221,573). The genome-wide association studies evaluating genotype-clinical phenotype correlations identified the strongest HLA association with a locus in *HLA*DQB1* (569). Single-nucleotide polymorphisms in more than 40 HLA and non-HLA gene loci across the entire genome appear to increase the individual susceptibility to systemic sclerosis. These include genes involved in the immune response (*STAT4, TBX21, IRF5, MIF, PTPN22, BANK1, BLK, TNFSF4, CD247*), fibrosis (*CTGF, serotonin 5-HT2A receptor, IL1-α, IL1-β, MMP*), and vascular disease (*ENG, SDF-1/CXCL12, HIF1A, uPAR, VEGF, eNOS/NOS3, KCNA5ET-1, fibrinogen*) (222,225,226). It is generally accepted that the inflammatory and autoimmune mechanisms predominate early in the course of the disease, while the fibrosis and vasculopathy are characteristic of the late stages of systemic sclerosis and have weaker genetic associations (244).

In addition to genetic polymorphisms, epigenetic modifications also alter the susceptibility and phenotype of the disease. Global DNA hypomethylation has been reported in $CD4^+$ T cells from patients with systemic sclerosis, and such epigenetic modifications can alter collagen gene expression (245,574). With the advent of personalized medicine, these epigenetic changes may potentially serve as therapeutic targets (575).

## Initial Injury

Endothelial cell injury leading to apoptosis in the microvasculature appears to be an early initiator of the cascade of events that eventually result in the clinical phenotype of systemic sclerosis. Early skin biopsies in patients with systemic sclerosis show endothelial cell damage and apoptosis, and these changes precede fibrosis by several months or years (576,577). Morphologic findings in the kidneys in patients with scleroderma renal crisis also indicate severe endothelial injury similar to that seen in HUS. The specific trigger for such endothelial injury in a genetically predisposed individual remains elusive. Potential culprits include cross-reactive antibodies against CMV and other viral epitopes, antiendothelial antibodies, and oxidative stress–induced reactive oxygen species (246,577,578).

Antiendothelial antibodies are frequently detected in the sera of the patients with systemic sclerosis and also in several other autoimmune disorders. Although they can induce antibody-dependent endothelial cell apoptosis, it is unclear if antiendothelial antibodies are just an epiphenomenon rather than pathogenic factors (192,579). Irrespective of the trigger, early endothelial injury causes activation of endothelial cells and release of various cytokines that influence other pathogenic pathways of tissue injury in systemic sclerosis.

## Vascular Abnormalities

### Endothelial Damage

Microvascular endothelial damage results in a chain of events including increased capillary permeability, platelet activation, coagulation abnormalities, and altered vasomotor activity. These changes eventually may lead to the typical morphologic and functional alterations seen in systemic sclerosis.

Nonspecific markers of endothelial injury in systemic sclerosis include elevated plasma levels of vWF, thrombomodulin, endothelin-1, thrombospondin, and VEGF (567,580–582). Overexpression of endothelin-1 protein in the glomeruli and arterioles of patients with systemic sclerosis and scleroderma renal crisis has also been documented (562). Activated endothelial cells show up-regulated expression of adhesion molecules (E-selectin, VCAM-1, and ICAM-1) that promotes adhesion of activated lymphocytes to the endothelium (564,583). The apoptosis of endothelial cells also triggers the release of fibrogenic mediators that activate fibroblasts and promote persistent myofibroblast differentiation (584). Endothelial cell damage is in turn exacerbated by cytokines and growth factors secreted by activated inflammatory cells, serum cytotoxic factors, and down-regulated complement regulatory proteins. Endothelial complement regulatory proteins normally protect endothelial cells from autologous complement. Down-regulation of proteins such as MCP, decay-accelerating factor, and CD59 has also been shown in punch biopsies of skin from patients with systemic sclerosis (585), potentially contributing to endothelial damage. Direct cell-cell interactions between the endothelium and activated lymphocytes and endothelial cell release of soluble mediators such as TGFβ and PDGF all point to a complex interplay between major pathogenic arms of disease process in systemic sclerosis.

### Increased Permeability

Studies of the nailfolds using in vivo capillary microscopy in systemic sclerosis patients have shown enlarged and deformed capillary loops surrounded by relatively avascular areas (586). Fluoresceinated tracers have revealed increased permeability of this capillary system (587). One study of skin lesions in systemic sclerosis revealed subendothelial edema as the earliest morphologic abnormality, followed by intravascular platelet aggregation and dermal accumulation of $CD4^+$ and $CD8^+$ subsets of lymphocytes (576). Endothelial cell activation results in increased

expression of adhesion molecules and vascular permeability (588). Tissue fibrosis and decreased intensity of inflammation were recognized at later stages during disease progression.

### Platelet Activation and Intravascular Coagulation

Various thrombotic lesions in the renal microvasculature shown in histologic sections of the kidney indicate platelet activation. Platelet activation in patients with systemic sclerosis was confirmed by demonstration of elevated levels of circulating platelet aggregates and impaired fibrinolysis (580). Most data seem to support the notion that platelet activation is secondary to endothelial injury; however, the role of a primary platelet abnormality in systemic sclerosis has also been suggested (589,590). Damaged endothelium may also trigger thrombosis through induced TF expression and decreased anticoagulant activity.

### Functional Vasoconstriction

Investigators have long known that Raynaud phenomenon is a common feature of systemic sclerosis, and it is exacerbated by cold. The exact mechanism causing the vasoconstriction that accompanies Raynaud phenomenon is not understood. The physiologic control of vascular tone seems to represent a dynamic interplay among neuropeptides, products of the endothelium, such as vasoconstrictor endothelin, vasodilator endothelial–dependent relaxation factor (NO), and $PGI_2$, and platelet release products such as serotonin and $TXA_2$. Complex abnormalities of normal vasoregulation were shown in patients with systemic sclerosis and Raynaud phenomenon. Elevated levels of vasoconstrictors endothelin-1 and $TXA_2$ in the circulation and platelet activation are well-known features in patients with systemic sclerosis (589,590). Neuropeptides, present in the sympathetic, parasympathetic, and sensory nervous system, may have vasoconstrictor or vasodilator effects. The exact role of neuropeptides in the increased vascular tone in Raynaud phenomenon is not known; however, deficiency of vasodilatory neuropeptides has been suggested (591). The renin-angiotensin system is considered to play a significant role only during scleroderma renal crisis with malignant hypertension (592)

Vasoconstriction may also occur in certain viscera in patients with systemic sclerosis (439,593). Cannon et al. (439) performed renal blood flow studies and angiography of the kidneys in patients with and without renal failure. Patients with proteinuria and milder hypertension had evidence of renal cortical vasoconstriction that can be accentuated by exposure to cold. These vasoconstrictive changes were more pronounced in the setting of severe hypertension and renal failure, accompanied by angiographic changes suggestive of vasospasm of large renal arteries and obliteration of interlobular arteries. This renal vasoconstriction was probably mainly attributable to the effects of angiotensin II.

The contribution of functional vasoconstriction to kidney damage is difficult to determine both in patients who have developed acute renal failure (scleroderma renal crisis) and in those who have not. In those patients with systemic sclerosis and acute renal failure, structural changes are invariably present in the renal vasculature, and these changes in themselves are sufficient to explain the renal decompensation. Activation of the renin-angiotensin system with its vasoconstrictive consequences could well augment these organic changes. In the case of patients without renal failure, repeated attacks of vasoconstriction corresponding to episodes of Raynaud phenomenon could possibly lead to tubular atrophy and other chronic changes seen at autopsy. Increased vascular resistance indices were demonstrated by color flow Doppler ultrasonography in patients who had systemic sclerosis without clinical symptoms of renal damage (594). The resistance indices were significantly elevated in all three vascular sites (main arteries, interlobar arteries, and cortical arteries) that were explored, but more so in vessels distal to the interlobar arteries. These findings provide evidence of abnormal renal vascular function in patients with systemic sclerosis without clinical evidence of renal damage. Finally, the ability of vasoconstriction to cause structural changes in arteries and arterioles is not known. Possibly, prolonged periods of intense vasoconstriction could cause structural arterial changes, but we consider this unlikely in the absence of more compelling demonstrations of extreme vasoconstriction over long periods.

## Abnormal Microvasculature: Impaired Angiogenesis and Vasculogenesis

Progressive endothelial injury and obliterative vasculopathy results in the paucity of capillaries in several organs including skin (563,576). This rarefaction of capillaries normally triggers angiogenesis from preexisting vessels. Despite the upregulation of angiogenic factors (VEGF, PDGF, ET-1, CCL2) in systemic sclerosis patients, the vascular repair processes are impaired, resulting in dilated and malformed capillaries such as nailfold capillary abnormalities and telangiectasias on the skin and mucous membranes (447,586,595). These proangiogenic factors are also potent activators of vascular smooth muscle cells and stromal fibroblasts, thus potentially exacerbating the chronic fibrotic disease manifestations. In addition to angiogenesis, the formation of new blood vessels, that is, vasculogenesis, is also aberrant, likely due to defective mesenchymal stem cells and endothelial progenitor cells derived from bone marrow in patients with systemic sclerosis (596,597). Animal models of systemic sclerosis have suggested that transcription factors such as Fra-2 and Fli1 play a role in the regulation of angiogenesis (437).

## Arterial Intimal Hyperplasia

In addition to microvascular injury, proliferative macrovascular changes contribute to the pathogenesis of systemic sclerosis. These macrovascular changes appear to contribute to the plexiform arteriopathy seen in pulmonary hypertension and the intimal hyperplasia and edema in scleroderma renal crisis (564). The fibrotic intimal hyperplasia with resultant luminal narrowing causes ischemia, potentially exacerbated by thrombotic events. These intimal changes may in fact precede Raynaud phenomenon. The pericytes in these blood vessels modulate the endothelial cells and might play an important role in angiogenesis. It has been demonstrated that the pericytes in systemic sclerosis express PDGF receptor β, a marker of activation, and may in fact contribute to smooth muscle hypertrophy observed in blood vessels (440,598). By also transdifferentiating into myofibroblasts, pericytes constitute the cellular link between the microvasculature and fibrosis.

## Inflammation and Immune Factors

Various features of activation of the humoral and cellular immune system frequently seen in patients with systemic sclerosis provide the conceptual basis for the immune hypothesis

of pathogenesis. The immune mediators are thought to promote both vascular changes and altered collagen production, which are the major features of systemic sclerosis. The list of these immune abnormalities in patients with systemic sclerosis is long and includes features of circulating B- and T-cell activation, various serum autoantibodies, increased serum levels of cytokines, up-regulation of various adhesion molecules on the vascular endothelium, lymphocytes, and fibroblasts, and increased expression of growth factors and growth factor receptors (599–601).

The early skin lesions of systemic sclerosis demonstrate perivascular cellular infiltrates composed of T cells, B cells, macrophages, and mast cells (576). Activated endothelial cells express adhesion molecules that promote perivascular inflammation (564,588). The T and B lymphocytes display an activated phenotype, and morphologic variation can be observed in relation to the clinical phase of disease such as early inflammatory or late fibroproliferative stage (567,576,602). The T lymphocytes in the skin and peripheral blood of systemic sclerosis patients show Th2 polarization with release of interleukin-4 and interleukin-13, both profibrotic cytokines with effects on TGFβ production (603). A more recently characterized IL-17–secreting Th17 $CD4^+$ T-cell subset may be pathogenetically important in systemic sclerosis and several other autoimmune disorders. Greater numbers of IL-17–producing helper T cells and higher IL-17 levels were observed in peripheral blood and bronchoalveolar lavage cells in systemic sclerosis patients (603b).

Although the autoantibodies in systemic sclerosis correlate with the clinical phenotype, they do not predict the clinical course of disease or outcome. Nevertheless, their presence indicates the involvement of the humoral immune system as does the presence of B-lymphocyte aggregates in the target tissues such as the skin and lung (162). B lymphocytes are efficient antigen-presenting cells and aid in the amplification of T-cell effector responses (604). Chronically activated B lymphocytes contribute to the pathogenesis of fibrosis by secreting interleukin-6 and TGFβ (599–601,604). Recent studies suggest a critical role of innate immunity in systemic sclerosis involving both toll-like receptor (TLR) and non-TLR signaling (605–607). TLRs are activated by pathogens and apoptotic material, stimulate the production of inflammatory mediators from macrophages and dendritic cells, and also mediate fibrosis. In addition, plasmacytoid dendritic cells respond to TLR stimulation and produce interferon-α, but the significance of the interferon signature in systemic sclerosis needs to be further investigated (605,606).

### Fibrosis

The increased deposition of the extracellular matrix is one of the major morphologic features in systemic sclerosis and accounts for late mortality in these patients. Changes in the composition of the extracellular matrix affect cellular functions such as cell migration, proliferation, and differentiation. Although fibrosis occurs in multiple organs including skin, lungs, gastrointestinal tract, and heart, most of the morphologic studies of abnormal matrix deposition have been carried out in skin, a frequently involved and easily accessible organ. The list of extracellular matrix components that are overexpressed in systemic sclerosis includes several collagens (e.g., types I, III, IV, V, VI), glycosaminoglycans, and noncollagenous glycoproteins such as fibronectin, laminin, and tenascin (608). Alpha-smooth muscle actin-expressing myofibroblasts accumulate in the dermis, and progressive loss of capillaries is evident. The myofibroblasts accumulated in fibrotic areas can be derived from modified fibroblasts as well as several other sources such as transdifferentiated epithelium or endothelium or pericytes, fibrocytes, and bone marrow stem cells (609). Fibrosis in systemic sclerosis represents dysregulated wound healing, and central to the enhanced extracellular matrix production are fibroblasts and their unopposed activation. Fibroblasts in systemic sclerosis may be inherently abnormal or are affected early in the course of disease as evidenced by gene expressional analysis of explanted dermal fibroblasts from lesional and nonlesional skin and from identical twins (610–612). Increased matrix synthesis is largely due to increased transcription of genes responsible for production of collagen and other matrix components by fibroblasts. Overexpression of collagen genes has been demonstrated in skin biopsies of patients with systemic sclerosis, and microRNA may play a role in posttranscriptional regulation as well (613,614) Activation of fibroblasts with increased matrix production may be induced by various cytokines and growth factors released by inflammatory cells or aggregated platelets. It has been suggested that activated mononuclear cells, frequently seen early in systemic sclerosis around the small blood vessels, may influence fibrotic and vascular events by direct cell-cell interaction and by the release of soluble cytokines. The infiltrating mononuclear cells are predominantly $CD4^+$ T cells and express the activation marker class II MHC antigen DR (615). Induced expression of adhesion molecules has been demonstrated in skin biopsies of patients with systemic sclerosis. Mast cells are also known to be present in early scleroderma skin lesions, frequently in close association with fibroblasts and small vessels. Several of the mast cell–derived products are potentially relevant to scleroderma; these include histamine, platelet-activating factor, tryptase, proteoglycans, and certain cytokines (616).

Both in vitro evidence and in vivo evidence support an important role of multifunctional growth factors, such as TGFβ, PDGF, CTGF, and ET-1 in skin lesions of systemic sclerosis (565,567). TGFβ plays a major role in systemic sclerosis by increasing collagen synthesis by fibroblasts and myofibroblasts, which demonstrate up-regulated TGF-β receptor expression (617). Intracellular downstream TGF-β signaling includes primarily the Smad pathway with a lesser role for Smad-independent pathways (567,618). Recent studies indicate that the tyrosine kinase c-Abl (of Philadelphia chromosome in chronic myelogenous leukemia) may mediate TGF β responses in fibroblasts, and systemic sclerosis patients may potentially benefit from its pharmacologic blockade by imatinib (619). TGF-β is in turn synthesized by fibroblasts and myofibroblasts, thus enmeshed in an autocrine loop of increasing fibrosis. T-cells, monocytes, macrophages, and platelets produce TGF-β as well, illuminating the complex interplay between inflammation and fibrosis. Several integrins also control TGF-β localization and activation. Among these, blocking TGF-β via inhibition of the αVβ6 integrin signaling has been recently entertained as a potential therapy for fibrosing lung disease in systemic sclerosis (39).

Other profibrogenic factors such as CTGF and ET-1 are also involved in autocrine loops with fibroblasts, and CTGF amplifies TGF-β signaling (607). PDGF is another growth factor that promotes extracellular matrix deposition and produces inflammatory cytokines (620). Produced by fibroblasts,

myofibroblasts, platelets, and macrophages, PDGF is an important player in fibrogenic responses. Anti-PDGF receptor antibodies that stimulate the PDGF receptor have been detected in patients with systemic sclerosis (621). Vasoconstriction and ischemia observed in systemic sclerosis cause oxidative stress and release of reactive oxygen species that also activate TGF-β and PDGF.

It appears that the normal antifibrotic mechanisms in wound healing may be compromised in systemic sclerosis. Peroxisome proliferator–activated receptor-γ (PPAR-γ), involved in insulin and lipid homeostasis, can block the TGF-β–induced stimulation of collagen synthesis in vitro and may be aberrantly expressed in systemic sclerosis (622,623).

Although the abnormalities in collagen deposition may well be substantial in the pathogenesis of skin and lung lesions in scleroderma, the relevance of these abnormalities to the pathogenesis of renal lesions in scleroderma renal crisis is uncertain. Abnormal collagen synthesis may be more important in the more chronic type of renal involvement with the coarser type of intimal arterial thickening.

### Animal Models

Several animal models of systemic sclerosis have been described; however, no currently available models exhibit all the aspects of systemic sclerosis (624,625). The systemic manifestations in most models are the result of genomic DNA alterations (as in transgenic or knockout animal models), but fibrosis reminiscent of systemic sclerosis can be induced by subcutaneous injections of bleomycin or by hematopoietic cell transplantation causing chronic sclerodermatous graft versus host disease. Severe tissue fibrosis is almost universal in these animal models, but small vessel vasculopathy can be studied only in UCD-200, UCD-206 chicken models, and the Fra-2 mouse model (626,627). One of the earlier and most extensively studied models of tight skin mice (TSK-1) have excessive extracellular matrix deposition, likely mediated by abnormal fibrillin activating the TGF-β cascade, and develop autoantibodies against systemic sclerosis-specific antigens (624,625). However, these animals differ from the human disease with thickening of the hypodermis rather than dermal fibrosis, the development of emphysematous changes instead of lung fibrosis, and the lack of vasculopathy and prominent inflammatory infiltrates. A recently described transgenic mouse that constitutively expresses the TGF-β receptor I in fibroblasts shows dermal fibrosis and extracellular matrix abnormalities indicating that active TGF-β signaling alone can result in the fibrotic phenotype of systemic sclerosis (628). The UCD-200 chicken model shows remarkable similarities to the morphologic, immunologic, and biochemical aspects of human systemic sclerosis. Endothelial cell apoptosis occurs in various organs (skin, esophagus, lung, and kidney) in this model during the initial and early inflammatory stage of the disease followed by mononuclear cell infiltrates and excessive collagen deposition in the skin and esophagus (629). In addition to developing an obliterative vasculopathy, the UCD-200 chicken model displays renal abnormalities. Glomerulonephritis is present in approximately 20% of the birds with glomerular deposition of IgG (626). *Fra-2*-transgenic mice demonstrate a vascular phenotype with TMA and pulmonary hypertension in addition to systemic fibrosis (624). The Fra-2 protein is a transcription factor implicated in cell proliferation, inflammation, and wound healing (630). Endothelial cell apoptosis precedes the loss of capillaries, and the complex interplay between epithelial cells, endothelial cells, and fibroblasts results in the full spectrum of the disease in these mice.

### Summary of Pathogenesis of Scleroderma Renal Crisis

The most plausible explanation for the renal vascular changes in scleroderma renal crisis relates to endothelial damage of the arteries and arterioles in the kidney. Endothelial damage may result in undue permeability of the endothelial barrier, so various constituents of the blood gain access to the intima and, in the case of smaller arteries and arterioles, to the media. Initially, one notes edema of the intima, which then appears to undergo changes leading to the cellular or fine fibrous intimal thickening seen so commonly. Fibrin often penetrates the wall as part of the excessive permeability and is sometimes a prominent feature; in the arterioles, fibrin may pervade the entire thickness, but in interlobular arteries, it is usually confined to the intima. Alternatively, coagulation may take place in the vascular lumen, followed by incorporation of fibrin into the wall by the intimal proliferative changes. The resultant picture could be one of fibrin deeply embedded in the intima. Endothelial damage may also initiate a chain of events leading to coagulation and release of factors that could stimulate smooth muscle cell or pericyte movement and proliferation into the intima. Microthrombi can be found in arterioles and small arteries, a finding suggesting an endothelial defect or a coagulation abnormality. The initiating factors and the precise mechanisms for endothelial damage are not fully characterized. Overall, both macrovascular intimal hyperplasia and microvascular injury likely play a role in scleroderma renal crisis. Abnormalities in cellular and humoral immunity with increased cytokine activity and vasoactive agents may be contributing factors.

## RADIATION NEPHROPATHY

Inadvertent or unavoidable irradiation of the kidney during x-ray treatment of malignant tumors may cause damage to the organ with impairment of its function and a rise in blood pressure. The advent of total-body irradiation approximately 30 years ago as treatment for some immunologically mediated diseases and as part of the conditioning treatment regimen utilized in preparation for hematopoietic stem cell transplant has expanded the settings in which radiation injury to the kidney may occur. More recently, radiation-induced renal toxicity has also been observed in patients who underwent treatment with various radiolabeled substances, such as monoclonal antibodies, antibody fragments, and low molecular weight oncophilic peptides. Awareness of the dangers of irradiation and the introduction of techniques specifically designed to minimize acute organ toxicity have fortunately reduced the number of cases of radiation nephritis, and this disorder is now rare.

Radiation nephropathy is discussed in this chapter because of the significant overlap of renal morphologic changes between radiation nephropathy and various forms of TMA. Clinical findings and renal morphologic changes secondary to external beam irradiation, total-body irradiation, and radionuclide treatment are included.

## Historical Review

Early writings have been well reviewed by Redd (631) and Mostofi (631b), the latter author recording 120 cases up to 1964. In the first decade of the 20th century, clinicians were aware of the occurrence of albuminuria and nitrogen retention in patients receiving roentgen-ray therapy, and they realized that care must be taken in its use. Domagk (631c) appears to have been the first to give an accurate picture of the irradiated human kidney; he described a 9-year-old girl whose abdomen was irradiated for tuberculous mesenteric lymph nodes. Four months after treatment, a raised temperature, oliguria, and albumin and casts in the urine were noted; death occurred 2 months after these symptoms first appeared. At autopsy, the kidneys were reduced in size and showed glomerular hyalinization or thickening of the Bowman capsule, tubular atrophy or necrosis, and hyaline material in the arterial walls. A short time later, Zuelzer et al. (632) described an unusual form of glomerulonephritis in three young children who had received heavy irradiation for either Wilms tumor or neuroblastoma; these investigators considered the condition in all probability to be radiation nephritis. Renal failure and hypertension developed within 3 months in these patients, and death occurred quickly. The glomeruli were severely involved by either capillary wall thickening or necrosis; tubular loss, interstitial fibrosis, and areas of necrosis in afferent arterioles and small arteries also were found. Luxton (633) followed this report with a large series of 27 patients whose kidneys had been damaged after irradiation of abdominal lymph nodes as part of the treatment for seminoma of the testicle. He recognized various clinical syndromes (discussed in the next section) and described the pathologic changes. His work was supplemented by that of Russell (633b) from the same hospital. Since that time, other smaller series and single case reports have appeared that amply confirm these principal observations, although some modifications to the conceptual formulations are needed to take account of the recorded clinical experience and the results of experimental studies performed since that time. In particular, the recognition of a syndrome of TMA associated with total-body irradiation as used in HSCT procedures has come to be recognized as a unique clinicopathologic entity, as discussed on page 793.

## Clinical Presentation and Clinical Course

The experience of Luxton is unique, and his initial article (633) and subsequent studies describing the long-term follow-up records of 54 patients treated with abdominal irradiation for malignant testicular tumors (49 cases) and ovarian tumors (5 cases) (633,634) set out distinct clinical syndromes (acute radiation nephritis, chronic radiation nephritis, asymptomatic proteinuria, benign hypertension, and malignant hypertension) as described below. Subsequent descriptions of radiation nephropathy due to external beam radiation therapy also confirmed the acute and chronic clinical presentations with characteristic renal pathologic findings (635,636).

### Acute Radiation Nephropathy (Acute Radiation Nephritis)

Twenty of the 54 patients studied were classified as having acute radiation nephritis. Luxton used the term *acute radiation nephritis* to describe a syndrome that developed over many months and whose pathologic manifestations, described later, lacked the inflammatory changes characteristic of other acute nephritides (633,634). For this last reason, most experts (631,637) prefer the terms *acute radiation nephropathy* and *chronic radiation nephropathy* for the syndromes that Luxton delineated.

Patients with acute radiation nephropathy, following a latent period of 6 to 12 months after irradiation, had a gradual onset of edema, hypertension, dyspnea on exertion, anemia, headaches, and urinary changes that included proteinuria and the presence of casts. The edema either was confined to the legs or was more generalized, with effusion into the pleural space and pericardial sac; it was absent in a few patients. Hypertension was constant and was commonly at its height within 6 months of the appearance of the first symptom. The raised blood pressure returned to normal levels in a certain proportion of patients; this took place even in some with malignant hypertension, which was present in almost half of these patients. Anemia was present in all patients and was of a severe normochromic, normocytic type. The blood urea level was raised early in some patients and either remained at a high level or fell.

Subsequent accounts confirmed Luxton's description, and one or two additional features were pointed out. Anemia is common, and in several reports (638–640), it was of the microangiopathic hemolytic type; deformed red blood cells were seen in blood smears, and platelets were decreased in numbers. Fibrin degradation products were present in the circulation in two accounts (639,640), and evidence of intravascular coagulation was claimed in one (639). Currently, these findings would be considered indicative of a TMA as the basis for the patients' renal dysfunction. Rare reports of the nephrotic syndrome associated with abdominal irradiation also have been described (641).

In general, the time required for acute-radiation nephropathy to develop is 6 to 12 months, but certain patients have had an earlier onset, such as 3 or 4 months (642,643), or a few weeks (644,645). All of these latter patients had been treated with chemotherapeutic agents such as actinomycin D, doxorubicin, bleomycin, vinblastine, dacarbazine, cyclophosphamide, and vincristine. These cases are of interest in view of the short interval between irradiation and the development of radiation nephropathy. Investigators have frequently suggested that chemotherapeutic agents may increase the risk of radiation damage to the kidney, as discussed in subsequent sections on dosage and HSCT.

In acute radiation nephropathy, Luxton related the immediate prognosis to the presence of malignant hypertension, although the exact parameters he used to define this entity were not given. Of a total of 20 patients studied, malignant hypertension developed in 8, and 6 of the 8 died within 3 to 12 months. The other two with malignant hypertension recovered spontaneously, although one died later with chronic renal failure, and the other had some degree of chronic renal insufficiency after 12 years of follow-up. Of the total group of 20, another 2 developed chronic renal failure and died from it 7 to 11 years after irradiation. One other patient died of a hypertensive cerebrovascular accident, and another died of widespread metastases of an original ovarian cancer. The remaining nine were alive and active after an average of 10 years, but all had evidence of chronic nephropathy. In this syndrome, signs of a poor prognosis were considered to be generalized edema, hypertensive retinopathy, and a blood urea level greater than 100 mg/100 mL during the first 3 months.

## Chronic Radiation Nephropathy (Chronic Radiation Nephritis)

Patients with chronic radiation "nephritis" (again, the term *nephropathy* is preferred to Luxton's use of the term *nephritis*) came from two main sources: (a) those who presented initially with acute radiation nephropathy and who continued with signs of chronic renal injury (e.g., the 14 patients described in the previous paragraph who did not die of malignant hypertension early in their course) and (b) those who gave no history of the acute syndrome but who had an indolent course and were found on later examination to have proteinuria and other evidence of renal damage. The symptoms in these two groups were referable to impairment of renal function, with some patients showing merely loss of energy and nocturia. Hypertension was present in some but not in others, and clearly, the prognosis was better in those patients with no rise in blood pressure. The long duration of chronic uremia was a feature of this syndrome, and blood urea levels of 100 to 250 mg/100 mL were borne for as long as 4 years.

Death in chronic renal failure could occur in either of the two groups of patients with chronic radiation nephropathy described in the foregoing paragraph. Although noting that the figures were small, Luxton gained the impression that patients whose cases followed an initial acute attack fared better than those without a preceding attack. Of the 14 patients whose chronic disease followed an acute attack, 9 were leading normal lives ≤12 years after irradiation; 5 of the 9 had impairment of renal function or hypertension (or both), whereas the other 4 showed only protein in the urine. The five deaths were due to chronic renal failure in three patients, hypertensive cerebrovascular accident in one, and spread of tumor in the other. As to the group of 10 patients in whom chronic nephropathy appeared without an antecedent acute attack, 3 died of chronic uremia on average 7 1/2 years after irradiation and 1 died of malignant disease. Malignant hypertension developed in one patient after 10 years, and five patients were alive 10 years after irradiation, two with slowly diminishing renal reserve and three with compensated renal failure.

## Asymptomatic Proteinuria

Thirteen of the 54 patients had renal dysfunction consisting only of proteinuria after an average follow-up of 11 years after radiotherapy. The quantitation of proteinuria was not provided, and the only additional information provided was a statement that "standard renal function tests were normal."

## Benign Hypertension

Six of Luxton's patients acquired a benign form of hypertension together with some proteinuria 2 1/2 to 5 years after irradiation. Two died of congestive failure, but the others were alive and without additional evidence of renal dysfunction at follow-up periods ranging from 9 to 13 years.

## Malignant Hypertension

Of all the patients studied by Luxton, 15 (28%) acquired malignant hypertension. This complication either occurred as part of the syndrome of acute radiation nephropathy or developed independently 18 months to 11 years after irradiation. Many deaths occurred in this group.

# Pathologic Findings

## Gross Appearance

The kidneys may be normal in size or contracted. Capsular thickening may be found in the more contracted forms, and the thickened renal capsule fuses with sclerotic tissue surrounding the kidney. The normal-sized kidneys, which correspond to those with little tubular loss and no interstitial fibrosis, have smooth subcapsular surfaces, and on cut surface, they may show nothing more than vague mottling. The contracted kidneys, corresponding to those with interstitial fibrosis, have capsules that may be impossible to strip from the cortex, which is reduced and firm. When radiation damage is confined to the main renal artery, the kidney would be expected to be reduced in size, as in renal artery stenosis of any cause. The subcapsular surface would be smooth in younger patients with no intrarenal vascular disease, but it could be granular in older patients.

## Light Microscopy

### Glomeruli

Most descriptions of glomerular alterations in clinical publications come from examination of the kidneys with advanced injury. These descriptions have not, in aggregate, resulted in the delineation of a distinctive histopathologic process. In the contracted kidneys, one may see considerable sclerosis of glomeruli, but even in this type of kidney, large numbers of glomeruli show little apparent change. In normal-sized kidneys, the glomeruli may show fibrinoid changes of tufts in continuity with similar changes in arterioles, segmental areas of glomerular scarring, and small fibrous crescents. Frequently, swelling of the glomerular capillary wall endothelium may progress to obliterate the capillary lumen. Thickening of the capillary walls is sometimes seen, and split, double-contoured glomerular basement membranes can be observed. The mesangium may be increased, primarily as a result of increased accumulations of matrix. *Mesangiolysis*, a term not in general use at the time some of these reports were written, can be identified in the illustrations of some of the early descriptions (632) (Fig. 18.37A). Accumulations of fibrin within glomerular capillaries can be demonstrated using special stains.

The most consistently observed changes—endothelial swelling and reactive changes, glomerular capillary basement membrane thickening and splitting, and mesangial thickening—are characteristic of a chronic mesangiocapillary or membranoproliferative form of glomerular injury. These changes may follow from diverse forms of primary injury, including the late manifestations of TMA. Indeed, some reports of radiation-associated glomerular injury clearly delineate changes consistent with the advanced stages of TMA (639,641), although this impression results primarily from the electron microscopic findings in these cases. The abnormalities seen by light microscopy have included segmental fibrinoid necrosis of the glomerular tufts, occlusive intracapillary accumulations of PAS-positive material with enmeshed erythrocytes, mesangiolysis, endothelial swelling, and splitting of glomerular capillary basement membranes, associated at times with correlative fibrinoid changes of the terminal afferent arterial vasculature. Only rarely have clinical laboratory findings been obtained to corroborate conclusively the presence of coagulation disorder consistent with TMA at the time of biopsy (639).

Appreciation of this potential congruence between TMA and radiation nephropathy is likely to be useful in unifying

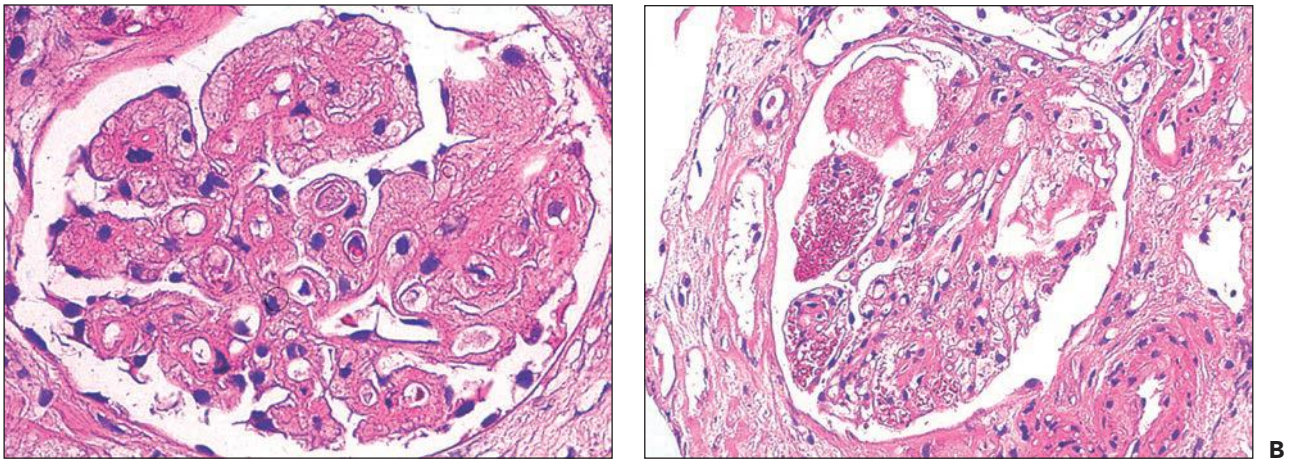

**FIGURE 18.37 Radiation nephropathy. A:** The glomerular capillary walls are diffusely thickened, and the capillary lumina are narrowed. Some of the glomerular capillary lumina are obliterated. (H&E) **B:** There is prominent segmental mesangiolysis with formation of capillary microaneurysms. (H&E) (Courtesy of Dr. Tibor Nadasdy.)

conceptually some of the various descriptions of radiation nephropathy in the older clinical literature with observations in experimental animals and with the special case of radiation nephropathy/TMA that clearly may occur in association with BMT.

### Blood Vessels

No vascular pathologic alterations are unique to radiation nephropathy. The arterioles and small interlobular arteries often show fibrinoid change without a cellular component in both contracted and normal-sized kidneys. These changes occur with more modest rises in blood pressure than is the case with nonirradiated kidneys and severe hypertension. These arteries may also demonstrate an intima expanded by the accumulation of pale-staining, at times mucoid-appearing, material that most likely represents a type of provisional matrix or loose connective tissue. Thrombotic lesions in the lumina may also be seen (Fig. 18.37B). Larger interlobular arteries often show fine collagenous or cellular intimal thickening and sometimes a denser type of sclerosis. This intimal thickening is usually a patchy process. Foam cells are often seen in the thickened intima of interlobular arteries (632,646). The origin of such cells remains unknown, but studies of foam cells in the arterial intima in human atherosclerosis have shown them to be predominately of macrophage origin, with a lesser population derived from smooth muscle cells. Arcuate, interlobar, and segmental arteries may show no changes, patchy fibrous intimal thickening, or more severe and extensive intimal sclerosing changes, especially when the kidneys are reduced in size. Because the microvascular injury may be focally distributed, its extent can be underappreciated in routine histologic sections.

### Tubules and Interstitium

As in the glomeruli and blood vessels, no distinctive tubular or interstitial pattern of injury characterizes any form of radiation nephropathy. Again, because much of the human material reported comes from cases with advanced injury, descriptions of the tubulointerstitial parenchyma emphasize the nonspecific features of interstitial fibrosis and tubular atrophy common to all forms of severe, chronic renal injury.

In the contracted kidneys demonstrating severe, chronic injury, considerable tubular loss is paralleled by extensive glomerular solidification. However, some degree of tubular loss and atrophy may occur in the absence of glomerular solidification, especially in laboratory animals subjected to irradiation. Nonspecific accumulations of mononuclear leukocytes are often seen.

In the nonscarred kidneys, tubular loss may be negligible or may occur focally in those areas where glomerular damage has occurred. The interstitium may show no changes or only a slight apparent increase in interstitial tissue. Edema with dilated peritubular capillaries may occur in patients dying early (647), comparable with the early stages of experimental irradiation of the canine kidney (648). Proteinaceous casts may be found in the lumina of tubules, and patchy hyaline droplet change occurs, especially in those cases with the greatest proteinuria. Other nonspecific changes, such as tubular cell vacuolization and desquamation, have been observed.

Some studies in the experimental literature, primarily those involving rodent and canine models, suggest an important and perhaps primary toxic effect on the tubules by radiation as the basis for subsequent radiation nephropathy (649–651). Evidence indicates an acute tubular injury, corresponding to acute tubular necrosis that may occur in the aftermath of high-dose irradiation (641). This injury appears to be followed by a reparative response of tubular regeneration and proliferation.

## Immunofluorescence Microscopy

Two reports noted no immunoglobulins, complement, or fibrinogen in the glomeruli (639,646). A patient with acute radiation nephritis complicated by the nephrotic syndrome (641) had segmental and irregular staining for IgM, C3, C4, and Clq in the glomerular capillary loops and mesangium; trace amounts of IgG and IgA were found in a similar distribution. Another report described IgG, IgM, and fibrinogen along the glomerular capillary walls with a focal and segmental distribution (638). IgM and C3 were detected in arteriolar walls. No distinctive pattern was identified.

### Electron Microscopy

In the glomeruli, one sees effacement of the visceral epithelial cell foot processes that is variable in its extent. The glomerular capillary walls usually show a widening of the subendothelial zone, which may contain electron-lucent or finely granular flocculent material. The glomerular endothelial cells are often swollen and contain increased numbers of organelles. In some accounts, these glomerular cells are described as detached from the endothelial aspect of the basement membrane. Mesangial cell cytoplasm or possibly entrapped endothelial cell cytoplasm is sometimes present in the capillary walls, and new glomerular basement membrane–like material may appear on the endothelial aspect. These changes are responsible for the double contours seen on silver staining. Mesangial cells may be swollen, and their cytoplasm may extend along the capillary loops. Fibrin has been seen between the endothelial cells and the glomerular basement membranes. Fewer accounts note changes in tubules and arteries, but in arteries, swollen and hypertrophic endothelium has been recorded along with fibrin or hyaline material in the subendothelial zone.

## Etiology and Pathogenesis

### Experimental Studies

Virtually, all the morphologic changes described in human radiation nephropathy can be reproduced in experimental models. However, the morphologic presentation of radiation nephropathy varies significantly between various species. In experimental animals, just like in humans, the radiation-induced renal functional deterioration is associated with structural changes affecting all four renal compartments. In rats, x-ray doses equivalent to those used in treating human subjects cause renal failure in both the local and total-body irradiation models after a median time of 30 to 40 weeks (652,653). The rats develop proteinuria, azotemia, and progressive hypertension eventually leading to renal failure which is the only significant cause of morbidity and mortality in this model. Progressive glomerular changes with dominant endothelial abnormalities similar to those described in the human kidneys with radiation nephropathy are the most characteristic findings in rats, mice, pigs, and nonhuman primates (637,650,651–652,654–656). The glomerular capillary endothelial changes include swelling, detachment from the underlying basement membrane, and accumulation of subendothelial electron-lucent material on electron microscopy with subsequent collapse of the capillary loops and eventual glomerular obsolescence. The glomerular endothelial changes are detectable in less than 6 weeks after irradiation in all species. In addition to endothelial changes, accumulation of platelet thrombi within the glomerular capillary loops is also observed in rats, mice, and pigs (637,649–651,657–659). Mesangial sclerosis has also been described in all animal models and mesangiolysis in some. The tubulointerstitial changes in rats show tubular epithelial degeneration, necrosis, and tubular atrophy that precede the morphologic changes in the arteries and arterioles suggesting direct toxicity of radiation to tubules (649–651,659,660). Fibrinoid necrosis of small arteries is a feature of radiation injury in dogs (661). Vascular occlusive lesions—thrombotic and nonthrombotic—seem to be prevalent only in some experimental models of radiation nephropathy (650). Widespread glomerulosclerosis, severe tubular loss, and extreme interstitial fibrosis were the long-term consequences of radiation injury to the kidney as shown in dogs that were irradiated in the neonatal period and died before the age of 4 years (662–664). These various experimental models show involvement of all four renal compartments of the kidney in the evolution of radiation nephropathy. Although some studies seem to support the prominence of glomerular endothelial injury in the pathogenesis, the relative contribution of the glomeruli, tubules, interstitium, and vessels in radiation nephropathy remains controversial. One traditional hypothesis of the pathogenesis of radiation nephropathy emphasizes the significance of vascular injury (665). The other hypothesis holds that parenchymal injury, primarily tubular cell injury, is the key event in the pathogenesis (666).

Mature pigs may serve as useful models for human radiation injury. In such models, when the pig kidneys are irradiated at doses of ≤9.8 Gy, one sees a measurable and progressive decline in renal hemodynamic parameters such as glomerular filtration rate and renal plasma flow (654). Serial renal biopsies in such animals have revealed principally injury to the glomerular peripheral capillary loops, initially characterized by leukocyte influx and attachment to the endothelium, and narrowing of the capillary lumina by the combined processes of endothelial cell swelling and intracapillary leukocyte stasis (654). These morphologic and functional changes can be identified as early as 3 to 6 weeks after irradiation. Immunohistochemical studies in this model using bromodeoxyuridine have detected an increase in glomerular cell proliferation as soon as 2 weeks after irradiation (667,668), before the appearance of overt morphologic injury. At subsequent periods of 2 months and later, the evolution of glomerular capillary wall abnormalities includes progressive widening of the subendothelial space with accumulations of electron-lucent material when studied by electron microscopy. Features suggestive of mesangiolytic injury can be identified in the illustrations of some of these studies. These morphologic changes most closely resemble those of TMA as encountered in humans, and they correspond to the descriptions of glomerular radiation injury in humans, mice, and some studies of rats given earlier in this section. In this model, detectable increases in tubular cell proliferation follow those of glomeruli by a period of weeks, morphologic evidence of tubular injury is much more focally distributed than that of glomeruli, and this injury appears reversible over time (668).

Although the precise molecular mechanisms responsible for the development of radiation nephropathy are not known, a number of factors playing potentially important roles in this process have been identified. In addition to angiotensin II, which seems to have a central role in the pathogenesis, there is some in vitro and in vivo evidence that inflammation and factors such as nitric oxide, TGF-β, connective tissue growth factor, and PAI-1 may also be involved (669–676). There are conflicting data about the role of chronic oxidative stress in the pathogenesis of radiation nephropathy. Some experimental data suggest the presence of oxidative damage to DNA for up to 24 weeks postirradiation (677), while others failed to detect any evidence of radiation-related oxidative stress in the kidney by the time there was physiologic evidence of renal injury (678). Recent experimental evidence also indicates that administration of dietary selenium is effective mitigating renal injury in a rat model of radiation nephropathy (679,680). Although the renoprotective effects of selenium in this model are not well

understood, the anti-inflammatory activity of selenium as well as selenoprotein-mediated antioxidant defenses may play a role.

## Hypertension

Hypertension often occurs in irradiation injury usually in association with radiation nephropathy. In addition to anemia, edema, proteinuria, hematuria, elevated serum creatinine, and decreased glomerular filtration rate, hypertension is one of the most common clinical manifestations of radiation nephropathy (681–683). Although the precise mechanism of radiation-associated hypertension is not readily understood, the renal origin seems undoubted. In several cases, removal of a single damaged kidney has resulted in a lowering of blood pressure, the first of these cases being reported by Dean and Abels (684). In this case, a woman received 4600 R (R, or roentgen, is a unit of radiation exposure introduced in 1928 but now replaced by other units of measurement; one roentgen = $2.58 \times 10^{-4}$ coulomb/kg of air (685)) to the left upper quadrant over 25 days, and hypertension developed 7 years later. At nephrectomy, the left kidney was shrunken in its lower third and showed obliterative arteriopathy in this part. Cogan and Ritter (686) described a 14-month-old boy who received a total dose of 5300 R over 36 days to the left renal area for a neuroblastoma; hypertension developed 3 months later. A left nephrectomy caused the blood pressure to fall. The excised kidney showed diffuse interstitial fibrosis, hyalinization and necrosis of the glomerular tufts, and intimal proliferative changes and thickening of arterial blood vessel walls. These investigators suggested that the hypertension was explainable by a "Goldblatt mechanism," presumably meaning that the arterial narrowing in the radiated kidney was responsible. A number of additional cases have also been reported with reversal of hypertension after removal of the irradiated kidney (645,687).

However, the precise mechanism of the hypertension in such cases is not known. Few reports mention intimal thickening of large arteries (688). We have seen a number of cases with prominent intimal thickening of the interlobular and arcuate arteries; however, there were cases that exhibited little change in large arteries.

Of great interest in this regard are cases in which irradiation is apparently responsible for narrowing of the main renal artery or for what has been called "hypoplasia" of the abdominal aorta and renal artery (689–692). In these cases, irradiation was usually performed during infancy or early childhood, and an interval of several years elapsed before hypertension became manifest. This interval was as long as 12 or 13 years in some instances. Unfortunately, reports on the pathologic features of the arterial changes are scanty, although in one of them (691), intimal and medial fibrosis with thrombosis in the narrowed renal artery was found. In two cases reported in one series (692), the patients were adults, and irradiation had been carried out at ages 15 and 27 years, respectively. Atheromatous plaques had developed in the proximal parts of the renal arteries. The immediate response to correction of the stenosis was good in some patients (690,691), a finding that suggests that the changes were more or less confined to the main renal artery or aorta with sparing of the intrarenal vasculature. Information on the pathologic features of the kidney is unfortunately sparse in this group, but ischemic changes were described in two patients (685), with no evidence of changes ascribable to radiation.

As described in Pathologic Changes (p. ) and Experimental Studies (p. ), it is quite apparent that the principal site of injury in clinically significant radiation nephropathy is the arterial and glomerular vasculature. The interstitial capillaries may also be prominently involved, but this feature is not as well established and is not always observed. As in other situations in which hypertension and lesions in small blood vessels coexist, the relationships between the two are complex. By analogy with the malignant phase of essential hypertension, one may assume that the fibrinoid necrosis of arterioles and small arteries that is frequently encountered in radiation injury is caused by the elevated blood pressure irrespective of mechanism. In contrast, necrosis in arterioles and small arteries may be found in the irradiated kidneys in the presence of only modest levels of blood pressure, a finding suggesting that the vascular changes are more likely related to the effects of radiation. The experimental evidence is confusing. First, investigators have shown that hypertension can develop in the rat after irradiation without any morphologically demonstrable vascular lesions (693). Second, Fisher and Hellstrom (694) showed that irradiation did exert an effect unrelated to any rise in blood pressure in the affected arteries. In this experiment, hypertension was produced in a series of rats by the application of a silver clip to one renal artery, the other renal artery being untouched. After irradiation of both the kidneys, vascular necroses were present on both sides. The arterial changes in the "nonclipped" kidney would be expected as a response to the elevated blood pressure, but such could not have been the case in the "clipped" kidney, which would have been protected against the high blood pressure by the clip on the renal artery. The necrotizing lesions in the clipped kidney must therefore have been caused by the radiation. Third, Asscher et al. (695) demonstrated that arteriolar and arterial necrosis can occur after irradiation at levels of blood pressure that, by themselves, are unlikely to cause necrosis. In these experiments, the authors irradiated part of a loop of mesentery and, after producing hypertension by renal artery constriction, compared the irradiated mesentery with the nonirradiated mesentery. Necrotizing arterial changes occurred in the irradiated but not in the untreated mesentery. "Sensitization" of the mesenteric arteries to the damaging effect of hypertension was not established until 2 months after the irradiation. That radiation alone was unable to produce the necroses was shown by the absence of these lesions in rats that were irradiated but did not have hypertension. Two possible explanations for the vascular damage as a result of combination of irradiation and hypertension were considered. The first was that irradiation could so weaken the arterial vessel wall that mechanical disruption by the increased intravascular pressure could occur. The second stemmed from Byrom's observation in the rat (696) that focal arterial spasm of cerebral and mesenteric vessels followed severe hypertension. It was suggested that this focal excessive vasoconstriction was a myogenic reaction to the increased intravascular pressure and that it led to arterial necrosis.

Asscher et al. (695) speculated that irradiation could render arterial walls more susceptible to hypertensive damage by exaggerating the myogenic response to variations in intravascular pressure. In this way, excessive vasoconstriction could occur as a response to what are merely physiologic variations in blood pressure, with consequent necrosis of the wall. Moreover, according to this concept, the increased susceptibility to vasoconstriction could cause ischemia, leading to the production of hypertension in the same way as occurs with organic occlusion of vessels.

These seemingly contradictory experimental studies do not really help us to determine the relationship between vascular lesions in the kidney and hypertension in humans, but they do show at least that radiation has a profound effect on arterial blood vessels with potential serious consequences for the kidney. More recent investigations of radiation-associated vascular injury have identified the endothelium as a principal site of injury, but they have not further clarified mechanisms that result in the fibrinoid vessel wall lesions and injury to arterial smooth muscle cells that were a focus of early studies. Studies of the potential activity of specific growth factors and cytokines now known to be important in arterial wall injury, repair, and sclerosis in various settings are noteworthy for their absence in studies of radiation nephropathy at the present time.

Other studies in animal models of radiation injury are beginning to dissect broader physiologic mechanisms for the development of hypertension. Administration of the angiotensin-converting enzyme inhibitor class of antihypertensive drugs or angiotensin II-receptor blockers is effective in the treatment or prophylaxis of experimental radiation nephropathy (697–700), whereas lowering of blood pressure to a similar degree with hydrochlorothiazide apparently has no similar beneficial effect (701). Studies by Juncos et al. (702) of the unilaterally radiated kidneys in rats revealed hypertension associated with elevated plasma renin levels at 12 weeks after radiation exposure. These studies suggest that radiation-associated hypertension may be mediated by alterations of the renin-angiotensin system, thus leading to a hyperreninemic state. However, no activation of the renin-angiotensin system was observed in a rat model of radiation nephropathy during the first 10 weeks postirradiation (653,698), and development of hypertension was also preceded by significant proteinuria and azotemia in this model. Therefore, the possibility that radiation nephropathy might be mediated by normal activity of the renin-angiotensin system has been raised. There is also experimental evidence to indicate that renal irradiation causes endothelial dysfunction prior to the onset of hypertension (665).

The preceding observations also suggest that, at present, the link between renal radiation injury and hypertension may invoke mechanisms not necessarily directly resulting from the effects of radiation on the target tissue, but they can be the consequence of chronic or fibrosing renal injury that may occur long after the acute injury. The evidence to support this conclusion is at least threefold: (a) in most clinically reported cases, the occurrence of hypertension takes place many years after radiation exposure; (b) in most case studies documenting the reversal of hypertension after excision of an irradiated kidney, the removed kidneys are typically described in whole or in part as shrunken, scarred, fibrotic end-stage organs; and (c) several studies implicate changes in the renin-angiotensin system that result from diminished blood flow in the development of postirradiation hypertension (i.e., Goldblatt-type mechanisms of injury), similar to changes that occur in other forms of advanced chronic renal injury.

A hyperreninemic state has been detected in some patients with radiation-associated hypertension, and this has been reversed with correction of blood pressure by removal of the damaged kidney, as published in isolated cases (645,691,703). In a prospective study of patients treated with high-dose abdominal irradiation for malignant diseases, approximately one half developed hypertension (704). The patients with hypertension demonstrated elevated peripheral plasma renin activity after oral administration of captopril compared with controls, and captopril renography was abnormal in five of the eight affected patients. Angiography in the five patients with an abnormal renogram demonstrated severe stenotic and tortuous changes in small intrarenal arterial vessels in the irradiated kidneys without stenosis of the main renal artery.

In aggregate, microvascular injury leading to diminished blood flow and a compensatory increase in renin and angiotensin could account for some proportion of cases of radiation-associated hypertension. Hypertension may also contribute to progressive vascular and parenchymal injury and the maintenance of the hypertension.

## Dosage of Irradiation

Early reports gave little idea of the level of dosage required to produce renal damage. Not until the investigations of Paterson (705) and Kunkler et al. (706) were published were limits of tolerance determined. Different methods of irradiation were considered with regard to the dose delivered to the kidney and the incidence of renal failure. Investigators showed with irradiation of the abdomen that (a) hypertension and renal failure may be caused when a homogeneous dose of 2300 R is delivered to the whole of both kidneys and (b) the risk of renal failure may be reduced when one third of the total volume of the kidneys is outside the irradiated field.

More current considerations of organ susceptibility to radiation have stressed the concept of tolerance dose. The minimal tolerance dose and the maximal tissue tolerance dose refer to a severe, life-threatening complication rate of 5% and 50%, respectively, occurring within 5 years of therapeutic radiation treatment (707). Few data define the tolerance doses of the human kidneys to a single dose of high-level radiation. Guidelines in this setting are frequently drawn from the studies of Glatstein et al. (637), who showed that in rats, a single dose of radiation of 19 Gy (a gray [Gy] being the Système International unit of absorbed radiation dose, where 1 Gy = 100 rad = 1 J of absorbed energy per kilogram of material (703)) to both the kidneys resulted in death from renal failure, whereas 90% of similarly irradiated rats survived at doses of 11 Gy. Although the perils of drawing exact correlations from the rat kidneys to the human kidneys are obvious, investigators have long recognized that dividing the total therapeutic radiation dose to be delivered into fractioned doses over a period of time considerably reduces the organ toxicity that would be engendered by a single dose of the same cumulative magnitude. Accordingly, more relevant tolerance doses are those that specify a specific regimen of fractionated dosing.

A recent comprehensive review of 12 studies reporting nephrotoxicity (i.e., increased serum creatinine or HUS after total-body irradiation) on 479 adult patients showed that on multivariate analysis, the radiation dose was the only significant factor associated with increased renal toxicity (708). Neither the dose rate nor the number of fractions played a significant role in the development of nephrotoxicity. However, when all the studies were considered except those with pediatric populations only ($n$ = 916 patients), the number of radiation fractions became a significant factor, in addition to the total dose and dose rate. A dose of 9.8 Gy was associated with a 5% risk of kidney toxicity, without nephrotoxic drugs, regardless of the fractionation scheme used (median dose, 12 Gy; range, 7.5 to 14; median fractions, 6; range 1 to 11, delivered once or twice daily).

The renal minimal tolerance dose has been considered to be 20 to 23 Gy when the kidneys are radiated bilaterally in fractionated protocols delivered over 3- to 5-week periods (709,710). Even the most acute radiation injuries of the kidney identified in humans take weeks to months to develop, and some features of chronic nephropathy or hypertension have been identified years and even decades after radiation exposure (710). Few clinical studies to ascertain tolerance doses have sufficient extended follow-up. A summary of such studies by Cassady (710) has identified a threshold radiation dose of 15 Gy, when fractionated protocols are used, for eventual development of detectable renal dysfunction. Whereas the pig and monkey can provide particularly good models of renal radiation injury, they have not yet been fully exploited to guide the establishment of criteria for tolerance doses using fractionated schedules.

Unilateral radiation to the left kidney in gastric cancer patients resulted in a progressive decrease in left (vs. right) renal function 12 to 18 months after chemoradiotherapy, with an associated increase in serum creatinine (711). The volume of the left kidney receiving greater than 20 Gy and the mean left kidney dose were associated with increased risk of renal injury. Sensitivity to radiation-induced kidney injury is also increased in neonates. Doses of 12 to 14 Gy at 1.25 to 1.5 Gy/fraction to an entire neonate kidney have been associated with a decreased glomerular filtration rate (712). For older children, the radiosensitivity of the kidney is similar to those seen in adults (710,713,714). A recent dose-volume analysis of radiation nephropathy in children showed that radiation-induced kidney functional impairment is rare with the current pediatric multimodal treatment approaches (715).

One prospective study of the deleterious effects of radiation on the kidney bears directly on the issue of radiation dose. In this study by Dewit et al. (716), several parameters of renal function (but not histopathologic features) were followed for 3 to 5 years in patients following therapeutic radiation for a variety of malignant diseases. In patients receiving the highest degree of radiation (40 Gy in fractionated doses over 5 1/2 weeks), creatinine clearances decreased approximately 20% over the 3- to 5-year interval. Patients receiving lesser doses of radiation (17 to 18 Gy in fractionated doses over 3 1/2 weeks) had no comparable detectable decline in renal function over the same follow-up period. Certain other tests of renal function, including concentrating capacity (measured by urine osmolality), demonstrated no significant alterations as a result of radiation in any of the dosage ranges studied.

An additional point, stressed by Dewit et al. (716) in summarizing their work and that of others, is that an inverse relationship appears to exist between radiation dose and the length of the latency period before overt nephrotoxicity can be detected. Thus, although the patients receiving high-dose radiation had detectable abnormalities within 3 to 5 years after injury, those patients receiving lower doses may not show such effects until some additional years later. For example, renal dysfunction attributed to the consequences of radiation has been identified at periods of up to 19 years after unilateral renal radiation at doses of 15 to 20 Gy occurring in patients receiving radiation therapy for gastric ulcers (717). This point makes it virtually impossible to determine precise lengths of time needed to determine tolerance doses for the kidney.

A longstanding but unresolved area of concern is the extent to which augmentation of radiation damage in the kidney by concomitant or sequential administration of chemotherapeutic agents or other drugs may occur (642,708,710,718,719). The mechanisms underlying such interactions are complex and poorly understood, but the effects are not due to simple additive cell killing of a common target cell population. Even when specific agents are directed against different cell populations, tissue sensitivity to both radiation (i.e., a lower tolerance dose) and the chemotherapeutic agents utilized may be enhanced. In other cases, chemotherapeutic agents may have direct renal toxicities, such as the tubular toxicities of cis-platinum, which may potentiate or add to the effect of radiation.

Because modern therapeutic radiation protocols frequently reduce radiation dosage or administer radiation sequentially when chemotherapy is being utilized in a given patient, serious complications of combined therapy are infrequent. Specific mechanistic interactions between chemotherapeutic agents and radiation in individual case reports are difficult to evaluate because of the uncontrolled, anecdotal nature of such evidence. Actinomycin D, used in conjunction with radiation to treat Wilms tumor, can potentiate radiation injury in some tissues, although such an effect in the kidney has not been substantiated (710). In rats, cisplatinum and the nitrosourea BCNU have both been shown to cause additional dose-related decreases in renal function beyond those attributable to radiation alone (719). In the comprehensive review by Cheng et al. (708), the use of cyclosporine and teniposide in pediatric patients was associated with radiation-induced nephrotoxicity following total-body irradiation. In patients without the concurrent use of these drugs, at doses ≤13 Gy, the incidence of kidney toxicity was less than 8%. A recent dose-volume analysis of radiation nephropathy in children showed that radiation-induced kidney functional impairment is rare with the current pediatric multimodal treatment approaches (715).

Only limited information is available on the nature of renal tissue injury that may develop in the aftermath of an atomic or nuclear explosion. A follow-up study of children exposed to the atomic bomb explosion at Hiroshima (720) showed no greater incidence of nephritis or urinary abnormality in these patients than in children not exposed. The most recent general report on mortality in the Life Span Study (LSS) cohort of atomic bomb survivors from Japan showed an increased risk of nonneoplastic diseases, including the circulatory diseases, but no increased risk for renal diseases (721). Early kidney failure due to high-dose irradiation (>20 Gy) has been described in radiation accidents in Russia (722).

## HEMATOPOIETIC STEM CELL TRANSPLANTATION–ASSOCIATED TMA

HSCT is a widely used therapy for hematologic, lymphoid, and some epithelial and mesenchymal malignant diseases, as well as for some nonneoplastic metabolic and genetic disorders. Although the hematopoietic stem cells can be derived from bone marrow (hence the historical term bone marrow transplantation–associated TMA), the most common source currently is peripheral blood. Total-body irradiation to ablate the recipient hematopoietic and lymphoid cells is an important aspect of HSCT. Several syndromes of acute renal insufficiency have been identified in these HSCT patients. Most of these syndromes occur early after HSCT (i.e., in the first 3 months) and are usually the result of sepsis, administration of nephrotoxic antibiotics

or other therapeutic agents, tumor lysis syndrome, or hepatorenal syndromes (723,724). They are not further discussed in this chapter. Of particular note is a clinical syndrome of late renal insufficiency, generally occurring greater than 3 months and with a peak incidence of 9 to 12 months after HSCT (724–731). This syndrome has long been recognized to demonstrate clinical and morphologic features of TMA (728,732). Historical synonyms for this syndrome include BMT-associated TMA, radiation nephropathy after total-body irradiation, BMT nephropathy, TTP, or HUS following BMT and HUS/TTP after BMT. Although the toxicity committee of the Blood and Marrow Transplant Clinical Trials Network recommended the term posttransplantation TMA for this disorder (733), we prefer the term hematopoietic stem cell transplant (HSCT)-associated TMA to avoid confusion with other TMAs that develop in patients with solid organ transplants. Therefore, the term HSCT-associated TMA is used throughout this chapter.

Hematopoietic stem cell transplant-associated TMA can occur with autologous or allogeneic HSCT, in the presence or absence of graft versus host disease, with or without total-body irradiation and exposure to cyclosporine, and a triggering infection may or not be present. A systematic review of the literature from 1966 to 2003 revealed an 8.2% incidence of TTP/HUS in 5423 allogeneic HSCT patients (728). However, the reported incidence varied by 125-fold from 0.5% to 63.6%. This great variation in the incidence underlines the difficulties of the clinical diagnosis of HSCT-associated TMA. These patients can be critically ill with numerous comorbidities such as opportunistic infections, drug toxicity, radiation-related injury, and acute GVHD all of which can mimic the laboratory and clinical features of TMA. The slightly different guidelines put forth by two expert panels to define the diagnostic criteria have highlighted the difficulties in rendering a clinical diagnosis of posttransplantation TMA (733,734).

## Clinical Presentation

The clinical presentation is either a rapid or a gradual decline in renal function manifested by increased BUN and serum creatinine concentrations. In addition to hemolytic anemia and thrombocytopenia, hypertension, hematuria and red blood cell casts, proteinuria, consumptive coagulopathy and congestive heart failure are frequent findings. Anemia is disproportionately worse than would be expected for the degree of azotemia. Individual patients may experience severe consequences as a result of these abnormalities, whereas in others, the disease process may be mild. However, there might be a significant discordance between the clinical and histologic diagnosis of TMA in this setting. Pathologic findings of TMA may be present in the kidney as an isolated lesion in the absence of clinical criteria for TMA, well documented in both autopsy and biopsy series (735–737). An autopsy study of 314 patients who died after HSCT reported a 20% incidence of renal TMA with little correlation between the clinical and tissue diagnosis of TMA (735). In contrast, another autopsy study of 20 patients with HSCT, 8 of whom had renal TMA, documented strong correlation between the clinical and morphologic diagnosis of TMA (738).

## Pathologic Findings

The morphologic features encountered in renal biopsies or autopsies of affected patients are typical of a thrombotic microangiopathic process such as HUS. These features are described in Pathologic Changes of TMA (p. 753–761) and are illustrated in Figs. 18.1 to 18.19, 18.37, and 18.38. Descriptions of the renal pathologic features in HSCT nephropathy are fairly uniform, despite variations in the conditioning regimen used to prepare patients for HSCT in different centers and in concurrent laboratory parameters of affected patients (737,739–741). Whether or not patients have clinical evidence of hemolytic anemia or thrombocytopenia, the renal lesions typically involve glomeruli and small arterial and arteriolar vessels. The light, immunofluorescent, and electron microscopic features are identical to the general description of TMA with progressive sclerotic changes observed with chronicity. Full morphologic resolution of the microthrombotic process has also been documented within months of the initial injury. In the series of Antignac et al. (742), in which two patients underwent repeat biopsy, the nephropathy was markedly progressive, with features of global

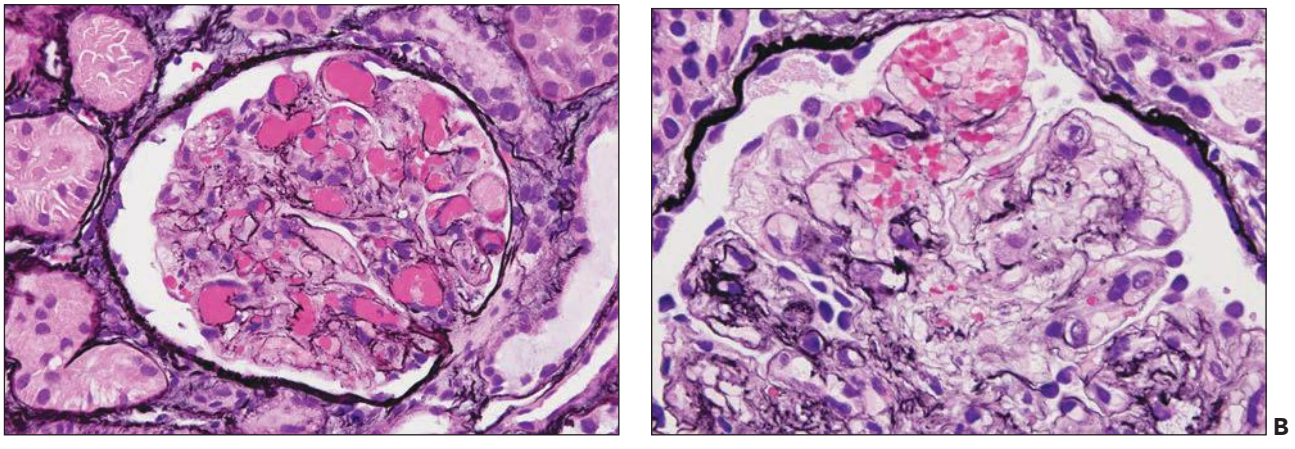


**FIGURE 18.38 Thrombotic microangiopathy in a 19-year-old patient 6 months after bone marrow transplantation.** **A:** A few of the glomerular capillaries show thrombotic lesions and many capillaries are congested. Focal reduplication of the glomerular capillary basement membranes is also noted. **B:** The finding of mesangiolysis and capillary microaneurysms is similar to that seen in radiation nephropathy. (Methenamine-silver.)

glomerular sclerosis, glomerular ischemia, and extensive tubular atrophy present in the second biopsy, but in which resolution of mesangiolytic changes and improvement in arteriolar lesions were also reported. In addition to renal involvement, HSCT-associated TMA can occasionally affect other organs such as lung and gastrointestinal tract (738,743).

## Etiology and Pathogenesis

The high incidence of this clinical and pathologic syndrome has led some investigators to identify it as a distinct entity, BMT nephropathy (now HSCT-associated TMA or HSCT nephropathy) (726,727). This syndrome was suspected to be a likely manifestation of radiation nephropathy at the time of its first description by Bergstein et al. (638), but in general, the difficulty in separating it from confounding contributions of concurrent drug therapy, infections, and other clinical variables present in these of extremely ill patients makes it difficult to establish this relationship conclusively (739,744).

However, the lines of evidence to support the role of radiation include (a) renal biopsy findings in affected patients that are virtually identical to those recognized as characteristic of radiation nephropathy as described in Pathologic Changes (p. ), (b) similar latencies after exposure to radiation between development of clinical radiation nephropathy and HSCT nephropathy, (c) overlapping clinical findings between patients with TMA after HSCT and patients with radiation nephropathy and concomitant evidence of a coagulation disorder, (d) occurrence of TMA in HSCT patients who underwent total-body irradiation and T-cell depletion but did not receive CNIs (745), and (e) an association between reduction of total radiation dose by partial shielding of the kidneys during total-body irradiation and dramatic lowering of the incidence of HSCT-associated HUS (746,747). In the series by Lawton et al. (747), this method for reducing the renal radiation dose resulted in a decline of HSCT-associated HUS from 26% to 6% in otherwise equivalent patient populations. This last observation in particular serves to establish the pathogenic role of renal irradiation in the development of this disorder. A variety of factors including radiation dose, volume and fractionation, and patient factors such as preexisting disease and other risk factors determine the renal tolerance and susceptibility to develop radiation nephropathy (707). It is generally recommended that during total-body irradiation, the kidneys be shielded to a biologically effective dose of less than 16 Gy; however, with a total of 12 Gy delivered in multiple fractions, the complication of radiation nephritis can be infrequent even without shielding the kidneys (748,749). Children and adults with comorbidities appear to tolerate lower doses of radiation.

However, note should be taken that TMA can also develop in HSCT patients who have not received total-body irradiation as part of their conditioning regimen (738,739,750). In a series published by Elliott et al. (750), TTP developed in 2 of 13 patients who underwent bone marrow transplantation after nonmyeloablative conditioning regimen. In our own series of HSCT-associated TMA, 4 of 8 patients with histologic evidence of renal TMA had no total-body irradiation as part of the conditioning regimen (738). The renal morphologic changes of TMA in patients with prior irradiation were indistinguishable from those who developed TMA without irradiation (738).

The pathogenesis of this disorder, as in radiation nephropathy unassociated with HSCT and total-body irradiation, remains obscure. Analysis of clinical series reported to date reveals no predisposition for the development of this late nephropathy resulting from episodes of acute renal failure occurring early in the clinical course; that is, this form of radiation nephropathy appears distinct from and is not potentiated by the renal effects of specific nephrotoxins, sepsis, or prior occurrence of a hepatorenal syndrome. Markers of endothelial injury, such as vWF and soluble thrombomodulin (TM) levels, were shown to be increased in patients with HSCT-associated TMA (751). UL vWF multimers, indicative of large-scale disruption of endothelial cells, have also been demonstrated in some patients with HSCT-associated TMA (750). Endothelial injury may result from multiple pathogenetic factors, including chemotherapy, infections, GVHD, and cyclosporine treatment potentiated by conditioning regimens (271,743,752).

Cyclosporine, a drug with known potential to cause HUS (209), is widely used to treat the graft versus host disease that frequently occurs in patients who have undergone HSCT. Accordingly, this drug, rather than total-body irradiation, has been suspected to contribute to or even to be the principal agent of HSCT nephropathy in some patients. However, early reviews in the literature showed that approximately one half of the affected patients who have undergone HSCT had no exposure to cyclosporine, a finding making it most unlikely that this drug is the principal nephrotoxin in this disorder (724,731). When CNIs were combined with sirolimus following allogeneic HSCT, the incidence of TMA (10.8%) was significantly higher than in those patients treated without sirolimus (753). In this study, only the use of sirolimus and grade II to IV acute graft versus host disease were associated with TMA in regression analyses.

It has also been hypothesized that GVHD and endothelial injury due to cytokines, cytotoxic donor T lymphocytes, or circulating antibodies may be involved in the pathogenesis of posttransplantation TMA (735,743,754). In a recent autopsy study of patients who underwent hematopoietic stem cell transplantation, the risk of TMA was significantly increased in the presence of graft versus host disease and was independent of cyclosporine use (735). In suspected cases of chronic graft versus host disease–induced posttransplantation TMA, glomerulitis and peritubular capillaritis with infiltrating cytotoxic T cells was demonstrated in addition to endothelial injury (755). In 4 of 7 cases, glomerular and peritubular capillary C4d staining was present, suggesting the involvement of chronic humoral-mediated graft versus host disease (755). There is also some evidence to suggest a potential link between VEGF and the development of GVHD and TMA. Lower levels of VEGF have been associated with more severe forms of GVHD, and therefore endothelial injury mediated by low levels of VEGF might play a role in the development of TMA (735,756). TMA in the kidney may merely represent renal manifestation of GVHD. This hypothesis of graft versus host disease-induced TMA has therapeutic implications as such patients may benefit from increasing rather than withdrawal of cyclosporine (735,743). Antibody-depleting therapies may be useful in the presence of C4d staining in kidney biopsies from HSCT patients with TMA.

## Animal Models

Some animal models, principally using rats and mice, have been designed to simulate the process of total-body irradiation used to prepare patients for HSCT (718,757,758). Several of these protocols further recapitulate the clinical protocols used

in patients by subsequent infusion of syngeneic bone marrow cells to avoid the confounding effects of hematopoietic failure that typically occur in this form of cytoreduction therapy. These studies have shown that such models can reproduce the glomerular and, at times, the hematologic features of glomerular injury and TMA described in human patients. These models have been used to dissect the additional effects on the kidney of several of the drugs commonly used in the course of HSCT. The results to date stand in some contrast to the human situation. Lawton et al. (718) concluded from studies in rodents that no additional nephrotoxic effects on renal function were noted beyond those of total-body irradiation alone for gentamicin, amphotericin, or cyclosporine for periods of ≤6 months after HSCT. In this study, busulfan was the only drug tested that exerted an additional nephrotoxic effect. This finding contrasts with the situation in human patients undergoing HSCT in whom the use of gentamicin and amphotericin has been strongly implicated in the acute renal failure that may occur in the first few months after HSCT (724), although in humans, this effect may be mediated through mechanisms of a hepatorenal syndrome rather than by direct cytotoxicity to renal cells. These findings indicate possibly important limitations in the use of these rodent protocols to model the human situation.

### Treatment

Treatment for radiation nephropathy in patients who have undergone HSCT remains supportive. Many patients exhibit only mild renal insufficiency, and such patients may recover renal function with improvement in concurrent hematologic abnormalities (731). More severe cases may require dialysis; in such patients, mortality may be high (731). Plasma exchange, the principal treatment in the idiopathic forms of TMA in adults, seems to be ineffective in patients with HSCT-associated TMA (759). The ineffectiveness of plasma exchange treatment in this setting has been attributed to the absence of ADAMTS13 deficiency in these patients (750,760). However, Vesely et al. (164) showed that severe ADAMTS13 deficiency does not identify all patients diagnosed with HUS/TTP who may respond to plasma exchange treatment.

Several studies in animal models and humans have confirmed the role of angiotensin-converting enzyme inhibitors and angiotensin II receptor antagonists in the prevention as well as treatment of HSCT and radiation nephropathy (697,761,762). While antihypertensive properties of these drugs are definitely beneficial, the primary mechanisms of renoprotection appear to be vasodilation of the glomerular arterioles with consequent lowering of intraglomerular pressures and through potential antioxidant properties (763). In addition to angiotensin II receptor blockers, amifostine, a potent free radical scavenger and antioxidant has been proposed as an agent to reduce radiation-induced nephropathy (764). Although no definite increase in markers of chronic oxidative stress was identified in the kidneys of a rat model after total-body irradiation (678), suppression of renin-angiotensin system has been proven to be clinically useful in randomized controlled studies to limit the radiation nephrotoxicity in hematopoietic transplantation (699,761). Individual studies suggest that administration of anticoagulant sulodexide (669) and the anti-inflammatory agent dexamethasone (765) also may diminish the nephrotoxic effect of radiation in experimental systems, but such findings have not yet been translated into clinical trials in human patients.

## RADIATION NEPHROPATHY DUE TO RADIONUCLIDE THERAPY

Radionuclide therapy targets tumors by delivering radioactive particles directly to tumor cells. This is achieved by attaching (chelating) beta, alpha, or gamma emitting radionuclides to monoclonal antibodies, antibody fragments, or low molecular weight oncophilic peptides. Delivery of radioactivity to the target tissue is governed by the specificity of the antibodies or oncophilic peptides to antigenic epitopes or receptors expressed by tumor cells. Radiation injury to the kidney has been observed in association with some of these treatment modalities (766,767).

Conventional radioimmunotherapy uses radiolabeled antibodies or *Fab* fragments that target tumor-associated epitopes, while the alternate approach, peptide-receptor radionuclide therapy (PRRT), utilizes radiolabeled peptides that bind to cell surface receptors expressed by tumors. Most clinical experience gained with PRRT so far has been with radiolabeled low molecular weight somatostatin-analogue (octreotide) octapeptides targeting tumors that overexpress somatostatin receptors. This particular therapy now has a well-established role in the treatment of metastatic neuroendocrine tumors (768). Binding of these octapeptides to somatostatin receptors is followed by receptor-mediated internalization and retention of the radiolabeled substances in lysosomes that facilitates accumulation of radioactivity within tumor cells. High activity indium 111 ($^{111}$I)-diethylenetriamine-pentaacetic acid (DTPA) octreotide and beta and/or gamma particle emitters such as Yttrium 90 ($^{90}$Y) conjugated via tetraazacyclododecane-tetraacetic acid (DOTA) to Tyr$^3$-octreotide ($^{90}$Y-DOTATOC) or to lanreotide ($^{90}$Y-DOTALAN), and of lutetium 177 ($^{177}$Lu) to Tyr$^3$-octreotate ($^{177}$Lu-DOTATATE) are in use (769). The binding activity of various somatostatin octapeptides to various subtypes of somatostatin receptors varies significantly, influencing the efficacy of the treatment. For example, the analogue octreotate (DOTA, Tyr$^3$-octreotate) has a ninefold higher activity for the somatostatin receptor subtype 2 as compared with octreotide (DOTA, Tyr$^3$-octreotide) (770). The main dose-limiting complications of PRRT include acute myelotoxicity and long-term renal toxicity. The renal uptake of radioactivity following intravenous administration of radiolabeled octapeptides is one of the highest among various organs making the kidney a potential target for radiation injury (771).

Nephrotoxicity secondary to treatment with the beta-emitter $^{90}$Y-DOTATOC has been reported by various groups (643,772,773) and is dose related (774,775). A study by Moll et al. (772) indicates that patients receiving a cumulative dose of greater than 200 mCi/m$^2$ of $^{90}$Y-DOTATOC for advanced neuroendocrine tumors may increase the risk of developing chronic renal failure. Renal biopsies from these patients with renal impairment show typical features of TMA with glomerular, arteriolar, and small arterial involvement. Dosimetry studies with $^{90}$Y-DOTATOC and $^{177}$Lu-DOTATATE have indicated that a biologic effective dose of less than 40 Gy is safe in the absence of preexisting renal disease, while a similar dose of greater than 45 Gy of $^{90}$Y-DOTATOC increases the risk of acute deterioration of renal function (774,776). No renal toxicity was observed in another trial that included 154 patients receiving cumulative doses up to 8.58 GBq (5.0 GBq/m$^2$) of $^{90}$Y-DOTALAN (486). Likewise, no renal toxicity has been

observed with $^{111}$In-DTPA-octreotide with cumulative doses up to 100 GBq and renal dose of 45 Gy (777).

No renal toxicity has been reported from clinical trials using various monoclonal antibodies, such as $^{131}$I-tositumomab or $^{90}$Y-ibritumomab (778–780). However, when rhenium 188–labeled monoclonal antibodies ($^{188}$Re-anti-CD66) were applied as part of combined chemotherapy and external beam total-body irradiation of 12 Gy prior to stem cell transplantation, 6 of 36 patients developed an increase in serum creatinine 6 to 12 months after therapy (781). Radiation nephropathy was diagnosed morphologically in one of six cases.

The higher rate of nephrotoxicity (e.g., radiation nephropathy) in patients treated with radionuclides chelated to small molecular weight peptides versus monoclonal antibodies is likely due to the high rate of renal catabolism of low molecular weight proteins (782). The low molecular weight proteins such as octreotide are filtered through the glomerular capillary basement membranes followed by tubular reabsorption. The cationic or neutral radiolabeled molecules filter more efficiently across the anionic GBM and are also better reabsorbed by proximal tubules. Multiple transport mechanisms appear to be involved in the proximal tubular reabsorption of these peptides (767). But in vitro studies suggest that the primary reabsorption mechanism is by receptor-mediated endocytosis, possibly involving megalin and cubilin receptors in proximal tubules (783). These peptides may also be actively secreted from the peritubular capillary blood into the tubular lumen (767). Proteolytic digestion of the peptides by tubular lysosomal enzymes results in breakdown products including radionuclide-chelated amino acids. Entrapment of these chelated amino acids within the kidney tubules occurs by binding to intracellular metal-binding proteins.

Several studies indicate that development of radiation nephropathy after radionuclide therapy likely depends on multiple factors, such as dose of radiation, the type of isotope, the characteristics of the carrier monoclonal antibody or peptide used, such as size, charge, and clearance pathways, individual renal volume, dose rate, and fractionation. The severity of nephrotoxicity can be reduced by accurate pretreatment renal dosimetry, especially in the setting of renal impairment (784). Alpha emitters, such as $^{111}$In, have high ionizing properties over a short range that result in a high relative biologic effectiveness as compared to a similar dose of x-rays (785). The mean particle range of Auger electrons emitted by $^{111}$In is less than one cell diameter, while beta particles emitted by $^{90}$Y have much deeper penetration range ($R_{95}$ 5.7 mm). It has been hypothesized that the absence of significant radiation nephrotoxicity in patients treated with $^{111}$In-DTPA compared to $^{90}$Y-DOTATOC is due to the limited range of damage inflicted by $^{111}$In.

The nephrotoxicity can be reduced by several methods that limit tubular reabsorption of and damage by these radiolabeled peptides. Structural changes to the peptide such as substitution, addition, or deletion of amino acids to alter the net charge can have significant effects on the renal uptake of these radionuclides (786–788). The pharmacokinetics of the peptides can also be altered by adding polyethylene glycol groups or albumin-binding sequences, with resultant renoprotection (789). The competition for the negative charges on the tubular basement membrane between the radiolabeled compound and other cationic compounds may also inhibit renal tubular reabsorption (790,791), and radiolabeled monoclonal antibodies could be inhibited significantly by cationic amino acids (792). In rats, coinjection of 400 mg/kg l-lysine inhibited renal uptake of $^{111}$In-DTPA octreotide by approximately 40% (793). There are several human studies indicating that renoprotection during radionuclide therapy can be achieved by coinfusions of cationic, basic amino acids such as lysine and arginine (776,794,795). It has been shown that the maximum cumulative dose of $^{90}$Y-DOTATOC and $^{177}$Lu-DOTA-TYR$^3$-octreotate can be safely increased when such renal protection is used (790,791). Currently, coinfusion of basic amino acids is a standard renoprotective regimen in PRRT therapy. Nevertheless, there are some data indicating that the long-term outcome of renal function in patients treated with PRRT may be less favorable even when renoprotective regimens are applied. A sustained decline in creatinine clearance with an average of 7.3% in patients treated with $^{90}$Y-DOTATOC and 3.8% in those treated with $^{177}$Lu-DOTATATE was observed with a median follow-up of 2.9 years and 2.3 years, respectively (796). Eleven of sixty-five patients in this study had more than 15% decline of creatinine clearance per year.

Although experimental, other protective modalities of proximal tubular reabsorption interference include disruption of endocytosis by reducing the energy supply using maleate that inhibits the citric acid cycle and blocking tubular transporters by using probenecid (797). The oxidative stress caused by radionuclide-induced release of free radicals can be countered by using radioprotectors such as amifostine (798). Dose fractionation may give the normal tissue a chance to repair between the irradiation cycles without significantly altering the tumor response (798).

Although somatostatin analogs are most commonly used, many other radiolabeled peptides have been studied including gastrin, cholecystokinin, and exendin analogs that target gastrin receptor, cholecystin-2 receptor, and glucagon-like peptide receptors, respectively, expressed by various neuroendocrine and nonneuroendocrine tumors (799,800). Several other new peptides such as bombesin, VIP, NPY ($Y_1$) can potentially be targeted in nonneuroendocrine tumors as prostate and breast carcinoma (769), expanding the therapeutic potential of PRRT, but also increasing the frequency of renal vulnerability.

In summary, radiation nephropathy occurs in patients with radionuclide therapy, especially those with PPRT. The toxicity depends on multiple factors, including the dose of radiation, type of radionuclide, and the physicochemical properties of the carrier molecules. Renal toxicity can be limited by various renoprotective modalities during therapy; however, gradual renal functional deterioration over long range may still be the consequence.

## REFERENCES


1. Moschcowitz E. An acute febrile pleiochromic anemia with hyaline thrombosis of the terminal arterioles and capillaries: an undescribed disease. *Arch Intern Med* 1925;36.
2. Baehr G, Klemperer P, Schifrin A. An acute febrile anemia and thrombocytopenic purpura with diffuse platelet thromboses of capillaries and arterioles. *Trans Assoc Am Physicians* 1936;65.
3. Singer K, Bornstein FP, Wile SA. Thrombotic thrombocytopenic purpura: hemorrhagic diathesis with generalized platelet thromboses. *Blood* 1947;2.
4. Symmers WS. Thrombotic microangiopathic haemolytic anaemia (thrombotic microangiopathy). *Br Med J* 1952;2(4790):897–903.

5. Gasser C, Gautier E, Steck A, et al. [Hemolytic-uremic syndrome: bilateral necrosis of the renal cortex in acute acquired hemolytic anemia]. *Schweiz Med Wochenschr* 1955;85(38–39):905–909.
6. Habib R, Mathieu H, Royer P. [Arteriole-capillary thrombotic disease of the kidney in children]. *Rev Fr Etud Clin Biol* 1958;3(8):891–894.
7. Amorosi EL, Ultmann JE. Thrombotic thrombocytopenic purpura: report of 16 cases and review of the literature. *Medicine* 1966;45:139.
8. Rock GA, Shumak KH, Buskard NA, et al. Comparison of plasma exchange with plasma infusion in the treatment of thrombotic thrombocytopenic purpura. Canadian Apheresis Study Group. *N Engl J Med* 1991;325(6):393–397.
9. George JN. How I treat patients with thrombotic thrombocytopenic purpura: 2010. *Blood* 2010;116(20):4060–4069.
10. Galbusera M, Noris M, Remuzzi G. Thrombotic thrombocytopenic purpura—then and now. *Semin Thromb Hemost* 2006;32(2):81–89.
11. Loirat C, Fremeaux-Bacchi V. Atypical hemolytic uremic syndrome. *Orphanet J Rare Dis* 2011;6:60.
12. Karpac CA, Li X, Terrell DR, et al. Sporadic bloody diarrhoea-associated thrombotic thrombocytopenic purpura-haemolytic uraemic syndrome: an adult and paediatric comparison. *Br J Haematol* 2008;141(5): 696–707.
13. Ruebner RL, Kaplan BS, Copelovitch L. A time for reappraisal of "atypical" hemolytic uremic syndrome: should all patients be treated the same? *Eur J Pediatr* 2012.
14. Schifferli A, von Vigier RO, Fontana M, et al. Hemolytic-uremic syndrome in Switzerland: a nationwide surveillance 1997–2003. *Eur J Pediatr* 2010;169(5):591–598.
15. Banerjee R, Hersh AL, Newland J, et al. *Streptococcus pneumoniae*-associated hemolytic uremic syndrome among children in North America. *Pediatr Infect Dis J* 2011;30(9):736–739.
16. Lee CS, Chen MJ, Chiou YH, et al. Invasive pneumococcal pneumonia is the major cause of paediatric haemolytic-uraemic syndrome in Taiwan. *Nephrology (Carlton)* 2012;17(1):48–52.
17. Rangel JM, Sparling PH, Crowe C, et al. Epidemiology of *Escherichia coli* O157:H7 outbreaks, United States, 1982–2002. *Emerg Infect Dis* 2005;11(4):603–609.
18. Banatvala N, Griffin PM, Greene KD, et al. The United States National Prospective Hemolytic Uremic Syndrome Study: microbiologic, serologic, clinical, and epidemiologic findings. *J Infect Dis* 2001;183(7):1063–1070.
19. Andreoli SP, Trachtman H, Acheson DW, et al. Hemolytic uremic syndrome: epidemiology, pathophysiology, and therapy. *Pediatr Nephrol* 2002;17(4):293–298.
20. Tozzi AE, Caprioli A, Minelli F, et al. Shiga toxin-producing *Escherichia coli* infections associated with hemolytic uremic syndrome, Italy, 1988–2000. *Emerg Infect Dis* 2003;9(1):106–108.
21. Thorpe CM. Shiga toxin-producing *Escherichia coli* infection. *Clin Infect Dis* 2004;38(9):1298–1303.
22. Jenkins C, Willshaw GA, Evans J, et al. Subtyping of virulence genes in verocytotoxin-producing *Escherichia coli* (VTEC) other than serogroup O157 associated with disease in the United Kingdom. *J Med Microbiol* 2003;52(Pt 11):941–947.
23. Lopez EL, Diaz M, Grinstein S, et al. Hemolytic uremic syndrome and diarrhea in Argentine children: the role of Shiga-like toxins. *J Infect Dis* 1989;160(3):469–475.
24. Tzschoppe M, Martin A, Beutin L. A rapid procedure for the detection and isolation of enterohaemorrhagic *Escherichia coli* (EHEC) serogroup O26, O103, O111, O118, O121, O145 and O157 strains and the aggregative EHEC O104:H4 strain from ready-to-eat vegetables. *Int J Food Microbiol* 2012;152(1–2):19–30.
25. Beutin L, Martin A. Outbreak of Shiga toxin-producing *Escherichia coli* (STEC) O104:H4 infection in Germany causes a paradigm shift with regard to human pathogenicity of STEC strains. *J Food Prot* 2012;75(2):408–418.
26. Kemper MJ. Outbreak of hemolytic uremic syndrome caused by *E. coli* O104:H4 in Germany: a pediatric perspective. *Pediatr Nephrol* 2012;27(2):161–164.
27. Srivastava RN, Moudgil A, Bagga A, et al. Hemolytic uremic syndrome in children in northern India. *Pediatr Nephrol* 1991;5(3):284–288.
28. Guerin PJ, Brasher C, Baron E, et al. *Shigella dysenteriae* serotype 1 in west Africa: intervention strategy for an outbreak in Sierra Leone. *Lancet* 2003;362(9385):705–706.
29. Okeke IN. Diarrheagenic *Escherichia coli* in sub-Saharan Africa: status, uncertainties and necessities. *J Infect Dev Ctries* 2009;3(11):817–842.
30. Olotu AI, Mithwani S, Newton CR. Haemolytic uraemic syndrome in children admitted to a rural district hospital in Kenya. *Trop Doct* 2008;38(3):165–167.
31. Houdouin V, Doit C, Mariani P, et al. A pediatric cluster of *Shigella dysenteriae* serotype 1 diarrhea with hemolytic uremic syndrome in 2 families from France. *Clin Infect Dis* 2004;38(9):e96–e99.
32. Ruggenenti P, Noris M, Remuzzi G. Thrombotic microangiopathy, hemolytic uremic syndrome, and thrombotic thrombocytopenic purpura. *Kidney Int* 2001;60(3):831–846.
33. Sellier-Leclerc AL, Fremeaux-Bacchi V, Dragon-Durey MA, et al. Differential impact of complement mutations on clinical characteristics in atypical hemolytic uremic syndrome. *J Am Soc Nephrol* 2007;18(8):2392–2400.
34. Noris M, Caprioli J, Bresin E, et al. Relative role of genetic complement abnormalities in sporadic and familial aHUS and their impact on clinical phenotype. *Clin J Am Soc Nephrol* 2010;5(10):1844–1859.
35. Constantinescu AR, Bitzan M, Weiss LS, et al. Non-enteropathic hemolytic uremic syndrome: causes and short-term course. *Am J Kidney Dis* 2004;43(6):976–982.
36. Noris M, Remuzzi G. Atypical hemolytic-uremic syndrome. *N Engl J Med* 2009;361(17):1676–1687.
37. Warwicker P, Goodship TH, Donne RL, et al. Genetic studies into inherited and sporadic hemolytic uremic syndrome. *Kidney Int* 1998;53(4):836–844.
38. Richards A, Buddles MR, Donne RL, et al. Factor H mutations in hemolytic uremic syndrome cluster in exons 18–20, a domain important for host cell recognition. *Am J Hum Genet* 2001;68(2):485–490.
39. Caprioli J, Bettinaglio P, Zipfel PF, et al. The molecular basis of familial hemolytic uremic syndrome: mutation analysis of factor H gene reveals a hot spot in short consensus repeat 20. *J Am Soc Nephrol* 2001;12(2):297–307.
40. Perez-Caballero D, Gonzalez-Rubio C, Gallardo ME, et al. Clustering of missense mutations in the C-terminal region of factor H in atypical hemolytic uremic syndrome. *Am J Hum Genet* 2001;68(2):478–484.
41. Neumann HP, Salzmann M, Bohnert-Iwan B, et al. Haemolytic uraemic syndrome and mutations of the factor H gene: a registry-based study of German speaking countries. *J Med Genet* 2003;40(9):676–681.
42. Dragon-Durey MA, Fremeaux-Bacchi V, Loirat C, et al. Heterozygous and homozygous factor h deficiencies associated with hemolytic uremic syndrome or membranoproliferative glomerulonephritis: report and genetic analysis of 16 cases. *J Am Soc Nephrol* 2004;15(3):787–795.
43. Sullivan M, Erlic Z, Hoffmann MM, et al. Epidemiological approach to identifying genetic predispositions for atypical hemolytic uremic syndrome. *Ann Hum Genet* 2010;74(1):17–26.
44. Dragon-Durey MA, Loirat C, Cloarec S, et al. Anti-Factor H autoantibodies associated with atypical hemolytic uremic syndrome. *J Am Soc Nephrol* 2005;16(2):555–563.
45. Skerka C, Jozsi M, Zipfel PF, et al. Autoantibodies in haemolytic uraemic syndrome (HUS). *Thromb Haemost* 2009;101(2):227–232.
46. Dragon-Durey MA, Blanc C, Marliot F, et al. The high frequency of complement factor H related CFHR1 gene deletion is restricted to specific subgroups of patients with atypical haemolytic uraemic syndrome. *J Med Genet* 2009;46(7):447–450.
47. Besbas N, Karpman D, Landau D, et al. A classification of hemolytic uremic syndrome and thrombotic thrombocytopenic purpura and related disorders. *Kidney Int* 2006;70(3):423–431.
48. Noris M, Remuzzi G. Hemolytic uremic syndrome. *J Am Soc Nephrol* 2005;16(4):1035–1050.
49. Scheiring J, Rosales A, Zimmerhackl LB. Clinical practice. Today's understanding of the haemolytic uraemic syndrome. *Eur J Pediatr* 2010;169(1):7–13.
50. Keir L, Coward RJ. Advances in our understanding of the pathogenesis of glomerular thrombotic microangiopathy. *Pediatr Nephrol* 2011;26(4):523–533.
51. Allen U, Licht C. Pandemic H1N1 influenza A infection and (atypical) HUS—more than just another trigger? *Pediatr Nephrol* 2011;26(1):3–5.
52. Sartelet H, Toupance O, Lorenzato M, et al. Sirolimus-induced thrombotic microangiopathy is associated with decreased expression of vascular endothelial growth factor in kidneys. *Am J Transplant* 2005;5(10):2441–2447.

53. Izzedine H, Rixe O, Billemont B, et al. Angiogenesis inhibitor therapies: Focus on kidney toxicity and hypertension. *Am J Kidney Dis* 2007;50(2):203–218.
54. Eremina V, Jefferson JA, Kowalewska J, et al. VEGF inhibition and renal thrombotic microangiopathy. *N Engl J Med* 2008;358(11):1129–1136.
55. Bollee G, Patey N, Cazajous G, et al. Thrombotic microangiopathy secondary to VEGF pathway inhibition by sunitinib. *Nephrol Dial Transplant* 2009;24(2):682–685.
56. Sharma AP, Greenberg CR, Prasad AN, et al. Hemolytic uremic syndrome (HUS) secondary to cobalamin C (cblC) disorder. *Pediatr Nephrol* 2007;22(12):2097–2103.
57. Van Hove JL, Van Damme-Lombaerts R, Grunewald S, et al. Cobalamin disorder Cbl-C presenting with late-onset thrombotic microangiopathy. *Am J Med Genet* 2002;111(2):195–201.
58. Johnson S, Taylor CM. What's new in haemolytic uraemic syndrome? *Eur J Pediatr* 2008;167(9):965–971.
59. Rodriguez R, Nakamura R, Palmer JM, et al. A phase II pilot study of tacrolimus/sirolimus GVHD prophylaxis for sibling donor hematopoietic stem cell transplantation using 3 conditioning regimens. *Blood* 2010;115(5):1098–1105.
60. Fakhouri F, Fremeaux-Bacchi V. Does hemolytic uremic syndrome differ from thrombotic thrombocytopenic purpura? *Nat Clin Pract Nephrol* 2007;3(12):679–687.
61. Fakhouri F, Roumenina L, Provot F, et al. Pregnancy-associated hemolytic uremic syndrome revisited in the era of complement gene mutations. *J Am Soc Nephrol* 2010;21(5):859–867.
62. Kaplan BS, Chesney RW, Drummond KN. Hemolytic uremic syndrome in families. *N Engl J Med* 1975;292(21):1090–1093.
63. Sullivan M, Rybicki LA, Winter A, et al. Age-related penetrance of hereditary atypical hemolytic uremic syndrome. *Ann Hum Genet* 2011;75(6):639–647.
64. Bender JM, Ampofo K, Byington CL, et al. Epidemiology of *Streptococcus pneumoniae*-induced hemolytic uremic syndrome in Utah children. *Pediatr Infect Dis J* 2010;29(8):712–716.
65. Waters AM, Kerecuk L, Luk D, et al. Hemolytic uremic syndrome associated with invasive pneumococcal disease: the United Kingdom experience. *J Pediatr* 2007;151(2):140–144.
66. Spinale JM, Ruebner RL, Kaplan BS, et al. Update on *Streptococcus pneumoniae* associated hemolytic uremic syndrome. *Curr Opin Pediatr* 2013;25(2):203–208.
67. Cabrera GR, Fortenberry JD, Warshaw BL, et al. Hemolytic uremic syndrome associated with invasive *Streptococcus pneumoniae* infection. *Pediatrics* 1998;101(4 Pt 1):699–703.
68. Nathanson S, Deschenes G. Prognosis of *Streptococcus pneumoniae*-induced hemolytic uremic syndrome. *Pediatr Nephrol* 2001;16(4):362–365.
69. Brandt J, Wong C, Mihm S, et al. Invasive pneumococcal disease and hemolytic uremic syndrome. *Pediatrics* 2002;110(2 Pt 1):371–376.
70. Menni F, Testa S, Guez S, et al. Neonatal atypical hemolytic uremic syndrome due to methylmalonic aciduria and homocystinuria. *Pediatr Nephrol* 2012;27(8):1401–1405.
71. Loirat C, Girma JP, Desconclois C, et al. Thrombotic thrombocytopenic purpura related to severe ADAMTS13 deficiency in children. *Pediatr Nephrol* 2009;24(1):19–29.
72. Fujimura Y, Matsumoto M. Registry of 919 patients with thrombotic microangiopathies across Japan: database of Nara Medical University during 1998–2008. *Intern Med* 2010;49(1):7–15.
73. Scully M, Yarranton H, Liesner R, et al. Regional UK TTP registry: correlation with laboratory ADAMTS 13 analysis and clinical features. *Br J Haematol* 2008;1(5):819–826.
74. Hovinga JA, Vesely SK, Terrell DR, et al. Survival and relapse in patients with thrombotic thrombocytopenic purpura. *Blood* 2010;115(8):1500–1511; quiz 662.
75. Frank C, Werber D, Cramer JP, et al. Epidemic profile of Shiga-toxin-producing *Escherichia coli* O104:H4 outbreak in Germany. *N Engl J Med* 2011;365(19):1771–1780.
76. Caprioli J, Noris M, Brioschi S, et al. Genetics of HUS: the impact of MCP, CFH, and IF mutations on clinical presentation, response to treatment, and outcome. *Blood* 2006;108(4):1267–1279.
77. Loos S, Ahlenstiel T, Kranz B, et al. An outbreak of Shiga toxin-producing *Escherichia coli* O104:H4 hemolytic uremic syndrome in Germany: presentation and short-term outcome in children. *Clin Infect Dis* 2012;55(6):753–759.
78. Gerber A, Karch H, Allerberger F, et al. Clinical course and the role of Shiga toxin-producing *Escherichia coli* infection in the hemolytic-uremic syndrome in pediatric patients, 1997–2000, in Germany and Austria: A prospective study. *J Infect Dis* 2002;186(4):493–500.
79. Greinacher A, Friesecke S, Abel P, et al. Treatment of severe neurological deficits with IgG depletion through immunoadsorption in patients with *Escherichia coli* O104:H4-associated haemolytic uraemic syndrome: A prospective trial. *Lancet* 2011;378(9797):1166–1173.
80. Piercefield EW, Bradley KK, Coffman RL, et al. Hemolytic uremic syndrome after an *Escherichia coli* O111 outbreak. *Arch Intern Med* 2010;170(18):1656–1663.
81. Krogvold L, Henrichsen T, Bjerre A, et al. Clinical aspects of a nationwide epidemic of severe haemolytic uremic syndrome (HUS) in children. *Scand J Trauma Resusc Emerg Med* 2011;19:44.
82. Nathanson S, Kwon T, Elmaleh M, et al. Acute neurological involvement in diarrhea-associated hemolytic uremic syndrome. *Clin J Am Soc Nephrol* 2010;5(7):1218–1228.
83. Argyle JC, Hogg RJ, Pysher TJ, et al. A clinicopathological study of 24 children with hemolytic uremic syndrome. A report of the Southwest Pediatric Nephrology Study Group. *Pediatr Nephrol* 1990;4(1):52–58.
84. Gallo EG, Gianantonio CA. Extrarenal involvement in diarrhoea-associated haemolytic-uraemic syndrome. *Pediatr Nephrol* 1995;9(1):117–119.
85. Ridolfi RL, Bell WR. Thrombotic thrombocytopenic purpura. Report of 25 cases and review of the literature. *Medicine* 1981;60(6):413–428.
86. Terrell DR, Williams LA, Vesely SK, et al. The incidence of thrombotic thrombocytopenic purpura-hemolytic uremic syndrome: all patients, idiopathic patients, and patients with severe ADAMTS-13 deficiency. *J Thromb Haemost* 2005;3(7):1432–1436.
87. Thoenes W, John HD. Endotheliotropic (hemolytic) nephroangiopathy and its various manifestation forms (thrombotic microangiopathy, primary malignant nephrosclerosis, hemolytic-uremic syndrome). *Klin Wochenschr* 1980;58(4):173–184.
88. Bohle A, Grabensee B, Fischer R, et al. On four cases of hemolytic-uremic syndrome without microangiopathy. *Clin Nephrol* 1985;24(2):88–92.
89. Coats MT, Murphy T, Paton JC, et al. Exposure of Thomsen-Friedenreich antigen in *Streptococcus pneumoniae* infection is dependent on pneumococcal neuraminidase A. *Microb Pathog* 2011;50(6):343–349.
90. Daram SR, Philipneri M, Puri N, et al. Thrombotic thrombocytopenic purpura without schistocytes on the peripheral blood smear. *South Med J* 2005;98(3):392–395.
91. Espie E, Grimont F, Mariani-Kurkdjian P, et al. Surveillance of hemolytic uremic syndrome in children less than 15 years of age, a system to monitor O157 and non-O157 Shiga toxin-producing *Escherichia coli* infections in France, 1996–2006. *Pediatr Infect Dis J* 2008;27(7):595–601.
92. Cummings KC, Mohle-Boetani JC, Werner SB, et al. Population-based trends in pediatric hemolytic uremic syndrome in California, 1994–1999: Substantial underreporting and public health implications. *Am J Epidemiol* 2002;155(10):941–948.
93. Lathrop S, Edge K, Bareta J. Shiga toxin-producing *Escherichia coli*, New Mexico, USA, 2004–2007. *Emerg Infect Dis* 2009;15(8):1289–1291.
94. Miller DP, Kaye JA, Shea K, et al. Incidence of thrombotic thrombocytopenic purpura/hemolytic uremic syndrome. *Epidemiology* 2004;15(2):208–215.
95. Rahman RC, Cobenas CJ, Drut R, et al. Hemorrhagic colitis in postdiarrheal hemolytic uremic syndrome: Retrospective analysis of 54 children. *Pediatr Nephrol* 2012;27(2):229–233.
96. Gorlova O, Martin JE, Rueda B, et al. Identification of novel genetic markers associated with clinical phenotypes of systemic sclerosis through a genome-wide association strategy. *PLoS Genet* 2011;7(7):e1002178.
97. Elliott EJ, Robins-Browne RM, O'Loughlin EV, et al. Nationwide study of haemolytic uraemic syndrome: clinical, microbiological, and epidemiological features. *Arch Dis Child* 2001;85(2):125–131.
98. Westra D, Volokhina E, van der Heijden E, et al. Genetic disorders in complement (regulating) genes in patients with atypical haemolytic uraemic syndrome (aHUS). *Nephrol Dial Transplant* 2010;25(7):2195–2202.
99. Esparza-Gordillo J, Goicoechea de Jorge E, Buil A, et al. Predisposition to atypical hemolytic uremic syndrome involves the concurrence of different susceptibility alleles in the regulators of complement activation gene cluster in 1q32. *Hum Mol Genet* 2005;14(5):703–712.

100. Bienaime F, Dragon-Durey MA, Regnier CH, et al. Mutations in components of complement influence the outcome of Factor I-associated atypical hemolytic uremic syndrome. *Kidney Int* 2010;77(4):339–349.
101. Maga TK, Nishimura CJ, Weaver AE, et al. Mutations in alternative pathway complement proteins in American patients with atypical hemolytic uremic syndrome. *Hum Mutat* 2010;31(6):E1445–E1460.
102. Geerdink LM, Westra D, van Wijk JA, et al. Atypical hemolytic uremic syndrome in children: complement mutations and clinical characteristics. *Pediatr Nephrol* 2012;27(8):1283–1291.
103. Lopez EL, Contrini MM, Glatstein E, et al. An epidemiologic surveillance of Shiga-like toxin-producing *Escherichia coli* infection in Argentinean children: risk factors and serum Shiga-like toxin 2 values. *Pediatr Infect Dis J* 2012;31(1):20–24.
104. Wendel AM, Johnson DH, Sharapov U, et al. Multistate outbreak of *Escherichia coli* O157:H7 infection associated with consumption of packaged spinach, August-September 2006: the Wisconsin investigation. *Clin Infect Dis* 2009;48(8):1079–1086.
105. Garg AX, Suri RS, Barrowman N, et al. Long-term renal prognosis of diarrhea-associated hemolytic uremic syndrome: a systematic review, meta-analysis, and meta-regression. *JAMA* 2003;290(10):1360–1370.
106. Milford DV, Taylor CM, Guttridge B, et al. Haemolytic uraemic syndromes in the British Isles 1985–8: Association with verocytotoxin producing *Escherichia coli*. Part 1: clinical and epidemiological aspects. *Arch Dis Child* 1990;65(7):716–721.
107. Decludt B, Bouvet P, Mariani-Kurkdjian P, et al. Haemolytic uraemic syndrome and Shiga toxin-producing *Escherichia coli* infection in children in France. The Societe de Nephrologie Pediatrique. *Epidemiol Infect* 2000;124(2):215–220.
108. Akashi S, Joh K, Tsuji A, et al. A severe outbreak of haemorrhagic colitis and haemolytic uraemic syndrome associated with *Escherichia coli* O157:H7 in Japan. *Eur J Pediatr* 1994;153(9):650–655.
109. Fukushima H, Hashizume T, Morita Y, et al. Clinical experiences in Sakai City Hospital during the massive outbreak of enterohemorrhagic *Escherichia coli* O157 infections in Sakai City, 1996. *Pediatr Int* 1999;41(2):213–217.
110. Gianantonio CA, Vitacco M, Mendilaharzu F, et al. The hemolytic-uremic syndrome. *Nephron* 1973;11(2):174–192.
111. Richardson SE, Karmali MA, Becker LE, et al. The histopathology of the hemolytic uremic syndrome associated with verocytotoxin-producing *Escherichia coli* infections. *Hum Pathol* 1988;19(9):1102–1108.
112. Fong JS, Kaplan BS. Impairment of platelet aggregation in hemolytic uremic syndrome: Evidence for platelet "exhaustion." *Blood* 1982;60(3):564–570.
113. Kaplan BS, Proesmans W. The hemolytic uremic syndrome of childhood and its variants. *Semin Hematol* 1987;24(3):148–160.
114. Lynn RM, O'Brien SJ, Taylor CM, et al. Childhood hemolytic uremic syndrome, United Kingdom and Ireland. *Emerg Infect Dis* 2005;11(4):590–596.
115. Lopez EL, Contrini MM, Devoto S, et al. Incomplete hemolytic-uremic syndrome in Argentinean children with bloody diarrhea. *J Pediatr* 1995;127(3):364–367.
116. Fraser ME, Fujinaga M, Cherney MM, et al. Structure of Shiga toxin type 2 (Stx2) from *Escherichia coli* O157:H7. *J Biol Chem* 2004;279(26):27511–27517.
117. O'Brien AD, Holmes RK. Shiga and Shiga-like toxins. *Microbiol Rev* 1987;51(2):206–220.
118. Allanore Y, Saad M, Dieude P, et al. Genome-wide scan identifies TNIP1, PSORS1C1, and RHOB as novel risk loci for systemic sclerosis. *PLoS Genet* 2011;7(7):e1002091.
119. Bolton DJ. Verocytotoxigenic (Shiga toxin-producing) *Escherichia coli*: Virulence factors and pathogenicity in the farm to fork paradigm. *Foodborne Pathogens Dis* 2011;8(3):357–365.
120. Melton-Celsa A, Mohawk K, Teel L, et al. Pathogenesis of Shiga-toxin producing *Escherichia coli*. *Curr Top Microbiol Immunol* 2012;357:67–103.
121. Rohde H, Qin J, Cui Y, et al. Open-source genomic analysis of Shiga-toxin-producing *E. coli* O104:H4. *N Engl J Med* 2011;365(8):718–724.
122. Mellmann A, Harmsen D, Cummings CA, et al. Prospective genomic characterization of the German enterohemorrhagic *Escherichia coli* O104:H4 outbreak by rapid next generation sequencing technology. *PLoS One* 2011;6(7):e22751.
123. Bielaszewska M, Mellmann A, Zhang W, et al. Characterisation of the *Escherichia coli* strain associated with an outbreak of haemolytic uraemic syndrome in Germany, 2011: a microbiological study. *Lancet Infect Dis* 2011;11(9):671–676.
124. Riley LW, Remis RS, Helgerson SD, et al. Hemorrhagic colitis associated with a rare *Escherichia coli* serotype. *N Engl J Med* 1983;308(12):681–685.
125. Karmali MA, Petric M, Lim C, et al. The association between idiopathic hemolytic uremic syndrome and infection by verotoxin-producing *Escherichia coli*. *J Infect Dis* 1985;151(5):775–782.
126. Karmali MA, Petric M, Lim C, et al. *Escherichia coli* cytotoxin, haemolytic-uraemic syndrome, and haemorrhagic colitis. *Lancet* 1983;2(8362):1299–1300.
127. Sanchez S, Martinez R, Alonso JM, et al. [Clinical and pathogenic aspects of infections due to *Escherichia coli* O157:H7 and other verocytotoxigenic *E. coli*]. *Enferm Infecc Microbiol Clin* 2010;28(6):370–374.
128. Brooks JT, Sowers EG, Wells JG, et al. Non-O157 Shiga toxin-producing *Escherichia coli* infections in the United States, 1983–2002. *J Infect Dis* 2005;192(8):1422–1429.
129. Bell BP, Goldoft M, Griffin PM, et al. A multistate outbreak of *Escherichia coli* O157:H7-associated bloody diarrhea and hemolytic uremic syndrome from hamburgers. The Washington experience. *JAMA* 1994;272(17):1349–1353.
130. Thomas A, Chart H, Cheasty T, et al. Vero cytotoxin-producing *Escherichia coli*, particularly serogroup O 157, associated with human infections in the United Kingdom: 1989–91. *Epidemiol Infect* 1993;110(3):591–600.
131. Buchholz U, Bernard H, Werber D, et al. German outbreak of *Escherichia coli* O104:H4 associated with sprouts. *N Engl J Med* 2011;365(19):1763–1770.
132. Wong CS, Mooney JC, Brandt JR, et al. Risk Factors for the hemolytic uremic syndrome in children infected with *Escherichia coli* O157:H7: A multivariable analysis. *Clin Infect Dis* 2012;55(1):33–41.
133. Rasko DA, Webster DR, Sahl JW, et al. Origins of the *E. coli* strain causing an outbreak of hemolytic-uremic syndrome in Germany. *N Engl J Med* 2011;365(8):709–717.
134. Muthing J, Schweppe CH, Karch H, et al. Shiga toxins, glycosphingolipid diversity, and endothelial cell injury. *Thromb Haemost* 2009;101(2):252–264.
135. Neil KP, Biggerstaff G, MacDonald JK, et al. A novel vehicle for transmission of *Escherichia coli* O157:H7 to humans: multistate outbreak of *E. coli* O157:H7 infections associated with consumption of ready-to-bake commercial prepackaged cookie dough: United States, 2009. *Clin Infect Dis* 2012;54(4):511–518.
136. Kuijper EJ, Soonawala D, Vermont C, et al. Household transmission of haemolytic uraemic syndrome associated with *Escherichia coli* O104:H4 in the Netherlands, May 2011. *Euro Surveil* 2011;16(25). Pii: 19897
137. Guh A, Phan Q, Nelson R, et al. Outbreak of *Escherichia coli* O157 associated with raw milk, Connecticut, 2008. *Clin Infect Dis* 2010;51(12):1411–1417.
138. Matsell DG, White CT. An outbreak of diarrhea-associated childhood hemolytic uremic syndrome: the Walkerton epidemic. *Kidney Int Suppl* 2009(112):S35–S37.
139. Locking ME, Pollock KG, Allison LJ, et al. *Escherichia coli* O157 infection and secondary spread, Scotland, 1999–2008. *Emerg Infect Dis* 2011;17(3):524–527.
140. Hauri A, Gotsch U, Strotmann I, et al. Secondary transmissions during the outbreak of Shiga toxin-producing *Escherichia coli* O104 in Hesse, Germany, 2011. *Euro Surveil* 2011;16(31). pii:19937
141. Koster F, Levin J, Walker L, et al. Hemolytic-uremic syndrome after shigellosis. Relation to endotoxemia and circulating immune complexes. *N Engl J Med* 1978;298(17):927–933.
142. Koster FT, Boonpucknavig V, Sujaho S, et al. Renal histopathology in the hemolytic-uremic syndrome following shigellosis. *Clin Nephrol* 1984;21(2):126–133.
143. Lopez EL, Prado-Jimenez V, O'Ryan-Gallardo M, et al. Shigella and Shiga toxin-producing *Escherichia coli* causing bloody diarrhea in Latin America. *Infect Dis Clin North Am* 2000;14(1):41–65, viii.
144. Mark Taylor C. Enterohaemorrhagic *Escherichia coli* and *Shigella dysenteriae* type 1-induced haemolytic uraemic syndrome. *Pediatr Nephrol* 2008;23(9):1425–1431.

145. Sallee M, Daniel L, Piercecchi MD, et al. Myocardial infarction is a complication of factor H-associated atypical HUS. *Nephrol Dial Transplant* 2010;25(6):2028–2032.
146. Kaplan BS, Garcia CD, Chesney RW, et al. Peripheral gangrene complicating idiopathic and recessive hemolytic uremic syndromes. *Pediatr Nephrol* 2000;14(10–11):985–989.
147. Siegler RL, Sherbotie JR, Denkers ND, et al. Clustering of post-diarrheal (Shiga toxin-mediated) hemolytic uremic syndrome in families. *Clin Nephrol* 2003;60(2):74–79.
148. Mattoo TK, Mahmood MA, al-Harbi MS, et al. Familial, recurrent hemolytic-uremic syndrome. *J Pediatr* 1989;114(5):814–816.
149. Kaplan BS, Kaplan P. Hemolytic uremic syndrome in families. In Kaplan BS, Trompeter RS, Moake JL, eds. *Hemolytic Uremic Syndrome and Thrombotic Thrombocytopenic Purpura*. New York: Marcel Dekker, 1992.
150. Siegler RL, Griffin PM, Barrett TJ, et al. Recurrent hemolytic uremic syndrome secondary to *Escherichia coli* O157:H7 infection. *Pediatrics* 1993;91(3):666–668.
151. Siegler RL, Pavia AT, Sherbotie JR. Recurrent hemolytic uremic syndrome. *Clin Pediatr* 2002;41(9):705–709.
152. Loirat C, Noris M, Fremeaux-Bacchi V. Complement and the atypical hemolytic uremic syndrome in children. *Pediatr Nephrol* 2008;23(11):1957–1972.
153. Bento D, Mapril J, Rocha C, et al. Triggering of atypical hemolytic uremic syndrome by influenza A (H1N1). *Ren Fail* 2010;32(6):753–756.
154. Trachtman H, Sethna C, Epstein R, et al. Atypical hemolytic uremic syndrome associated with H1N1 influenza A virus infection. *Pediatr Nephrol* 2011;26(1):145–146.
155. Caltik A, Akyuz SG, Erdogan O, et al. Hemolytic uremic syndrome triggered with a new pandemic virus: Influenza A (H1N1). *Pediatr Nephrol* 2011;26(1):147–148.
156. George JN. The association of pregnancy with thrombotic thrombocytopenic purpura-hemolytic uremic syndrome. *Curr Opin Hematol* 2003;10(5):339–344.
157. Terrell DR, Motto DG, Kremer Hovinga JA, et al. Blood group O and black race are independent risk factors for thrombotic thrombocytopenic purpura associated with severe ADAMTS13 deficiency. *Transfusion* 2011;51(10):2237–2243.
158. George JN, Vesely SK, James JA. Overlapping features of thrombotic thrombocytopenic purpura and systemic lupus erythematosus. *South Med J* 2007;100(5):512–514.
159. Scully M, Gattens M, Khair K, et al. The use of intermediate purity factor VIII concentrate BPL 8Y as prophylaxis and treatment in congenital thrombotic thrombocytopenic purpura. *Br J Haematol* 2006;135(1):101–104.
160. Schiff DE, Roberts WD, Willert J, et al. Thrombocytopenia and severe hyperbilirubinemia in the neonatal period secondary to congenital thrombotic thrombocytopenic purpura and ADAMTS13 deficiency. *J Pediatr Hematol Oncol* 2004;26(8):535–538.
161. Loirat C, Sellier-Leclerc AL, Fremeaux-Bacchi V, et al. [Hereditary hemolytic uremic syndromes and thrombotic thrombocytopenic purpura]. *Nephrol Ther* 2006;2(Suppl 2):S149–S161.
162. Lafyatis R, O'Hara C, Feghali-Bostwick CA, et al. B cell infiltration in systemic sclerosis-associated interstitial lung disease. *Arthritis Rheum* 2007;56(9):3167–3168.
163. Zheng XL, Wu HM, Shang D, et al. Multiple domains of ADAMTS13 are targeted by autoantibodies against ADAMTS13 in patients with acquired idiopathic thrombotic thrombocytopenic purpura. *Haematologica* 2010;95(9):1555–1562.
164. Vesely SK, George JN, Lammle B, et al. ADAMTS13 activity in thrombotic thrombocytopenic purpura-hemolytic uremic syndrome: Relation to presenting features and clinical outcomes in a prospective cohort of 142 patients. *Blood* 2003;102(1):60–68.
165. Scully M, Hunt BJ, Benjamin S, et al. Guidelines on the diagnosis and management of thrombotic thrombocytopenic purpura and other thrombotic microangiopathies. *Br J Haematol* 2012;158(3):323–325.
166. Coppo P, Wolf M, Veyradier A, et al. Prognostic value of inhibitory anti-ADAMTS13 antibodies in adult-acquired thrombotic thrombocytopenic purpura. *Br J Haematol* 2006;132(1):66–74.
167. Peyvandi F, Lavoretano S, Palla R, et al. ADAMTS13 and anti-ADAMTS13 antibodies as markers for recurrence of acquired thrombotic thrombocytopenic purpura during remission. *Haematologica* 2008;93(2):232–239.
168. Lotta LA, Mariani M, Consonni D, et al. Different clinical severity of first episodes and recurrences of thrombotic thrombocytopenic purpura. *Br J Haematol* 2010;151(5):488–494.
169. Cserti CM, Landaw S, Uhl L. Do infections provoke exacerbations and relapses of thrombotic thrombocytopenic purpura? *J Clin Apher* 2007;22(1):21–25.
170. Swisher KK, Doan JT, Vesely SK, et al. Pancreatitis preceding acute episodes of thrombotic thrombocytopenic purpura-hemolytic uremic syndrome: Report of five patients with a systematic review of published reports. *Haematologica* 2007;92(7):936–943.
171. Vesely SK, Li X, McMinn JR, et al. Pregnancy outcomes after recovery from thrombotic thrombocytopenic purpura-hemolytic uremic syndrome. *Transfusion* 2004;44(8):1149–1158.
172. Booth KK, Terrell DR, Vesely SK, et al. Systemic infections mimicking thrombotic thrombocytopenic purpura. *Am J Hematol* 2011;86(9):743–751.
173. Kosugi N, Tsurutani Y, Isonishi A, et al. Influenza A infection triggers thrombotic thrombocytopenic purpura by producing the anti-ADAMTS13 IgG inhibitor. *Intern Med* 2010;49(7):689–693.
174. Douglas KW, Pollock KG, Young D, et al. Infection frequently triggers thrombotic microangiopathy in patients with preexisting risk factors: A single-institution experience. *J Clin Apher* 2010;25(2):47–53.
175. Chu QD, Medeiros LJ, Fisher AE, et al. Thrombotic thrombocytopenic purpura and HIV infection. *South Med J* 1995;88(1):82–86.
176. Thompson CE, Damon LE, Ries CA, et al. Thrombotic microangiopathies in the 1980s: Clinical features, response to treatment, and the impact of the human immunodeficiency virus epidemic. *Blood* 1992;80(8):1890–1895.
177. Ucar A, Fernandez HF, Byrnes JJ, et al. Thrombotic microangiopathy and retroviral infections: a 13-year experience. *Am J Hematol* 1994;45(4):304–309.
178. Sutor GC, Schmidt RE, Albrecht H. Thrombotic microangiopathies and HIV infection: report of two typical cases, features of HUS and TTP, and review of the literature. *Infection* 1999;27(1):12–15.
179. Becker S, Fusco G, Fusco J, et al. HIV-associated thrombotic microangiopathy in the era of highly active antiretroviral therapy: an observational study. *Clin Infect Dis* 2004;39 Suppl 5:S267-S275.
180. Gervasoni C, Ridolfo AL, Vaccarezza M, et al. Thrombotic microangiopathy in patients with acquired immunodeficiency syndrome before and during the era of introduction of highly active antiretroviral therapy. *Clin Infect Dis* 2002;35(12):1534–1540.
181. Bell WR, Chulay JD, Feinberg JE. Manifestations resembling thrombotic microangiopathy in patients with advanced human immunodeficiency virus (HIV) disease in a cytomegalovirus prophylaxis trial (ACTG 204). *Medicine* 1997;76(5):369–380.
182. Pene F, Vigneau C, Auburtin M, et al. Outcome of severe adult thrombotic microangiopathies in the intensive care unit. *Intensive Care Med* 2005;31(1):71–78.
183. Outschoorn UM, Ferber A. Outcomes in the treatment of thrombotic thrombocytopenic purpura with splenectomy: a retrospective cohort study. *Am J Hematol* 2006;81(12):895–900.
184. Gunther K, Garizio D, Nesara P. ADAMTS13 activity and the presence of acquired inhibitors in human immunodeficiency virus-related thrombotic thrombocytopenic purpura. *Transfusion* 2007;47(9):1710–1716.
185. Benjamin M, Terrell DR, Vesely SK, et al. Frequency and significance of HIV infection among patients diagnosed with thrombotic thrombocytopenic purpura. *Clin Infect Dis* 2009;48(8):1129–1137.
186. Malak S, Wolf M, Millot GA, et al. Human immunodeficiency virus-associated thrombotic microangiopathies: clinical characteristics and outcome according to ADAMTS13 activity. *Scand J Immunol* 2008;68(3):337–344.
187. Hart D, Sayer R, Miller R, et al. Human immunodeficiency virus associated thrombotic thrombocytopenic purpura—favourable outcome with plasma exchange and prompt initiation of highly active antiretroviral therapy. *Br J Haematol* 2011;153(4):515–519.
188. Moore RD. Schistocytosis and a thrombotic microangiopathy-like syndrome in hospitalized HIV-infected patients. *Am J Hematol* 1999;60(2):116–120.

189. Park YA, Hay SN, Brecher ME. ADAMTS13 activity levels in patients with human immunodeficiency virus-associated thrombotic microangiopathy and profound CD4 deficiency. *J Clin Apher* 2009;24(1):32–36.
190. Brecher ME, Hay SN, Park YA. Is it HIV TTP or HIV-associated thrombotic microangiopathy? *J Clin Apher* 2008;23(6):186–190.
191. Alpers CE. Light at the end of the TUNEL: HIV-associated thrombotic microangiopathy. *Kidney Int* 2003;63(1):385–396.
192. Mihai C, Tervaert JW. Anti-endothelial cell antibodies in systemic sclerosis. *Ann Rheum Dis* 2010;69(2):319–324.
193. Maslo C, Peraldi MN, Desenclos JC, et al. Thrombotic microangiopathy and cytomegalovirus disease in patients infected with human immunodeficiency virus. *Clin Infect Dis* 1997;24(3):350–355.
194. Starck M, Abedinpour F, Dendorfer U, et al. Acquired thrombotic thrombocytopenic purpura as the presenting symptom of systemic lupus erythematosus. Successful treatment with plasma exchange and immunosuppression—report of two cases. *Eur J Haematol* 2005;75(5):436–440.
195. Gungor T, Furlan M, Lammle B, et al. Acquired deficiency of von Willebrand factor-cleaving protease in a patient suffering from acute systemic lupus erythematosus. *Rheumatology (Oxford)* 2001;40(8):940–942.
196. Mannucci PM, Vanoli M, Forza I, et al. Von Willebrand factor cleaving protease (ADAMTS-13) in 123 patients with connective tissue diseases (systemic lupus erythematosus and systemic sclerosis). *Haematologica* 2003;88(8):914–918.
197. Hunt BJ, Tueger S, Pattison J, et al. Microangiopathic haemolytic anaemia secondary to lupus nephritis: an important differential diagnosis of thrombotic thrombocytopenic purpura. *Lupus* 2007;16(5):358–362.
198. Kojouri K, Vesely SK, George JN. Quinine-associated thrombotic thrombocytopenic purpura-hemolytic uremic syndrome: frequency, clinical features, and long-term outcomes. *Ann Intern Med* 2001;135(12):1047–1051.
199. Bennett CL, Connors JM, Carwile JM, et al. Thrombotic thrombocytopenic purpura associated with clopidogrel. *N Engl J Med* 2000;342(24):1773–1777.
200. Medina PJ, Sipols JM, George JN. Drug-associated thrombotic thrombocytopenic purpura-hemolytic uremic syndrome. *Curr Opin Hematol* 2001;8(5):286–293.
201. Blake-Haskins JA, Lechleider RJ, Kreitman RJ. Thrombotic microangiopathy with targeted cancer agents. *Clin Cancer Res* 2011;17(18):5858–5866.
202. Park YA, Hay SN, King KE, et al. Is it quinine TTP/HUS or quinine TMA? ADAMTS13 levels and implications for therapy. *J Clin Apher* 2009;24(3):115–119.
203. Knower MT, Bowton DL, Owen J, et al. Quinine-induced disseminated intravascular coagulation: case report and review of the literature. *Intensive Care Med* 2003;29(6):1007–1011.
204. Ntukidem N, Arce-Lara C, Otterson GA, et al. Capped-dose mitomycin C: a pooled safety analysis from three prospective clinical trials. *Cancer Chemother Pharmacol* 2010;65(2):319–324.
205. George JN. The thrombotic thrombocytopenic purpura and hemolytic uremic syndromes: overview of pathogenesis (experience of The Oklahoma TTP-HUS Registry, 1989–2007). *Kidney Int Suppl* 2009;(112):S8–S10.
206. Stroncek DF, Vercellotti GM, Hammerschmidt DE, et al. Characterization of multiple quinine-dependent antibodies in a patient with episodic hemolytic uremic syndrome and immune agranulocytosis. *Blood* 1992;80(1):241–248.
207. Karkowski L, Wolf M, Lescampf J, et al. [Clopidogrel induced thrombotic thrombocytopenic purpura]. *Rev Med Intern* 2011;32(12):762–765.
208. Zakarija A, Kwaan HC, Moake JL, et al. Ticlopidine- and clopidogrel-associated thrombotic thrombocytopenic purpura (TTP): Review of clinical, laboratory, epidemiological, and pharmacovigilance findings (1989–2008). *Kidney Int Suppl* 2009(112):S20–S24.
209. Zakarija A, Bennett C. Drug-induced thrombotic microangiopathy. *Semin Thromb Hemost* 2005;31(6):681–690.
210. Bennett CL, Kim B, Zakarija A, et al. Two mechanistic pathways for thienopyridine-associated thrombotic thrombocytopenic purpura: A report from the SERF-TTP Research Group and the RADAR Project. *J Am Coll Cardiol* 2007;50(12):1138–1143.
211. Pisoni R, Ruggenenti P, Remuzzi G. Drug-induced thrombotic microangiopathy: incidence, prevention and management. *Drug Saf* 2001;24(7):491–501.
212. Dlott JS, Danielson CF, Blue-Hnidy DE, et al. Drug-induced thrombotic thrombocytopenic purpura/hemolytic uremic syndrome: a concise review. *Ther Apher Dial* 2004;8(2):102–111.
213. Izzedine H, Isnard-Bagnis C, Launay-Vacher V, et al. Gemcitabine-induced thrombotic microangiopathy: a systematic review. *Nephrol Dial Transplant* 2006;21(11):3038–3045.
214. El-Ghazal R, Podoltsev N, Marks P, et al. Mitomycin—C-induced thrombotic thrombocytopenic purpura/hemolytic uremic syndrome: cumulative toxicity of an old drug in a new era. *Clin Colorectal Cancer* 2011;10(2):142–145.
215. Hong MJ, Lee HG, Hur M, et al. Slow, but complete, resolution of mitomycin-induced refractory thrombotic thrombocytopenic purpura after rituximab treatment. *Korean J Hematol* 2011;46(1):45–48.
216. Cattell V. Mitomycin-induced hemolytic uremic kidney. An experimental model in the rat. *Am J Pathol* 1985;121(1):88–95.
217. Proia AD, Harden EA, Silberman HR. Mitomycin-induced hemolytic-uremic syndrome. *Arch Pathol Lab Med* 1984;108(12):959–962.
218. Crocker J, Jones EL. Haemolytic-uraemic syndrome complicating long-term mitomycin C and 5-fluorouracil therapy for gastric carcinoma. *J Clin Pathol* 1983;36(1):24–29.
219. Eremina V, Quaggin SE. Biology of anti-angiogenic therapy-induced thrombotic microangiopathy. *Semin Nephrol* 2010;30(6):582–590.
220. Frangie C, Lefaucheur C, Medioni J, et al. Renal thrombotic microangiopathy caused by anti-VEGF-antibody treatment for metastatic renal-cell carcinoma. *Lancet Oncol* 2007;8(2):177–178.
221. Gilchrist FC, Bunn C, Foley PJ, et al. Class II HLA associations with autoantibodies in scleroderma: a highly significant role for HLA-DP. *Genes Immun* 2001;2(2):76–81.
222. Broen JC, Coenen MJ, Radstake TR. Genetics of systemic sclerosis: An update. *Curr Rheumatol Rep* 2012;14(1):11–21.
223. Izzedine H, Sene D, Hadoux J, et al. Thrombotic microangiopathy related to anti-VEGF agents: intensive versus conservative treatment? *Ann Oncol* 2011;22(2):487–490.
224. Izzedine H, Brocheriou I, Deray G, et al. Thrombotic microangiopathy and anti-VEGF agents. *Nephrol Dial Transplant* 2007;22(5):1481–1482.
225. Romano E, Manetti M, Guiducci S, et al. The genetics of systemic sclerosis: an update. *Clin Exp Rheumatol* 2011;29(2 Suppl 65):S75–S86.
226. Martin JE, Bossini-Castillo L, Martin J. Unraveling the genetic component of systemic sclerosis. *Hum Genet* 2012;131(7):1023–1037.
227. Al Aly Z, Philoctete Ashley JM, Gellens ME, et al. Thrombotic thrombocytopenic purpura in a patient treated with imatinib mesylate: true association or mere coincidence? *Am J Kidney Dis* 2005;45(4):762–768.
228. Moore H, Romeril K. Multiple myeloma presenting with a fever of unknown origin and development of thrombotic thrombocytopenic purpura post-bortezomib. *Intern Med J* 2011;41(4):348–350.
229. Reynolds JC, Agodoa LY, Yuan CM, et al. Thrombotic microangiopathy after renal transplantation in the United States. *Am J Kidney Dis* 2003;42(5):1058–1068.
230. Meehan SM, Kremer J, Ali FN, et al. Thrombotic microangiopathy and peritubular capillary C4d expression in renal allograft biopsies. *Clin J Am Soc Nephrol* 2011;6(2):395–403.
231. Satoskar AA, Pelletier R, Adams P, et al. De novo thrombotic microangiopathy in renal allograft biopsies-role of antibody-mediated rejection. *Am J Transplant* 2010;10(8):1804–1811.
232. Shulman H, Striker G, Deeg HJ, et al. Nephrotoxicity of cyclosporin A after allogeneic marrow transplantation: glomerular thromboses and tubular injury. *N Engl J Med* 1981;305(23):1392–1395.
233. Van Buren D, Van Buren CT, Flechner SM, et al. De novo hemolytic uremic syndrome in renal transplant recipients immunosuppressed with cyclosporine. *Surgery* 1985;98(1):54–62.
234. Young BA, Marsh CL, Alpers CE, et al. Cyclosporine-associated thrombotic microangiopathy/hemolytic uremic syndrome following kidney and kidney-pancreas transplantation. *Am J Kidney Dis* 1996;28(4):561–571.
235. Lefaucheur C, Nochy D, Amrein C, et al. Renal histopathological lesions after lung transplantation in patients with cystic fibrosis. *Am J Transplant* 2008;8(9):1901–1910.
236. Zarifian A, Meleg-Smith S, O'Donovan R, et al. Cyclosporine-associated thrombotic microangiopathy in renal allografts. *Kidney Int* 1999;55(6):2457–2466.

237. Pham PT, Peng A, Wilkinson AH, et al. Cyclosporine and tacrolimus-associated thrombotic microangiopathy. *Am J Kidney Dis* 2000;36(4):844–850.
238. Holman MJ, Gonwa TA, Cooper B, et al. FK506-associated thrombotic thrombocytopenic purpura. *Transplantation* 1993;55(1):205–206.
239. Carson JM, Newman ED, Farber JL, et al. Tacrolimus-induced thrombotic microangiopathy: Natural history of a severe, acute vasculopathy. *Clin Nephrol* 2012;77(1):79–84.
240. Saikali JA, Truong LD, Suki WN. Sirolimus may promote thrombotic microangiopathy. *Am J Transplant* 2003;3(2):229–230.
241. Robson M, Cote I, Abbs I, et al. Thrombotic micro-angiopathy with sirolimus-based immunosuppression: potentiation of calcineurin-inhibitor-induced endothelial damage? *Am J Transplant* 2003;3(3):324–327.
242. Crew RJ, Radhakrishnan J, Cohen DJ, et al. De novo thrombotic microangiopathy following treatment with sirolimus: report of two cases. *Nephrol Dial Transplant* 2005;20(1):203–209.
243. Fortin MC, Raymond MA, Madore F, et al. Increased risk of thrombotic microangiopathy in patients receiving a cyclosporin-sirolimus combination. *Am J Transplant* 2004;4(6):946–952.
244. Ramos-Casals M, Fonollosa-Pla V, Brito-Zeron P, et al. Targeted therapy for systemic sclerosis: how close are we? *Nat Rev Rheumatol* 2010;6(5):269–278.
245. Lei W, Luo Y, Yan K, et al. Abnormal DNA methylation in CD4+ T cells from patients with systemic lupus erythematosus, systemic sclerosis, and dermatomyositis. *Scand J Rheumatol* 2009;38(5):369–374.
246. Sgonc R, Gruschwitz MS, Boeck G, et al. Endothelial cell apoptosis in systemic sclerosis is induced by antibody-dependent cell-mediated cytotoxicity via CD95. *Arthritis Rheum* 2000;43(11):2550–2562.
247. Le Quintrec M, Lionet A, Kamar N, et al. Complement mutation-associated de novo thrombotic microangiopathy following kidney transplantation. *Am J Transplant* 2008;8(8):1694–1701.
248. Lorcy N, Rioux-Leclercq N, Lombard ML, et al. Three kidneys, two diseases, one antibody? *Nephrol Dial Transplant* 2011;26(11):3811–3813.
249. Waiser J, Budde K, Rudolph B, et al. De novo hemolytic uremic syndrome postrenal transplant after cytomegalovirus infection. *Am J Kidney Dis* 1999;34(3):556–559.
250. Asaka M, Ishikawa I, Nakazawa T, et al. Hemolytic uremic syndrome associated with influenza A virus infection in an adult renal allograft recipient: case report and review of the literature. *Nephron* 2000;84(3):258–266.
251. Murer L, Zacchello G, Bianchi D, et al. Thrombotic microangiopathy associated with parvovirus B 19 infection after renal transplantation. *J Am Soc Nephrol* 2000;11(6):1132–1137.
252. De Keyzer K, Van Laecke S, Peeters P, et al. De novo thrombotic microangiopathy induced by cytomegalovirus infection leading to renal allograft loss. *Am J Nephrol* 2010;32(5):491–496.
253. Ardalan MR, Shoja MM, Tubbs RS, et al. Parvovirus B19 microepidemic in renal transplant recipients with thrombotic microangiopathy and allograft vasculitis. *Exp Clin Transplant* 2008;6(2):137–143.
254. Baid-Agrawal S, Farris AB, 3rd, Pascual M, et al. Overlapping pathways to transplant glomerulopathy: chronic humoral rejection, hepatitis C infection, and thrombotic microangiopathy. *Kidney Int* 2011;80(8):879–885.
255. Wong W, Denton M, Rennke HG, et al. Hepatitis C, proteinuria, and renal insufficiency. *Am J Kidney Dis* 2004;44(5):924–929.
256. Bresin E, Daina E, Noris M, et al. Outcome of renal transplantation in patients with non-Shiga toxin-associated hemolytic uremic syndrome: prognostic significance of genetic background. *Clin J Am Soc Nephrol* 2006;1(1):88–99.
257. Loirat C, Fremeaux-Bacchi V. Hemolytic uremic syndrome recurrence after renal transplantation. *Pediatr Transplant* 2008;12(6):619–629.
258. Noris M, Remuzzi G. Thrombotic microangiopathy after kidney transplantation. *Am J Transplant* 2010;10(7):1517–1523.
259. Zuber J, Le Quintrec M, Sberro-Soussan R, et al. New insights into postrenal transplant hemolytic uremic syndrome. *Nat Rev Nephrol* 2011;7(1):23–35.
260. Chan MR, Thomas CP, Torrealba JR, et al. Recurrent atypical hemolytic uremic syndrome associated with factor I mutation in a living related renal transplant recipient. *Am J Kidney Dis* 2009;53(2):321–326.
261. Schwimmer J, Nadasdy TA, Spitalnik PF, et al. De novo thrombotic microangiopathy in renal transplant recipients: a comparison of hemolytic uremic syndrome with localized renal thrombotic microangiopathy. *Am J Kidney Dis* 2003;41(2):471–479.
262. Said T, Al-Otaibi T, Al-Wahaib S, et al. Posttransplantation calcineurin inhibitor-induced hemolytic uremic syndrome: single-center experience. *Transplant Proc* 2010;42(3):814–816.
263. Lovric S, Kielstein JT, Kayser D, et al. Combination of everolimus with calcineurin inhibitor medication resulted in post-transplant haemolytic uraemic syndrome in lung transplant recipients—A case series. *Nephrol Dial Transplant* 2011;26(9):3032–3038.
264. Schwarz A, Haller H, Schmitt R, et al. Biopsy-diagnosed renal disease in patients after transplantation of other organs and tissues. *Am J Transplant* 2010;10(9):2017–2025.
265. Moro J, Almenar L, Martinez-Dolz L, et al. mTOR inhibitors and their secondary effects in cardiac transplant recipients: a descriptive study. *Transplant Proc* 2007;39(7):2365–2367.
266. Izquierdo MT, Almenar L, Martinez-Dolz L, et al. [Hemolytic uremic syndrome due to sirolimus in a heart transplant recipient. Case report]. *Rev Esp Cardiol* 2007;60(1):85–86.
267. Shindoh J, Sugawara Y, Akamatsu N, et al. Thrombotic microangiopathy after living-donor liver transplantation. *Am J Transplant* 2012;12(3):728–736.
268. Rangel EB, Gonzalez AM, Linhares MM, et al. Thrombotic microangiopathy after simultaneous pancreas-kidney transplantation. *Clin Transplant* 2007;21(2):241–245.
269. Dierickx D, Monbaliu D, De Rycke A, et al. Thrombotic microangiopathy following intestinal transplantation: a single center experience. *Transplant Proc* 2010;42(1):79–81.
270. Ruggenenti P. Post-transplant hemolytic-uremic syndrome. *Kidney Int* 2002;62(3):1093–1104.
271. Zoja C, Furci L, Ghilardi F, et al. Cyclosporin-induced endothelial cell injury. *Lab Invest* 1986;55(4):455–462.
272. Remuzzi G, Bertani T. Renal vascular and thrombotic effects of cyclosporine. *Am J Kidney Dis* 1989;13(4):261–272.
273. Garcia-Maldonado M, Kaufman CE, Comp PC. Decrease in endothelial cell-dependent protein C activation induced by thrombomodulin by treatment with cyclosporine. *Transplantation* 1991;51(3):701–705.
274. Carlsen E, Flatmark A, Prydz H. Cytokine-induced procoagulant activity in monocytes and endothelial cells. Further enhancement by cyclosporine. *Transplantation* 1988;46(4):575–580.
275. Nankivell BJ, Chapman JR, Bonovas G, et al. Oral cyclosporine but not tacrolimus reduces renal transplant blood flow. *Transplantation* 2004;77(9):1457–1459.
276. Fujimura Y, Matsumoto M, Kokame K, et al. Pregnancy-induced thrombocytopenia and TTP, and the risk of fetal death, in Upshaw-Schulman syndrome: a series of 15 pregnancies in 9 genotyped patients. *Br J Haematol* 2009;144(5):742–754.
277. Calderazzo JC, Kempfer A, Powazniak Y, et al. A new ADAMTS13 missense mutation (D1362V) in thrombotic thrombocytopenic purpura diagnosed during pregnancy. *Thromb Haemost* 2012;108(2):401–403.
278. Ducloy-Bouthors AS, Caron C, Subtil D, et al. Thrombotic thrombocytopenic purpura: Medical and biological monitoring of six pregnancies. *Eur J Obstet Gynecol Reprod Biol* 2003;111(2):146–152.
279. Martin JN, Jr., Bailey AP, Rehberg JF, et al. Thrombotic thrombocytopenic purpura in 166 pregnancies: 1955–2006. *Am J Obstet Gynecol* 2008;199(2):98–104.
280. Salmon JE, Heuser C, Triebwasser M, et al. Mutations in complement regulatory proteins predispose to preeclampsia: a genetic analysis of the PROMISSE cohort. *PLoS Med* 2011;8(3):e1001013.
281. Hulstein JJ, van Runnard Heimel PJ, Franx A, et al. Acute activation of the endothelium results in increased levels of active von Willebrand factor in hemolysis, elevated liver enzymes and low platelets (HELLP) syndrome. *J Thromb Haemost* 2006;4(12):2569–2575.
282. Brown JH, Tellez J, Wilson V, et al. Postpartum aHUS secondary to a genetic abnormality in factor H acquired through liver transplantation. *Am J Transplant* 2012;12(6):1632–1636.
283. Kanodia KV, Vanikar AV, Shah PR, et al. C1q nephropathy with acute hemolytic uremic syndrome. *Saudi J Kidney Dis* 2012;23(3):556–558.

284. El Karoui K, Hill GS, Karras A, et al. A clinicopathologic study of thrombotic microangiopathy in IgA nephropathy. *J Am Soc Nephrol* 2012;23(1):137–148.
285. Chang A, Kowalewska J, Smith KD, et al. A clinicopathologic study of thrombotic microangiopathy in the setting of IgA nephropathy. *Clin Nephrol* 2006;66(6):397–404.
286. Kuppachi S, Chander P, Yoo J. Membranous nephropathy and thrombotic thrombocytopenic purpura treated with rituximab. *J Nephrol* 2009;22(4):561–564.
287. Duvic C, Desrame J, Herody M, et al. Acute poststreptococcal glomerulonephritis associated with thrombotic microangiopathy in an adult. *Clin Nephrol* 2000;54(2):169–173.
288. Charney DA, Nassar G, Truong L, et al. "Pauci-Immune" proliferative and necrotizing glomerulonephritis with thrombotic microangiopathy in patients with systemic lupus erythematosus and lupus-like syndrome. *Am J Kidney Dis* 2000;35(6):1193–1206.
289. Tan PH, Yadin O, Kleinman KS, et al. Simultaneous postinfectious glomerulonephritis and thrombotic microangiopathy: a renal biopsy study. *Am J Kidney Dis* 1998;31(3):513–520.
290. Stefanidis I, Helmchen U, Schmitt H, et al. Coincidence of haemolytic uraemic syndrome and c-ANCA-associated rapidly progressive glomerulonephritis. *Nephrol Dial Transplant* 1998;13(7):1818–1821.
291. Herzenberg AM, Telford JJ, De Luca LG, et al. Thrombotic microangiopathy associated with cryoglobulinemic membranoproliferative glomerulonephritis and hepatitis C. *Am J Kidney Dis* 1998;31(3):521–526.
292. Stave GM, Croker BP. Thrombotic microangiopathy in anti-glomerular basement membrane glomerulonephritis. *Arch Pathol Lab Med* 1984; 108(9):747–751.
293. Gnappi E, Allinovi M, Vaglio A, et al. Membrano-proliferative glomerulonephritis, atypical hemolytic uremic syndrome, and a new complement factor H mutation: report of a case. *Pediatr Nephrol* 2012;27(10):1995–1999.
294. Caprioli J, Castelletti F, Bucchioni S, et al. Complement factor H mutations and gene polymorphisms in haemolytic uraemic syndrome: the C-257T, the A2089G and the G2881T polymorphisms are strongly associated with the disease. *Hum Mol Genet* 2003;12(24):3385–3395.
295. Ranque B, Mouthon L. Geoepidemiology of systemic sclerosis. *Autoimmun Rev* 2010;9(5):A311–A318.
296a. Mayes MD, Lacey JV, Jr., Beebe-Dimmer J, et al. Prevalence, incidence, survival, and disease characteristics of systemic sclerosis in a large US population. *Arthritis Rheum* 2003;48(8):2246–2255.
296b. Reveille JD. Ethnicity and race and systemic sclerosis: how it affects susceptibility, severity, antibody genetics, and clinical manifestations. *Curr Rheumatol Rep* 2003;5(2):160–167.
297. Bohle A, Helmchen U, Grund KE, et al. Malignant nephrosclerosis in patients with hemolytic uremic syndrome (primary malignant nephrosclerosis). *Curr Top Pathol* 1977;65:81–113.
298. Zhang B, Xing C, Yu X, et al. Renal thrombotic microangiopathies induced by severe hypertension. *Hypertens Res* 2008;31(3):479–483.
299. Shibagaki Y, Fujita T. Thrombotic microangiopathy in malignant hypertension and hemolytic uremic syndrome (HUS)/ thrombotic thrombocytopenic purpura (TTP): can we differentiate one from the other? *Hypertens Res* 2005;28(1):89–95.
300. Shavit L, Reinus C, Slotki I. Severe renal failure and microangiopathic hemolysis induced by malignant hypertension—case series and review of literature. *Clin Nephrol* 2010;73(2):147–152.
301. van den Born BJ, Lowenberg EC, van der Hoeven NV, et al. Endothelial dysfunction, platelet activation, thrombogenesis and fibrinolysis in patients with hypertensive crisis. *J Hypertens* 2011;29(5):922–927.
302. Francis KK, Kalyanam N, Terrell DR, et al. Disseminated malignancy misdiagnosed as thrombotic thrombocytopenic purpura: a report of 10 patients and a systematic review of published cases. *Oncologist* 2007;12(1):11–19.
303. Alonso JV, Fonseca J, Lopera EL, et al. A report of disseminated adenocarcinoma presenting as thrombotic thrombocytopenic purpura. *Hematol Rep* 2011;3(2):e14.
304. Vasko R, Koziolek M, Fuzesi L, et al. Fulminant plasmapheresis-refractory thrombotic microangiopathy associated with advanced gastric cancer. *Ther Apher Dial* 2010;14(2):222–225.
305. Fontana S, Gerritsen HE, Kremer Hovinga J, et al. Microangiopathic haemolytic anaemia in metastasizing malignant tumours is not associated with a severe deficiency of the von Willebrand factor-cleaving protease. *Br J Haematol* 2001;113(1):100–102.
306. Lohrmann HP, Brech W, Widmer K, et al. [Hemolytic-uremic syndrome in adults]. *Dtsch Med Wochenschr* 1973;98(2):54–61.
307. John HD, Thoenes W. The glomerular lesions in endotheliotropic hemolytic nephroangiopathy (hemolytic uremic syndrome, malignant nephrosclerosis, post partum renal insufficiency). *Pathol Res Pract* 1982;173(3):236–259.
308. Morel-Maroger L, Kanfer A, Solez K, et al. Prognostic importance of vascular lesions in acute renal failure with microangiopathic hemolytic anemia (hemolytic-uremic syndrome): clinicopathologic study in 20 adults. *Kidney Int* 1979;15(5):548–558.
309. Taylor CM, Chua C, Howie AJ, et al. Clinico-pathological findings in diarrhoea-negative haemolytic uraemic syndrome. *Pediatr Nephrol* 2004;19(4):419–425.
310. Renaud C, Niaudet P, Gagnadoux MF, et al. Haemolytic uraemic syndrome: prognostic factors in children over 3 years of age. *Pediatr Nephrol* 1995;9(1):24–29.
311. Inward CD, Howie AJ, Fitzpatrick MM, et al. Renal histopathology in fatal cases of diarrhoea-associated haemolytic uraemic syndrome. British Association for Paediatric Nephrology. *Pediatr Nephrol* 1997;11(5):556–559.
312. Yajima T. [The bacterial invasion and tissue reactions of rabbits inoculated subcutaneously with BCG vaccine added with hyaluronidase]. *Kekkaku* 1956;31(8):499–502.
313. Pearce RM. An experimental glomerular lesion caused by venom (*Crotalus adamanteus*). *J Exp Med* 1909;11(4):532–540.
314. Koitabashi Y, Rosenberg BF, Shapiro H, et al. Mesangiolysis: An important glomerular lesion in thrombotic microangiopathy. *Mod Pathol* 1991;4(2):161–166.
315. Morita T, Yamamoto T, Churg J. Mesangiolysis: an update. *Am J Kidney Dis* 1998;31(4):559–573.
316. Orbison JL. Morphology of thrombotic thrombocytopenic purpura with demonstration of aneurysms. *Am J Pathol* 1952;28(1):129–143.
317. Umlas J. Glomeruloid structures in thrombohemolytic thrombocytopenic purpura, glomerulonephritis, and disseminated intravascular coagulation. *Hum Pathol* 1972;3(3):437–441.
318. Habib R, Lévy M, Gagnadoux M-F, et al. Prognosis of the hemolytic uremic syndrome in children. *Adv Nephrol* 1982;11:99–128.
319. Asada Y, Sumiyoshi A, Hayashi T, et al. Immunohistochemistry of vascular lesion in thrombotic thrombocytopenic purpura, with special reference to factor VIII related antigen. *Thromb Res* 1985;38(5):469–479.
320. Hosler GA, Cusumano AM, Hutchins GM. Thrombotic thrombocytopenic purpura and hemolytic uremic syndrome are distinct pathologic entities: A review of 56 autopsy cases. *Arch Pathol Lab Med* 2003;127(7):834–839.
321. Tsai HM, Chandler WL, Sarode R, et al. von Willebrand factor and von Willebrand factor-cleaving metalloprotease activity in *Escherichia coli* O157:H7-associated hemolytic uremic syndrome. *Pediatr Res* 2001;49(5):653–659.
322. Katoh M, Shigematsu H. Renal involvement of thrombotic thrombocytopenic purpura: special reference to the glomeruloid structures. *Pathol Int* 1999;49(7):638–642.
323. Rennke HG. Secondary membranoproliferative glomerulonephritis. *Kidney Int* 1995;47(2):643–656.
324. Haas M. Transplant glomerulopathy: it's not always about chronic rejection. *Kidney Int* 2011;80(8):801–803.
325. Cosio FG, Roche Z, Agarwal A, et al. Prevalence of hepatitis C in patients with idiopathic glomerulopathies in native and transplant kidneys. *Am J Kidney Dis* 1996;28(5):752–758.
326. Gallay BJ, Alpers CE, Davis CL, et al. Glomerulonephritis in renal allografts associated with hepatitis C infection: a possible relationship with transplant glomerulopathy in two cases. *Am J Kidney Dis* 1995;26(4):662–667.
327. Mocan H, Mocan MZ. Postpartum hemolytic uremic syndrome with a more severe liver involvement. *Clin Nephrol* 1998;49(5):319–320.
328. Zhou GY. Postpartum hemolytic uremic syndrome with multiple organ involvement in a severe case. *Nefrologia* 2012;32(3):408–410.
329. Sane DC, Streer NP, Owen J. Myocardial necrosis in patients with thrombotic thrombocytopenic purpura: pathophysiology and rationale for specific therapy. *Eur J Haematol* 2009;82(2):83–92.

330. Praprotnik S, Ferluga D, Vizjak A, et al. Microthrombotic/microangiopathic manifestations of the antiphospholipid syndrome. *Clin Rev Allergy Immunol* 2009;36(2–3):109–125.
331. Robson WL, Leung AK, Montgomery MD. Causes of death in hemolytic uremic syndrome. *Child Nephrol Urol* 1991;11(4):228–233.
332. Fitzpatrick MM, Walters MD, Trompeter RS, et al. Atypical (non-diarrhea-associated) hemolytic-uremic syndrome in childhood. *J Pediatr* 1993;122(4):532–537.
333. Gianviti A, Tozzi AE, De Petris L, et al. Risk factors for poor renal prognosis in children with hemolytic uremic syndrome. *Pediatr Nephrol* 2003;18(12):1229–1235.
334. Siegler RL, Pavia AT, Hansen FL, et al. Atypical hemolytic-uremic syndrome: a comparison with postdiarrheal disease. *J Pediatr* 1996;128(4):505–511.
335. Lopez EL, Devoto S, Fayad A, et al. Association between severity of gastrointestinal prodrome and long-term prognosis in classic hemolytic-uremic syndrome. *J Pediatr* 1992;120(2 Pt 1):210–215.
336. Small G, Watson AR, Evans JH, et al. Hemolytic uremic syndrome: defining the need for long-term follow-up. *Clin Nephrol* 1999;52(6):352–356.
337. Huseman D, Gellermann J, Vollmer I, et al. Long-term prognosis of hemolytic uremic syndrome and effective renal plasma flow. *Pediatr Nephrol* 1999;13(8):672–677.
338. Spizzirri FD, Rahman RC, Bibiloni N, et al. Childhood hemolytic uremic syndrome in Argentina: long-term follow-up and prognostic features. *Pediatr Nephrol* 1997;11(2):156–160.
339. Walters MD, Matthei IU, Kay R, et al. The polymorphonuclear leucocyte count in childhood haemolytic uraemic syndrome. *Pediatr Nephrol* 1989;3(2):130–134.
340. Siegler RL, Pavia AT, Christofferson RD, et al. A 20-year population-based study of postdiarrheal hemolytic uremic syndrome in Utah. *Pediatrics* 1994;94(1):35–40.
341. De Petris L, Gianviti A, Giordano U, et al. Blood pressure in the long-term follow-up of children with hemolytic uremic syndrome. *Pediatr Nephrol* 2004;19(11):1241–1244.
342. Siegler R, Oakes R. Hemolytic uremic syndrome; pathogenesis, treatment, and outcome. *Curr Opin Pediatr* 2005;17(2):200–204.
343. Garg AX, Clark WF, Salvadori M, et al. Microalbuminuria three years after recovery from *Escherichia coli* O157 hemolytic uremic syndrome due to municipal water contamination. *Kidney Int* 2005;67(4):1476–1482.
344. Garg AX, Clark WF, Salvadori M, et al. Absence of renal sequelae after childhood *Escherichia coli* O157:H7 gastroenteritis. *Kidney Int* 2006;70(4):807–812.
345. Basile DP, Donohoe D, Roethe K, et al. Renal ischemic injury results in permanent damage to peritubular capillaries and influences long-term function. *Am J Physiol Renal Physiol* 2001;281(5):F887–F899.
346. Eckardt KU, Bernhardt WM, Weidemann A, et al. Role of hypoxia in the pathogenesis of renal disease. *Kidney Int Suppl* 2005(99):S46–S51.
347. Remuzzi G, Ruggenenti P, Codazzi D, et al. Combined kidney and liver transplantation for familial haemolytic uraemic syndrome. *Lancet* 2002;359(9318):1671–1672.
348. Saland JM, Emre SH, Shneider BL, et al. Favorable long-term outcome after liver-kidney transplant for recurrent hemolytic uremic syndrome associated with a factor H mutation. *Am J Transplant* 2006;6(8):1948–1952.
349. Khanh BT, Bhathena D, Vazquez M, et al. Role of heparin therapy in the outcome of adult hemolytic uremic syndrome. *Nephron* 1976;16(4):292–301.
350. Bell WR, Braine HG, Ness PM, et al. Improved survival in thrombotic thrombocytopenic purpura-hemolytic uremic syndrome. Clinical experience in 108 patients. *N Engl J Med* 1991;325(6):398–403.
351. Matsumae T, Takebayashi S, Naito S. The clinico-pathological characteristics and outcome in hemolytic-uremic syndrome of adults. *Clin Nephrol* 1996;45(3):153–162.
352. Melnyk AM, Solez K, Kjellstrand CM. Adult hemolytic-uremic syndrome. A review of 37 cases. *Arch Intern Med* 1995;155(19):2077–2084.
353. Tostivint I, Mougenot B, Flahault A, et al. Adult haemolytic and uraemic syndrome: causes and prognostic factors in the last decade. *Nephrol Dial Transplant* 2002;17(7):1228–1234.
354. Mehrazma M, Hooman N, Otukesh H. Prognostic value of renal pathological findings in children with atypical hemolytic uremic syndrome. *Iran J Kidney Dis* 2011;5(6):380–385.
355. Gagnadoux MF, Habib R, Gubler MC, et al. Long-term (15–25 years) outcome of childhood hemolytic-uremic syndrome. *Clin Nephrol* 1996;46(1):39–41.
356. Winter KR, Stoffregen WC, Dean-Nystrom EA. Shiga toxin binding to isolated porcine tissues and peripheral blood leukocytes. *Infect Immun* 2004;72(11):6680–6684.
357. Sandvig K, Garred O, Prydz K, et al. Retrograde transport of endocytosed Shiga toxin to the endoplasmic reticulum. *Nature* 1992;358(6386):510–512.
358. Mukhopadhyay S, Linstedt AD. Manganese blocks intracellular trafficking of Shiga toxin and protects against Shiga toxicosis. *Science* 2012;335(6066):332–335.
359. Tam PJ, Lingwood CA. Membrane cytosolic translocation of verotoxin A1 subunit in target cells. *Microbiology* 2007;153(Pt 8):2700–2710.
360. Endo Y, Tsurugi K, Yutsudo T, et al. Site of action of a Vero toxin (VT2) from *Escherichia coli* O157:H7 and of Shiga toxin on eukaryotic ribosomes. RNA N-glycosidase activity of the toxins. *Eur J Biochem* 1988;171(1–2):45–50.
361. Johannes L, Romer W. Shiga toxins–from cell biology to biomedical applications. *Nat Rev Microbiol* 2010;8(2):105–116.
362. Pijpers AH, van Setten PA, van den Heuvel LP, et al. Verocytotoxin-induced apoptosis of human microvascular endothelial cells. *J Am Soc Nephrol* 2001;12(4):767–778.
363. Cherla RP, Lee SY, Tesh VL. Shiga toxins and apoptosis. *FEMS Microbiol Lett* 2003;228(2):159–166.
364. Bielaszewska M, Karch H. Consequences of enterohaemorrhagic *Escherichia coli* infection for the vascular endothelium. *Thromb Haemost* 2005;94(2):312–318.
365. Petruzziello TN, Mawji IA, Khan M, et al. Verotoxin biology: Molecular events in vascular endothelial injury. *Kidney Int Suppl* 2009(112):S17–S19.
366. Matussek A, Lauber J, Bergau A, et al. Molecular and functional analysis of Shiga toxin-induced response patterns in human vascular endothelial cells. *Blood* 2003;102(4):1323–1332.
367. Morigi M, Micheletti G, Figliuzzi M, et al. Verotoxin-1 promotes leukocyte adhesion to cultured endothelial cells under physiologic flow conditions. *Blood* 1995;86(12):4553–4558.
368. Zoja C, Angioletti S, Donadelli R, et al. Shiga toxin-2 triggers endothelial leukocyte adhesion and transmigration via NF-kappaB dependent up-regulation of IL-8 and MCP-1. *Kidney Int* 2002;62(3):846–856.
369. Zanchi C, Zoja C, Morigi M, et al. Fractalkine and CX3CR1 mediate leukocyte capture by endothelium in response to Shiga toxin. *J Immunol* 2008;181(2):1460–1469.
370. Morigi M, Galbusera M, Binda E, et al. Verotoxin-1-induced up-regulation of adhesive molecules renders microvascular endothelial cells thrombogenic at high shear stress. *Blood* 2001;98(6):1828–1835.
371. Van de Kar NC, Monnens LA, Van Hinsbergh VW. Tumor necrosis factor and interleukin 1 induce expression of the glycolipid verotoxin receptor in human endothelial cells. Implications for the pathogenesis of the haemolytic uraemic syndrome. *Behring Inst Mitt* 1993(92):202–209.
372. van Setten PA, Monnens LA, Verstraten RG, et al. Effects of verocytotoxin-1 on nonadherent human monocytes: Binding characteristics, protein synthesis, and induction of cytokine release. *Blood* 1996;88(1):174–183.
373. Nolasco LH, Turner NA, Bernardo A, et al. Hemolytic uremic syndrome-associated Shiga toxins promote endothelial-cell secretion and impair ADAMTS13 cleavage of unusually large von Willebrand factor multimers. *Blood* 2005;106(13):4199–4209.
374. Liu F, Huang J, Sadler JE. Shiga toxin (Stx)1B and Stx2B induce von Willebrand factor secretion from human umbilical vein endothelial cells through different signaling pathways. *Blood* 2011;118(12):3392–3398.
375. Huang J, Haberichter SL, Sadler JE. The B subunits of Shiga-like toxins induce regulated VWF secretion in a phospholipase D1 dependent manner. *Blood* 2012;120(5)1143–1149.
376. Nestoridi E, Kushak RI, Tsukurov O, et al. Role of the renin angiotensin system in TNF-alpha and Shiga-toxin-induced tissue factor expression. *Pediatr Nephrol* 2008;23(2):221–231.

377. Morigi M, Galbusera M, Gastoldi S, et al. Alternative pathway activation of complement by Shiga toxin promotes exuberant C3a formation that triggers microvascular thrombosis. *J Immunol* 2011;187(1):172–180.
378. Thurman JM, Marians R, Emlen W, et al. Alternative pathway of complement in children with diarrhea-associated hemolytic uremic syndrome. *Clin J Am Soc Nephrol* 2009;4(12):1920–1924.
379. Stahl AL, Sartz L, Karpman D. Complement activation on platelet-leukocyte complexes and microparticles in enterohemorrhagic *Escherichia coli*-induced hemolytic uremic syndrome. *Blood* 2011;117(20):5503–5513.
380. Petruzziello-Pellegrini TN, Yuen DA, Page AV, et al. The CXCR4/CXCR7/SDF-1 pathway contributes to the pathogenesis of Shiga toxin-associated hemolytic uremic syndrome in humans and mice. *J Clin Invest* 2012;122(2):759–776.
381. Nakajima H, Kiyokawa N, Katagiri YU, et al. Kinetic analysis of binding between Shiga toxin and receptor glycolipid Gb3Cer by surface plasmon resonance. *J Biol Chem* 2001;276(46):42915–42922.
382. Siegler RL. Postdiarrheal Shiga toxin-mediated hemolytic uremic syndrome. *JAMA* 2003;290(10):1379–1381.
383. Brigotti M, Carnicelli D, Ravanelli E, et al. Molecular damage and induction of proinflammatory cytokines in human endothelial cells exposed to Shiga toxin 1, Shiga toxin 2, and alpha-sarcin. *Infect Immun* 2007;75(5):2201–2207.
384. Beutin L, Krause G, Zimmermann S, et al. Characterization of Shiga toxin-producing *Escherichia coli* strains isolated from human patients in Germany over a 3-year period. *J Clin Microbiol* 2004;42(3):1099–1108.
385. Bielaszewska M, Friedrich AW, Aldick T, et al. Shiga toxin activatable by intestinal mucus in *Escherichia coli* isolated from humans: predictor for a severe clinical outcome. *Clin Infect Dis* 2006;43(9):1160–1167.
386. Friedrich AW, Bielaszewska M, Zhang WL, et al. *Escherichia coli* harboring Shiga toxin 2 gene variants: Frequency and association with clinical symptoms. *J Infect Dis* 2002;185(1):74–84.
387. Acheson DW, Moore R, De Breucker S, et al. Translocation of Shiga toxin across polarized intestinal cells in tissue culture. *Infect Immun* 1996;64(8):3294–3300.
388. Hurley BP, Thorpe CM, Acheson DW. Shiga toxin translocation across intestinal epithelial cells is enhanced by neutrophil transmigration. *Infect Immun* 2001;69(10):6148–6155.
389. Thorpe CM, Hurley BP, Lincicome LL, et al. Shiga toxins stimulate secretion of interleukin-8 from intestinal epithelial cells. *Infect Immun* 1999;67(11):5985–5993.
390. te Loo DM, Monnens LA, van Der Velden TJ, et al. Binding and transfer of verocytotoxin by polymorphonuclear leukocytes in hemolytic uremic syndrome. *Blood* 2000;95(11):3396–3402.
391. Te Loo DM, van Hinsbergh VW, van den Heuvel LP, et al. Detection of verocytotoxin bound to circulating polymorphonuclear leukocytes of patients with hemolytic uremic syndrome. *J Am Soc Nephrol* 2001;12(4):800–806.
392. Brigotti M, Tazzari PL, Ravanelli E, et al. Clinical relevance of shiga toxin concentrations in the blood of patients with hemolytic uremic syndrome. *Pediatr Infect Dis J* 2011;30(6):486–490.
393. Obrig TG, Louise CB, Lingwood CA, et al. Endothelial heterogeneity in Shiga toxin receptors and responses. *J Biol Chem* 1993;268(21):15484–15488.
394. Zoja C, Corna D, Farina C, et al. Verotoxin glycolipid receptors determine the localization of microangiopathic process in rabbits given verotoxin-1. *J Lab Clin Med* 1992;120(2):229–238.
395. Hughes AK, Stricklett PK, Kohan DE. Cytotoxic effect of Shiga toxin-1 on human proximal tubule cells. *Kidney Int* 1998;54(2):426–437.
396. Louise CB, Obrig TG. Shiga toxin-associated hemolytic-uremic syndrome: Combined cytotoxic effects of Shiga toxin, interleukin-1 beta, and tumor necrosis factor alpha on human vascular endothelial cells in vitro. *Infect Immun* 1991;59(11):4173–4179.
397. Louise CB, Obrig TG. Shiga toxin-associated hemolytic uremic syndrome: Combined cytotoxic effects of shiga toxin and lipopolysaccharide (endotoxin) on human vascular endothelial cells in vitro. *Infect Immun* 1992;60(4):1536–1543.
398. Siegler RL, Pysher TJ, Lou R, et al Response to Shiga toxin-1, with and without lipopolysaccharide, in a primate model of hemolytic uremic syndrome. *Am J Nephrol* 2001;21(5):420–425.
399. Coratelli P, Buongiorno E, Passavanti G. Endotoxemia in hemolytic uremic syndrome. *Nephron* 1988;50(4):365–367.
400. Bitzan M, Moebius E, Ludwig K, et al. High incidence of serum antibodies to *Escherichia coli* O157 lipopolysaccharide in children with hemolytic-uremic syndrome. *J Pediatr* 1991;119(3):380–385.
401. Heyderman RS, Fitzpatrick MM, Barclay GR. Haemolytic-uraemic syndrome. *Lancet* 1994;343(8904):1042.
402. Fernandez-Brando RJ, Bentancor LV, Mejias MP, et al. Antibody response to Shiga toxins in Argentinean children with enteropathic hemolytic uremic syndrome at acute and long-term follow-up periods. *PLoS One* 2011;6(4):e19136.
403. Ludwig K, Karmali MA, Sarkim V, et al. Antibody response to Shiga toxins Stx2 and Stx1 in children with enteropathic hemolytic-uremic syndrome. *J Clin Microbiol* 2001;39(6):2272–2279.
404. Karmali MA, Mascarenhas M, Petric M, et al. Age-specific frequencies of antibodies to *Escherichia coli* verocytotoxins (Shiga toxins) 1 and 2 among urban and rural populations in southern Ontario. *J Infect Dis* 2003;188(11):1724–1729.
405. Devaux P, Christiansen D, Fontaine M, et al. Control of C3b and C5b deposition by CD46 (membrane cofactor protein) after alternative but not classical complement activation. *Eur J Immunol* 1999;29(3):815–822.
406. Delvaeye M, Noris M, De Vriese A, et al. Thrombomodulin mutations in atypical hemolytic-uremic syndrome. *N Engl J Med* 2009;361(4):345–357.
407. Skerka C, Zipfel PF, Muller D, et al. The autoimmune disease DEAP-hemolytic uremic syndrome. *Semin Thromb Hemost* 2010;36(6):625–632.
408. Ermini L, Goodship TH, Strain L, et al. Common genetic variants in complement genes other than CFH, CD46 and the CFHRs are not associated with aHUS. *Mol Immunol* 2012;49(4):640–648.
409. Kerr H, Richards A. Complement-mediated injury and protection of endothelium: lessons from atypical haemolytic uraemic syndrome. *Immunobiology* 2012;217(2):195–203.
410. Saunders RE, Abarrategui-Garrido C, Fremeaux-Bacchi V, et al. The interactive Factor H-atypical hemolytic uremic syndrome mutation database and website: update and integration of membrane cofactor protein and Factor I mutations with structural models. *Hum Mutat* 2007;28(3):222–234.
411. Manuelian T, Hellwage J, Meri S, et al. Mutations in factor H reduce binding affinity to C3b and heparin and surface attachment to endothelial cells in hemolytic uremic syndrome. *J Clin Invest* 2003;111(8):1181–1190.
412. Heinen S, Jozsi M, Hartmann A, et al. Hemolytic uremic syndrome: a factor H mutation (E1172Stop) causes defective complement control at the surface of endothelial cells. *J Am Soc Nephrol* 2007;18(2):506–514.
413. Stahl AL, Vaziri-Sani F, Heinen S, et al. Factor H dysfunction in patients with atypical hemolytic uremic syndrome contributes to complement deposition on platelets and their activation. *Blood* 2008;111(11):5307–5315.
414. Jozsi M, Strobel S, Dahse HM, et al. Anti factor H autoantibodies block C-terminal recognition function of factor H in hemolytic uremic syndrome. *Blood* 2007;110(5):1516–1518.
415. Jozsi M, Licht C, Strobel S, et al. Factor H autoantibodies in atypical hemolytic uremic syndrome correlate with CFHR1/CFHR3 deficiency. *Blood* 2008;111(3):1512–1514.
416. Moore I, Strain L, Pappworth I, et al. Association of factor H autoantibodies with deletions of CFHR1, CFHR3, CFHR4, and with mutations in CFH, CFI, CD46, and C3 in patients with atypical hemolytic uremic syndrome. *Blood* 2010;115(2):379–387.
417. Abarrategui-Garrido C, Martinez-Barricarte R, Lopez-Trascasa M, et al. Characterization of complement factor H-related (CFHR) proteins in plasma reveals novel genetic variations of CFHR1 associated with atypical hemolytic uremic syndrome. *Blood* 2009;114(19):4261–4271.
418. Westra D, Vernon KA, Volokhina EB, et al. Atypical hemolytic uremic syndrome and genetic aberrations in the complement factor H-related 5 gene. *J Hum Genet* 2012;57(7):459–464.
419. Provaznikova D, Rittich S, Malina M, et al. Manifestation of atypical hemolytic uremic syndrome caused by novel mutations in MCP. *Pediatr Nephrol* 2012;27(1):73–81.

420. Fremeaux-Bacchi V, Moulton EA, Kavanagh D, et al. Genetic and functional analyses of membrane cofactor protein (CD46) mutations in atypical hemolytic uremic syndrome. *J Am Soc Nephrol* 2006;17(7):2017–2025.
421. Kavanagh D, Richards A, Noris M, et al. Characterization of mutations in complement factor I (CFI) associated with hemolytic uremic syndrome. *Mol Immunol* 2008;45(1):95–105.
422. Goicoechea de Jorge E, Harris CL, Esparza-Gordillo J, et al. Gain-of-function mutations in complement factor B are associated with atypical hemolytic uremic syndrome. *Proc Natl Acad Sci U S A* 2007;104(1):240–245.
423. Roumenina LT, Jablonski M, Hue C, et al. Hyperfunctional C3 convertase leads to complement deposition on endothelial cells and contributes to atypical hemolytic uremic syndrome. *Blood* 2009;114(13):2837–2845.
424a. Volokhina E, Westra D, Xue X, et al. Novel C3 mutation p.Lys65Gln in aHUS affects complement factor H binding. *Pediatr Nephrol* 2012;27(9):1519–1524.
424b. Fremeaux-Bacchi V, Kemp EJ, Goodship JA, et al. The development of atypical HUS is influenced by susceptibility factors in factor H and membrane cofactor protein-evidence from two independent cohorts. *J Med Genet* 2005;42:852–856.
424c. Noris M, Bucchioni S, Galbusera M, et al. Complement factor H mutation in familial thrombotic thrombocytopenic purpura with ADAMTS13 deficiency and renal involvement. *J Am Soc Nephrol* 2005;(5):1177–1183.
425. Huang YH, Lin TY, Wong KS, et al. Hemolytic uremic syndrome associated with pneumococcal pneumonia in Taiwan. *Eur J Pediatr* 2006;165(5):332–335.
426. Lee CF, Liu SC, Lue KH, et al. Pneumococcal pneumonia with empyema and hemolytic uremic syndrome in children: report of three cases. *J Microbiol Immunol Infect* 2006;39(4):348–352.
427. Chautemps N, Milesi C, Cambonie G, et al. [Pneumococcal hemolytic uremic syndrome: about 2 cases]. *Arch Pediatr Fr* 2008;15(7):1206–1210.
428. Hopkins CK, Yuan S, Lu Q, et al. A severe case of atypical hemolytic uremic syndrome associated with pneumococcal infection and T activation treated successfully with plasma exchange. *Transfusion* 2008;48(11):2448–2452.
429. Prestidge C, Wong W. Ten years of pneumococcal-associated haemolytic uraemic syndrome in New Zealand children. *J Paediatr Child Health* 2009;45(12):731–735.
430. Copelovitch L, Kaplan BS. *Streptococcus pneumoniae*—associated hemolytic uremic syndrome: Classification and the emergence of serotype 19A. *Pediatrics* 2010;125(1):e174–e182.
431. Chen SY, Wu CY, Tsai IJ, et al. Nonenteropathic hemolytic uremic syndrome: The experience of a medical center. *Pediatr Neonatol* 2011;52(2):73–77.
432. Pelras S, Delmas Y, Lamireau D, et al. Severe transient ADAMTS13 deficiency in pneumococcal-associated hemolytic uremic syndrome. *Pediatr Nephrol* 2011;26(4):631–635.
433. Quiviger S, Flechelles O, Cecile W, et al. [*Streptococcus pneumoniae*-induced hemolytic uremic syndrome: a serotype-3-associated case]. *Arch Pediatr* 2012;19(6):599–602.
434. Copelovitch L, Kaplan BS. *Streptococcus pneumoniae*-associated hemolytic uremic syndrome. *Pediatr Nephrol* 2008;23(11):1951–1956.
435. Moake J. Thrombotic thrombocytopenia purpura (TTP) and other thrombotic microangiopathies. *Best Pract Res Clin Haematol* 2009;22(4):567–576.
436. Dong JF, Moake JL, Nolasco L, et al. ADAMTS-13 rapidly cleaves newly secreted ultralarge von Willebrand factor multimers on the endothelial surface under flowing conditions. *Blood* 2002;100(12):4033–4039.
437. Trojanowska M. Cellular and molecular aspects of vascular dysfunction in systemic sclerosis. *Nat Rev Rheumatol* 2010;6(8):453–460.
438a. Tsai HM. Deficiency of ADAMTS13 in thrombotic thrombocytopenic purpura. *Int J Hematol* 2002;76(Suppl 2):132–138.
438b. Banno F, Chauhan AK, Kokame K, et al. The distal carboxyl-terminal domains of ADAMTS13 are required for regulation of in *vivo* thrombus formation. *Blood* 2009;113(21):5323–5329.
438c. Turner N, Nolasco L, Tao Z et al. Human endothelial cells synthesize and release ADAMTS-13. *J Thromb Haemost* 2006;4(6):1396–1404.
439. Cannon PJ, Hassar M, Case DB, et al. The relationship of hypertension and renal failure in scleroderma (progressive systemic sclerosis) to structural and functional abnormalities of the renal cortical circulation. *Medicine* 1974;53(1):1–46.
440. Helmbold P, Fiedler E, Fischer M, et al. Hyperplasia of dermal microvascular pericytes in scleroderma. *J Cutan Pathol* 2004;31(6):431–440.
441. George JN. The thrombotic thrombocytopenic purpura and hemolytic uremic syndromes: Evaluation, management, and long-term outcomes experience of the Oklahoma TTP-HUS Registry, 1989–2007. *Kidney Int Suppl* 2009;(112):S52–S54.
442. Levy GG, Nichols WC, Lian EC, et al. Mutations in a member of the ADAMTS gene family cause thrombotic thrombocytopenic purpura. *Nature* 2001;413(6855):488–494.
443. Zheng X, Chung D, Takayama TK, et al. Structure of von Willebrand factor-cleaving protease (ADAMTS13), a metalloprotease involved in thrombotic thrombocytopenic purpura. *J Biol Chem* 2001;276(44):41059–41063.
444. Padilla A, Moake JL, Bernardo A, et al. P-selectin anchors newly released ultralarge von Willebrand factor multimers to the endothelial cell surface. *Blood* 2004;103(6):2150–2156.
445. Arya M, Anvari B, Romo GM, et al. Ultralarge multimers of von Willebrand factor form spontaneous high-strength bonds with the platelet glycoprotein Ib-IX complex: studies using optical tweezers. *Blood* 2002;99(11):3971–3977.
446. Moake JL. Thrombotic microangiopathies. *N Engl J Med* 2002; 347(8):589–600.
447. Manetti M, Guiducci S, Ibba-Manneschi L, et al. Mechanisms in the loss of capillaries in systemic sclerosis: angiogenesis versus vasculogenesis. *J Cell Mol Med* 2010;14(6A):1241–1254.
448. Remuzzi G, Galbusera M, Noris M, et al. von Willebrand factor cleaving protease (ADAMTS13) is deficient in recurrent and familial thrombotic thrombocytopenic purpura and hemolytic uremic syndrome. *Blood* 2002;100(3):778–785.
449. Bianchi V, Robles R, Alberio L, et al. Von Willebrand factor-cleaving protease (ADAMTS13) in thrombocytopenic disorders: a severely deficient activity is specific for thrombotic thrombocytopenic purpura. *Blood* 2002;100(2):710–713.
450. Veyradier A, Obert B, Houllier A, et al. Specific von Willebrand factor-cleaving protease in thrombotic microangiopathies: a study of 111 cases. *Blood* 2001;98(6):1765–1772.
451. Veyradier A, Obert B, Haddad E, et al. Severe deficiency of the specific von Willebrand factor-cleaving protease (ADAMTS 13) activity in a subgroup of children with atypical hemolytic uremic syndrome. *J Pediatr* 2003;142(3):310–317.
452. Remuzzi G. Is ADAMTS-13 deficiency specific for thrombotic thrombocytopenic purpura? No. *J Thromb Haemost* 2003;1(4):632–634.
453. Studt JD, Kremer Hovinga JA, Alberio L, et al. Von Willebrand factor-cleaving protease (ADAMTS-13) activity in thrombotic microangiopathies: Diagnostic experience 2001/2002 of a single research laboratory. *Swiss Med Wkly* 2003;133(23–24):325–332.
454. Veyradier A, Girma JP. Assays of ADAMTS-13 activity. *Semin Hematol* 2004;41(1):41–47.
455. Veyradier A, Lavergne JM, Ribba AS, et al. Ten candidate ADAMTS13 mutations in six French families with congenital thrombotic thrombocytopenic purpura (Upshaw-Schulman syndrome). *J Thromb Haemost* 2004;2(3):424–429.
456. George JN, Terrell DR, Vesely SK, et al. Thrombotic microangiopathic syndromes associated with drugs, HIV infection, hematopoietic stem cell transplantation and cancer. *Presse Med* 2012;41(3 Pt 2): e177–e188.
457. Raife T, Atkinson B, Montgomery R, et al. Severe deficiency of VWF-cleaving protease (ADAMTS13) activity defines a distinct population of thrombotic microangiopathy patients. *Transfusion* 2004;44(2): 146–150.
458. Bettoni G, Palla R, Valsecchi C, et al. ADAMTS13 activity and autoantibodies classes and subclasses as prognostic predictors in acquired thrombotic thrombocytopenic purpura. *J Thromb Haemost* 2012;10(8):1556–1565.
459. Ruiz-Torres MP, Casiraghi F, Galbusera M, et al. Complement activation: The missing link between ADAMTS-13 deficiency and microvascular thrombosis of thrombotic microangiopathies. *Thromb Haemost* 2005;93(3):443–452.
460. Réti M, Farkas P, Csuka D, et al. Complement activation in thrombotic thrombocytopenic purpura. *J Thromb Haemost* 2012;10(5):791–798.

461. Karpman D, Manea M, Vaziri-Sani F, et al. Platelet activation in hemolytic uremic syndrome. *Semin Thromb Hemost* 2006;32(2):128–145.
462. Stahl AL, Svensson M, Morgelin M, et al. Lipopolysaccharide from enterohemorrhagic *Escherichia coli* binds to platelets through TLR4 and CD62 and is detected on circulating platelets in patients with hemolytic uremic syndrome. *Blood* 2006;108(1):167–176.
463. Licht C, Pluthero FG, Li L, et al. Platelet-associated complement factor H in healthy persons and patients with atypical HUS. *Blood* 2009;114(20):4538–4545.
464. Cambien B, Wagner DD. A new role in hemostasis for the adhesion receptor P-selectin. *Trends Mol Med* 2004;10(4):179–186.
465. Bergstein JM, Riley M, Bang NU. Role of plasminogen-activator inhibitor type 1 in the pathogenesis and outcome of the hemolytic uremic syndrome. *N Engl J Med* 1992;327(11):755–759.
466. Chandler WL, Jelacic S, Boster DR, et al. Prothrombotic coagulation abnormalities preceding the hemolytic-uremic syndrome. *N Engl J Med* 2002;346(1):23–32.
467. Xu Y, Hagege J, Mougenot B, et al. Different expression of the plasminogen activation system in renal thrombotic microangiopathy and the normal human kidney. *Kidney Int* 1996;50(6):2011–2019.
468. Kamitsuji H, Nonami K, Murakami T, et al. Elevated tissue factor circulating levels in children with hemolytic uremic syndrome caused by verotoxin-producing *E. coli*. *Clin Nephrol* 2000;53(5):319–324.
469. Stahl AL, Sartz L, Nelsson A, et al. Shiga toxin and lipopolysaccharide induce platelet-leukocyte aggregates and tissue factor release, a thrombotic mechanism in hemolytic uremic syndrome. *PLoS One* 2009;4(9):e6990.
470. Milford DV, Staten J, MacGreggor I, et al. Prognostic markers in diarrhoea-associated haemolytic-uraemic syndrome: initial neutrophil count, human neutrophil elastase and von Willebrand factor antigen. *Nephrol Dial Transplant* 1991;6(4):232–237.
471. Fitzpatrick MM, Shah V, Trompeter RS, et al. Interleukin-8 and polymorphoneutrophil leucocyte activation in hemolytic uremic syndrome of childhood. *Kidney Int* 1992;42(4):951–956.
472. Sakiri R, Ramegowda B, Tesh VL. Shiga toxin type 1 activates tumor necrosis factor-alpha gene transcription and nuclear translocation of the transcriptional activators nuclear factor-kappaB and activator protein-1. *Blood* 1998;92(2):558–566.
473. Ferraris V, Acquier A, Ferraris JR, et al. Oxidative stress status during the acute phase of haemolytic uraemic syndrome. *Nephrol Dial Transplant* 2011;26(3):858–864.
474. King AJ. Acute inflammation in the pathogenesis of hemolytic-uremic syndrome. *Kidney Int* 2002;61(4):1553–1564.
475. Ruiz-Irastorza G, Crowther M, Branch W, et al. Antiphospholipid syndrome. *Lancet* 2010;376(9751):1498–1509.
476. Miyakis S, Lockshin MD, Atsumi T, et al. International consensus statement on an update of the classification criteria for definite antiphospholipid syndrome (APS). *J Thromb Haemost* 2006;4(2):295–306.
477. Cervera R, Font J, Gomez-Puerta JA, et al. Validation of the preliminary criteria for the classification of catastrophic antiphospholipid syndrome. *Ann Rheum Dis* 2005;64(8):1205–1209.
478. Cervera R, Espinosa G. Update on the catastrophic antiphospholipid syndrome and the "CAPS Registry". *Semin Thromb Hemost* 2012;38(4):333–338.
479. Asherson RA, Cervera R, de Groot PG, et al. Catastrophic antiphospholipid syndrome: International consensus statement on classification criteria and treatment guidelines. *Lupus* 2003;12(7):530–534.
480. Fischer MJ, Rauch J, Levine JS. The antiphospholipid syndrome. *Semin Nephrol* 2007;27(1):35–46.
481. Tektonidou MG. Renal involvement in the antiphospholipid syndrome (APS)-APS nephropathy. *Clin Rev Allergy Immunol* 2009;36(2–3):131–140.
482. Fakhouri F, Noel LH, Zuber J, et al. The expanding spectrum of renal diseases associated with antiphospholipid syndrome. *Am J Kidney Dis* 2003;41(6):1205–1211.
483. Nochy D, Daugas E, Droz D, et al. The intrarenal vascular lesions associated with primary antiphospholipid syndrome. *J Am Soc Nephrol* 1999;10(3):507–518.
484. Asherson RA. New subsets of the antiphospholipid syndrome in 2006: "PRE-APS" (probable APS) and microangiopathic antiphospholipid syndromes ("MAPS"). *Autoimmun Rev* 2006;6(2):76–80.
485. Tektonidou MG, Sotsiou F, Moutsopoulos HM. Antiphospholipid syndrome (APS) nephropathy in catastrophic, primary, and systemic lupus erythematosus-related APS. *J Rheumatol* 2008;35(10):1983–1988.
486. Espinosa G, Bucciarelli S, Cervera R, et al. Thrombotic microangiopathic haemolytic anaemia and antiphospholipid antibodies. *Ann Rheum Dis* 2004;63(6):730–736.
487. Sinico RA, Cavazzana I, Nuzzo M, et al. Renal involvement in primary antiphospholipid syndrome: retrospective analysis of 160 patients. *Clin J Am Soc Nephrol* 2010;5(7):1211–1217.
488. Farrugia E, Torres VE, Gastineau D, et al. Lupus anticoagulant in systemic lupus erythematosus: a clinical and renal pathological study. *Am J Kidney Dis* 1992;20(5):463–471.
489. Bhandari S, Harnden P, Brownjohn AM, et al. Association of anticardiolipin antibodies with intraglomerular thrombi and renal dysfunction in lupus nephritis. *Q J Med* 1998;91(6):401–409.
490. Daugas E, Nochy D, Huong DL, et al. Antiphospholipid syndrome nephropathy in systemic lupus erythematosus. *J Am Soc Nephrol* 2002;13(1):42–52.
491. Tektonidou MG, Sotsiou F, Nakopoulou L, et al. Antiphospholipid syndrome nephropathy in patients with systemic lupus erythematosus and antiphospholipid antibodies: Prevalence, clinical associations, and long-term outcome. *Arthritis Rheum* 2004;50(8):2569–2579.
492. Moroni G, Ventura D, Riva P, et al. Antiphospholipid antibodies are associated with an increased risk for chronic renal insufficiency in patients with lupus nephritis. *Am J Kidney Dis* 2004;43(1):28–36.
493. Pierangeli SS, Chen PP, Raschi E, et al. Antiphospholipid antibodies and the antiphospholipid syndrome: pathogenic mechanisms. *Semin Thromb Hemost* 2008;34(3):236–250.
494. Yang CD, Hwang KK, Yan W, et al. Identification of anti-plasmin antibodies in the antiphospholipid syndrome that inhibit degradation of fibrin. *J Immunol* 2004;172(9):5765–5773.
495. Montiel-Manzano G, Romay-Penabad Z, Papalardo de Martinez E, et al. In vivo effects of an inhibitor of nuclear factor-kappa B on thrombogenic properties of antiphospholipid antibodies. *Ann N Y Acad Sci* 2007;1108:540–553.
496. Nojima J, Suehisa E, Kuratsune H, et al. Platelet activation induced by combined effects of anticardiolipin and lupus anticoagulant IgG antibodies in patients with systemic lupus erythematosus—possible association with thrombotic and thrombocytopenic complications. *Thromb Haemost* 1999;81(3):436–441.
497. Vega-Ostertag M, Casper K, Swerlick R, et al. Involvement of p38 MAPK in the up-regulation of tissue factor on endothelial cells by antiphospholipid antibodies. *Arthritis Rheum* 2005;52(5):1545–1554.
498. Pierangeli SS, Girardi G, Vega-Ostertag M, et al. Requirement of activation of complement C3 and C5 for antiphospholipid antibody-mediated thrombophilia. *Arthritis Rheum* 2005;52(7):2120–2124.
499. de Groot PG, Derksen RH. Pathophysiology of the antiphospholipid syndrome. *J Thromb Haemost* 2005;3(8):1854–1860.
500. Taylor FB, Jr., Tesh VL, DeBault L, et al. Characterization of the baboon responses to Shiga-like toxin: descriptive study of a new primate model of toxic responses to Stx-1. *Am J Pathol* 1999;154(4):1285–1299.
501. Siegler RL, Pysher TJ, Tesh VL, et al Response to single and divided doses of Shiga toxin-1 in a primate model of hemolytic uremic syndrome. *J Am Soc Nephrol* 2001;12(7):1458–1467.
502. Woods JB, Schmitt CK, Darnell SC, et al. Ferrets as a model system for renal disease secondary to intestinal infection with *Escherichia coli* O157:H7 and other Shiga toxin-producing *E. coli*. *J Infect Dis* 2002;185(4):550–554.
503. Raife T, Friedman KD, Fenwick B. Lepirudin prevents lethal effects of Shiga toxin in a canine model. *Thromb Haemost* 2004;92(2):387–393.
504. Gunzer F, Hennig-Pauka I, Waldmann KH, et al. Gnotobiotic piglets develop thrombotic microangiopathy after oral infection with enterohemorrhagic *Escherichia coli*. *Am J Clin Pathol* 2002;118(3):364–375.
505. Ikeda M, Ito S, Honda M. Hemolytic uremic syndrome induced by lipopolysaccharide and Shiga-like toxin. *Pediatr Nephrol* 2004;19(5):485–489.
506. Keepers TR, Psotka MA, Gross LK, et al. A murine model of HUS: shiga toxin with lipopolysaccharide mimics the renal damage and physiologic response of human disease. *J Am Soc Nephrol* 2006;17(12):3404–3414.

507. Motto DG, Chauhan AK, Zhu G, et al. Shigatoxin triggers thrombotic thrombocytopenic purpura in genetically susceptible ADAMTS13-deficient mice. *J Clin Invest* 2005;115(10):2752–2761.
508. Chauhan AK, Walsh MT, Zhu G, et al. The combined roles of ADAMTS13 and VWF in murine models of TTP, endotoxemia, and thrombosis. *Blood* 2008;111(7):3452–3457.
509. Gruppo RA, Rother RP. Eculizumab for congenital atypical hemolytic-uremic syndrome. *N Engl J Med* 2009;360(5):544–546.
510. Zuber J, Le Quintrec M, Krid S, et al. Eculizumab for atypical hemolytic uremic syndrome recurrence in renal transplantation. *Am J Transplant* 2012;12(12):3337–3354.
511. Lapeyraque AL, Malina M, Fremeaux-Bacchi V, et al. Eculizumab in severe Shiga-toxin-associated HUS. *N Engl J Med* 2011;364(26):2561–2563.
512. Walker JG, Pope J, Baron M, et al. The development of systemic sclerosis classification criteria. *Clin Rheumatol* 2007;26(9):1401–1409.
513. Hachulla E, Launay D. Diagnosis and classification of systemic sclerosis. *Clin Rev Allergy Immunol* 2011;40(2):78–83.
514. LeRoy EC, Medsger TA, Jr. Criteria for the classification of early systemic sclerosis. *J Rheumatol* 2001;28(7):1573–1576.
515. Poormoghim H, Lucas M, Fertig N, et al. Systemic sclerosis sine scleroderma: Demographic, clinical, and serologic features and survival in forty-eight patients. *Arthritis Rheum* 2000;43(2):444–451.
516. Iaccarino L, Gatto M, Bettio S, et al. Overlap connective tissue disease syndromes. *Autoimmun Rev* 2013;12(3):363-373.
517. Batal I, Domsic RT, Medsger TA, et al. Scleroderma renal crisis: A pathology perspective. *Int J Rheumatol* 2010;2010:543704.
518. Shanmugam VK, Steen VD. Renal disease in scleroderma: An update on evaluation, risk stratification, pathogenesis and management. *Curr Opin Rheumatol* 2012;24(6):669–676.
519. Helfrich DJ, Banner B, Steen VD, et al. Normotensive renal failure in systemic sclerosis. *Arthritis Rheum* 1989;32(9):1128–1134.
520. Cozzi F, Marson P, Cardarelli S, et al. Prognosis of scleroderma renal crisis: a long-term observational study. *Nephrol Dial Transplant* 2012;27(12):4398–4403.
521. Penn H, Howie AJ, Kingdon EJ, et al. Scleroderma renal crisis: Patient characteristics and long-term outcomes. *Q J Med* 2007;100(8): 485–494.
522. Furst DE, Fernandes AW, Iorga SR, et al. Epidemiology of systemic sclerosis in a large US managed care population. *J Rheumatol* 2012;39(4):784–786.
523. Barnes J, Mayes MD. Epidemiology of systemic sclerosis: incidence, prevalence, survival, risk factors, malignancy, and environmental triggers. *Curr Opin Rheumatol* 2012;24(2):165–170.
524. Panda S. Scleroderma in children: Emerging management issues. *Indian J Dermatol Venereol Leprol* 2010;76(4):348–356.
525. Chifflot H, Fautrel B, Sordet C, et al. Incidence and prevalence of systemic sclerosis: a systematic literature review. *Semin Arthritis Rheum* 2008;37(4):223–235.
526. Hoffmann-Vold AM, Midtvedt O, Molberg O, et al. Prevalence of systemic sclerosis in south-east Norway. *Rheumatology (Oxford)* 2012;51(9):1600–1605.
527. Johnson SR, Feldman BM, Hawker GA. Classification criteria for systemic sclerosis subsets. *J Rheumatol* 2007;34(9):1855–1863.
528. Scussel-Lonzetti L, Joyal F, Raynauld JP, et al. Predicting mortality in systemic sclerosis: Analysis of a cohort of 309 French Canadian patients with emphasis on features at diagnosis as predictive factors for survival. *Medicine* 2002;81(2):154–167.
529. Villalta D, Imbastaro T, Di Giovanni S, et al. Diagnostic accuracy and predictive value of extended autoantibody profile in systemic sclerosis. *Autoimmun Rev* 2012;12(2):114–120.
530. Grassegger A, Pohla-Gubo G, Frauscher M, et al. Autoantibodies in systemic sclerosis (scleroderma): clues for clinical evaluation, prognosis and pathogenesis. *Wien Med Wochenschr* 2008;158(1–2):19–28.
531. Graf SW, Hakendorf P, Lester S, et al. South Australian Scleroderma Register: autoantibodies as predictive biomarkers of phenotype and outcome. *Int J Rheum Dis* 2012;15(1):102–109.
532. Walker UA, Tyndall A, Czirjak L, et al. Clinical risk assessment of organ manifestations in systemic sclerosis: a report from the EULAR Scleroderma Trials And Research group database. *Ann Rheum Dis* 2007;66(6):754–763.
533. Della Rossa A, Valentini G, Bombardieri S, et al. European multicentre study to define disease activity criteria for systemic sclerosis. I. Clinical and epidemiological features of 290 patients from 19 centres. *Ann Rheum Dis* 2001;60(6):585–591.
534. Ferri C, Valentini G, Cozzi F, et al. Systemic sclerosis: Demographic, clinical, and serologic features and survival in 1,012 Italian patients. *Medicine* 2002;81(2):139–153.
535. Steen VD, Medsger TA, Jr. Severe organ involvement in systemic sclerosis with diffuse scleroderma. *Arthritis Rheum* 2000;43(11):2437–2444.
536. Teixeira L, Mouthon L, Mahr A, et al. Mortality and risk factors of scleroderma renal crisis: a French retrospective study of 50 patients. *Ann Rheum Dis* 2008;67(1):110–116.
537. Steen VD, Medsger TA, Jr, Osial TA, Jr, et al. Factors predicting development of renal involvement in progressive systemic sclerosis. *Am J Med* 1984;76(5):779–786.
538. Steen VD. Scleroderma renal crisis. *Rheum Dis Clin North Am* 2003;29(2):315–333.
539. Saeed F, Parke A. Normotensive scleroderma renal crisis in a patient with progressive systemic sclerosis: case report and review of literature. *Conn Med* 2011;75(2):83–87.
540. Seiberlich B, Hunzelmann N, Krieg T, et al. Intermediate molecular weight proteinuria and albuminuria identify scleroderma patients with increased morbidity. *Clin Nephrol* 2008;70(2):110–117.
541. Nikpour M, Hissaria P, Byron J, et al. Prevalence, correlates and clinical usefulness of antibodies to RNA polymerase III in systemic sclerosis: a cross-sectional analysis of data from an Australian cohort. *Arthritis Res Ther* 2011;13(6):R211.
542. Nguyen B, Assassi S, Arnett FC, et al. Association of RNA polymerase III antibodies with scleroderma renal crisis. *J Rheumatol* 2010;37(5):1068.
543. Hesselstrand R, Scheja A, Wuttge DM. Scleroderma renal crisis in a Swedish systemic sclerosis cohort: survival, renal outcome, and RNA polymerase III antibodies as a risk factor. *Scand J Rheumatol* 2012;41(1): 39–43.
544. Al-Dhaher FF, Pope JE, Ouimet JM. Determinants of morbidity and mortality of systemic sclerosis in Canada. *Semin Arthritis Rheum* 2010;39(4):269–277.
545. 545. Joven BE, Almodovar R, Carmona L, et al. Survival, causes of death, and risk factors associated with mortality in Spanish systemic sclerosis patients: results from a single university hospital. *Semin Arthritis Rheum* 2010;39(4):285–293.
546. Hashimoto A, Tejima S, Tono T, et al. Predictors of survival and causes of death in Japanese patients with systemic sclerosis. *J Rheumatol* 2011;38(9):1931–1939.
547. Sampaio-Barros PD, Bortoluzzo AB, Marangoni RG, et al. Survival, causes of death, and prognostic factors in systemic sclerosis: Analysis of 947 brazilian patients. *J Rheumatol* 2012;39(10):1971–1978.
548. Elhai M, Meune C, Avouac J, et al. Trends in mortality in patients with systemic sclerosis over 40 years: a systematic review and meta-analysis of cohort studies. *Rheumatology (Oxford)* 2012;51(6):1017–1026.
549. Batal I, Domsic RT, Shafer A, et al. Renal biopsy findings predicting outcome in scleroderma renal crisis. *Hum Pathol* 2009;40(3): 332–340.
550. Salyer WR, Salyer DC, Heptinstall RH. Scleroderma and microangiopathic hemolytic anemia. *Ann Intern Med* 1973;78(6):895–897.
551. Trostle DC, Bedetti CD, Steen VD, et al Renal vascular histology and morphometry in systemic sclerosis. A case–control autopsy study. *Arthritis Rheum* 1988;31(3):393–400.
552. Pardo V, Fisher ER, Perez-Stable E, et al. Ultrastructural studies in hypertension. II. Renal vascular changes in progressive systemic sclerosis. *Lab Invest* 1966;15(9):1434–1441.
553. Lapenas D, Rodnan GP, Cavallo T. Immunopathology of the renal vascular lesion of progressive systemic sclerosis (scleroderma). *Am J Pathol* 1978;91(2):243–258.
554. Sinclair RA, Antonovych TT, Mostofi FK. Renal proliferative arteriopathies and associated glomerular changes: a light and electron microscopic study. *Hum Pathol* 1976;7(5):565–588.
555. Silva FG, Pirani CL. Electron microscopic study of medical diseases of the kidney: Update–1988. *Mod Pathol* 1988;1(4):292–315.
556. Oliver JA, Cannon PJ. The kidney in scleroderma. *Nephron* 1977;18(3):141–150.

557. Volhard F, Fahr T. Die brightsche Nierenkrankheit. Berlin, Germany: Julius Springer, 1914.
558. Kovalchik MT, Guggenheim SJ, Silverman MH, et al. The kidney in progressive systemic sclerosis: a prospective study. *Ann Intern Med* 1978;89(6):881–887.
559. Akoglu H, Atilgan GK, Ozturk R, et al. A "silent" course of normotensive scleroderma renal crisis: case report and review of the literature. *Rheumatol Int* 2009;29(10):1223–1229.
560. Fleischmajer R, Gould AB. Serum renin and renin substrate levels in scleroderma. *Proc Soc Exp Biol Med* 1975;150(2):374–379.
561. Schmidt J, Launay D, Soudan B, et al. [Assessment of plasma endothelin level measurement in systemic sclerosis]. *Rev Med Intern* 2007;28(6):371–376.
562. Mouthon L, Mehrenberger M, Teixeira L, et al. Endothelin-1 expression in scleroderma renal crisis. *Hum Pathol* 2011;42(1):95–102.
563. Gabrielli A, Avvedimento EV, Krieg T. Scleroderma. *N Engl J Med* 2009;360(19):1989–2003.
564. Geyer M, Muller-Ladner U. The pathogenesis of systemic sclerosis revisited. *Clin Rev Allergy Immunol* 2011;40(2):92–103.
565. Ihn H. Scleroderma, fibroblasts, signaling, and excessive extracellular matrix. *Curr Rheumatol Rep* 2005;7(2):156–162.
566. Jimenez SA, Derk CT. Following the molecular pathways toward an understanding of the pathogenesis of systemic sclerosis. *Ann Intern Med* 2004;140(1):37–50.
567. Katsumoto TR, Whitfield ML, Connolly MK. The pathogenesis of systemic sclerosis. *Annu Rev Pathol* 2011;6:509–537.
568. Loubiere LS, Lambert NC, Madeleine MM, et al. HLA allelic variants encoding DR11 in diffuse and limited systemic sclerosis in Caucasian women. *Rheumatology (Oxford)* 2005;44(3):318–322.
569. Radstake TR, Gorlova O, Rueda B, et al. Genome-wide association study of systemic sclerosis identifies CD247 as a new susceptibility locus. *Nat Genet* 2010;42(5):426–429.
570. Nelson JL, Furst DE, Maloney S, et al. Microchimerism and HLA-compatible relationships of pregnancy in scleroderma. *Lancet* 1998;351(9102):559–562.
571. Artlett CM, Smith JB, Jimenez SA. Identification of fetal DNA and cells in skin lesions from women with systemic sclerosis. *N Engl J Med* 1998;338(17):1186–1191.
572. Arnett FC, Cho M, Chatterjee S, et al. Familial occurrence frequencies and relative risks for systemic sclerosis (scleroderma) in three United States cohorts. *Arthritis Rheum* 2001;44(6):1359–1362.
573. Kuwana M, Inoko H, Kameda H, et al. Association of human leukocyte antigen class II genes with autoantibody profiles, but not with disease susceptibility in Japanese patients with systemic sclerosis. *Intern Med* 1999;38(4):336–344.
574. Wang Y, Fan PS, Kahaleh B. Association between enhanced type I collagen expression and epigenetic repression of the FLI1 gene in scleroderma fibroblasts. *Arthritis Rheum* 2006;54(7):2271–2279.
575. De Santis M, Selmi C. The therapeutic potential of epigenetics in autoimmune diseases. *Clin Rev Allergy Immunol* 2012;42(1):92–101.
576. Prescott RJ, Freemont AJ, Jones CJ, et al. Sequential dermal microvascular and perivascular changes in the development of scleroderma. *J Pathol* 1992;166(3):255–263.
577. Sgonc R, Gruschwitz MS, Dietrich H, et al. Endothelial cell apoptosis is a primary pathogenetic event underlying skin lesions in avian and human scleroderma. *J Clin Invest* 1996;98(3):785–792.
578. Jun JB, Kuechle M, Harlan JM, et al. Fibroblast and endothelial apoptosis in systemic sclerosis. *Curr Opin Rheumatol* 2003;15(6):756–760.
579. Worda M, Sgonc R, Dietrich H, et al. In vivo analysis of the apoptosis-inducing effect of anti-endothelial cell antibodies in systemic sclerosis by the chorionallantoic membrane assay. *Arthritis Rheum* 2003;48(9):2605–2614.
580. Cerinic MM, Valentini G, Sorano GG, et al. Blood coagulation, fibrinolysis, and markers of endothelial dysfunction in systemic sclerosis. *Semin Arthritis Rheum* 2003;32(5):285–295.
581. Scheja A, Akesson A, Geborek P, et al. Von Willebrand factor propeptide as a marker of disease activity in systemic sclerosis (scleroderma). *Arthritis Res* 2001;3(3):178–182.
582. Sticherling M. The role of endothelin in connective tissue diseases. *Rheumatology (Oxford)* 2006;45(Suppl 3):8–10.
583. Kalin R, Righi A, Del Rosso A, et al. Activin, a grape seed-derived proanthocyanidin extract, reduces plasma levels of oxidative stress and adhesion molecules (ICAM-1, VCAM-1 and E-selectin) in systemic sclerosis. *Free Radic Res* 2002;36(8):819–825.
584. Laplante P, Raymond MA, Gagnon G, et al. Novel fibrogenic pathways are activated in response to endothelial apoptosis: Implications in the pathophysiology of systemic sclerosis. *J Immunol* 2005;174(9):5740–5749.
585. Venneker GT, van den Hoogen FH, Boerbooms AM, et al. Aberrant expression of membrane cofactor protein and decay-accelerating factor in the endothelium of patients with systemic sclerosis. A possible mechanism of vascular damage. *Lab Invest* 1994;70(6):830–835.
586. Maricq HR, LeRoy EC, D'Angelo WA, et al. Diagnostic potential of in vivo capillary microscopy in scleroderma and related disorders. *Arthritis Rheum* 1980;23(2):183–189.
587. Bollinger A, Jager K, Siegenthaler W. Microangiopathy of progressive systemic sclerosis. Evaluation by dynamic fluorescence videomicroscopy. *Arch Intern Med* 1986;146(8):1541–1545.
588. Szekanecz Z, Koch AE. Vascular involvement in rheumatic diseases: 'vascular rheumatology'. *Arthritis Res Ther* 2008;10(5):224.
589. Pauling JD, O'Donnell VB, McHugh NJ. The contribution of platelets to the pathogenesis of Raynaud's phenomenon and systemic sclerosis. *Platelets* 2013;24(7):503–515.
590. Ramirez GA, Franchini S, Rovere-Querini P, et al. The role of platelets in the pathogenesis of systemic sclerosis. *Front Immunol* 2012;3:160.
591. Fonseca C, Abraham D, Ponticos M. Neuronal regulators and vascular dysfunction in Raynaud's phenomenon and systemic sclerosis. *Curr Vasc Pharmacol* 2009;7(1):34–39.
592. Guiducci S, Fatini C, Rogai V, et al. Angiotensin-converting enzyme in systemic sclerosis: from endothelial injury to a genetic polymorphism. *Ann N Y Acad Sci* 2006;1069:10–19.
593. Quarto Di Palo F, Rivolta R, Berruti V, et al. Superior mesenteric artery blood flow in systemic sclerosis patients. *Rheumatology (Oxford)* 2002;41(7):730–734.
594. Rivolta R, Mascagni B, Berruti V, et al. Renal vascular damage in systemic sclerosis patients without clinical evidence of nephropathy. *Arthritis Rheum* 1996;39(6):1030–1034.
595. Distler O, Distler JH, Scheid A, et al. Uncontrolled expression of vascular endothelial growth factor and its receptors leads to insufficient skin angiogenesis in patients with systemic sclerosis. *Circ Res* 2004;95(1):109–116.
596. Kuwana M, Okazaki Y, Yasuoka H, et al. Defective vasculogenesis in systemic sclerosis. *Lancet* 2004;364(9434):603–610.
597. Cipriani P, Guiducci S, Miniati I, et al. Impairment of endothelial cell differentiation from bone marrow-derived mesenchymal stem cells: new insight into the pathogenesis of systemic sclerosis. *Arthritis Rheum* 2007;56(6):1994–2004.
598. Rajkumar VS, Sundberg C, Abraham DJ, et al. Activation of microvascular pericytes in autoimmune Raynaud's phenomenon and systemic sclerosis. *Arthritis Rheum* 1999;42(5):930–941.
599. Harris ML, Rosen A. Autoimmunity in scleroderma: the origin, pathogenetic role, and clinical significance of autoantibodies. *Curr Opin Rheumatol* 2003;15(6):778–784.
600. Hasegawa M, Fujimoto M, Takehara K, et al. Pathogenesis of systemic sclerosis: Altered B cell function is the key linking systemic autoimmunity and tissue fibrosis. *J Dermatol Sci* 2005;39(1):1–7.
601. Sato S, Fujimoto M, Hasegawa M, et al. Altered B lymphocyte function induces systemic autoimmunity in systemic sclerosis. *Mol Immunol* 2004;41(12):1123–1133.
602. Kalogerou A, Gelou E, Mountantonakis S, et al. Early T cell activation in the skin from patients with systemic sclerosis. *Ann Rheum Dis* 2005;64(8):1233–1235.
603a. Mavalia C, Scaletti C, Romagnani P, et al. Type 2 helper T-cell predominance and high CD30 expression in systemic sclerosis. *Am J Pathol* 1997;151(6):1751–1758.
603b. Meloni F, Solari N, Cavagna L, et al. Frequency of Th1, Th2 and Th17 producing T lymphocytes in bronchoalveolar lavage of patients with systemic sclerosis. *Clin Exp Rheumatol* 2009;27(5):765–772.
604. Hasegawa M. B lymphocytes: Shedding new light on the pathogenesis of systemic sclerosis. *J Dermatol* 2010;37(1):3–10.

605. Lafyatis R, York M. Innate immunity and inflammation in systemic sclerosis. *Curr Opin Rheumatol* 2009;21(6):617–622.
606. van Bon L, Cossu M, Radstake TR. An update on an immune system that goes awry in systemic sclerosis. *Curr Opin Rheumatol* 2011;23(6):505–510.
607. Bhattacharyya S, Wei J, Varga J. Understanding fibrosis in systemic sclerosis: Shifting paradigms, emerging opportunities. *Nat Rev Rheumatol* 2012;8(1):42–54.
608. Varga J, Bashey RI. Regulation of connective tissue synthesis in systemic sclerosis. *Int Rev Immunol* 1995;12(2–4):187–199.
609. Hinz B, Phan SH, Thannickal VJ, et al. Recent developments in myofibroblast biology: Paradigms for connective tissue remodeling. *Am J Pathol* 2012;180(4):1340–1355.
610. Whitfield ML, Finlay DR, Murray JI, et al. Systemic and cell type-specific gene expression patterns in scleroderma skin. *Proc Natl Acad Sci U S A* 2003;100(21):12319–12324.
611. Zhou X, Tan FK, Xiong M, et al. Monozygotic twins clinically discordant for scleroderma show concordance for fibroblast gene expression profiles. *Arthritis Rheum* 2005;52(10):3305–3314.
612. Zhou X, Tan FK, Xiong M, et al. Systemic sclerosis (scleroderma): Specific autoantigen genes are selectively overexpressed in scleroderma fibroblasts. *J Immunol* 2001;167(12):7126–7133.
613. Maurer B, Stanczyk J, Jungel A, et al. MicroRNA-29, a key regulator of collagen expression in systemic sclerosis. *Arthritis Rheum* 2010;62(6):1733–1743.
614. Moinzadeh P, Denton CP, Abraham D, et al. Biomarkers for skin involvement and fibrotic activity in scleroderma. *J Eur Acad Dermatol Venereol* 2012;26(3):267–276.
615. Chizzolini C. T lymphocyte and fibroblast interactions: the case of skin involvement in systemic sclerosis and other examples. *Springer Semin Immunopathol* 1999;21(4):431–450.
616. Gruber BL. Mast cells in the pathogenesis of fibrosis. *Curr Rheumatol Rep* 2003;5(2):147–153.
617. Varga J, Whitfield ML. Transforming growth factor-beta in systemic sclerosis (scleroderma). *Front Biosci (Schol Ed)* 2009;1:226–235.
618. Trojanowska M. Noncanonical transforming growth factor beta signaling in scleroderma fibrosis. *Curr Opin Rheumatol* 2009;21(6):623–629.
619. Daniels CE, Wilkes MC, Edens M, et al. Imatinib mesylate inhibits the profibrogenic activity of TGF-beta and prevents bleomycin-mediated lung fibrosis. *J Clin Invest* 2004;114(9):1308–1316.
620. Heldin CH, Westermark B. Mechanism of action and in vivo role of platelet-derived growth factor. *Physiol Rev* 1999;79(4):1283–1316.
621. Baroni SS, Santillo M, Bevilacqua F, et al. Stimulatory autoantibodies to the PDGF receptor in systemic sclerosis. *N Engl J Med* 2006;354(25):2667–2676.
622. Trojanowska M, Varga J. Molecular pathways as novel therapeutic targets in systemic sclerosis. *Curr Opin Rheumatol* 2007;19(6):568–573.
623. Wei J, Bhattacharyya S, Varga J. Peroxisome proliferator-activated receptor gamma: Innate protection from excessive fibrogenesis and potential therapeutic target in systemic sclerosis. *Curr Opin Rheumatol* 2010;22(6):671–676.
624. Beyer C, Schett G, Distler O, et al. Animal models of systemic sclerosis: Prospects and limitations. *Arthritis Rheum* 2010;62(10):2831–2844.
625. Christner PJ, Jimenez SA. Animal models of systemic sclerosis: Insights into systemic sclerosis pathogenesis and potential therapeutic approaches. *Curr Opin Rheumatol* 2004;16(6):746–752.
626. Gershwin ME, Abplanalp H, Castles JJ, et al. Characterization of a spontaneous disease of white leghorn chickens resembling progressive systemic sclerosis (scleroderma). *J Exp Med* 1981;153(6):1640–1659.
627. Maurer B, Busch N, Jungel A, et al. Transcription factor fos-related antigen-2 induces progressive peripheral vasculopathy in mice closely resembling human systemic sclerosis. *Circulation* 2009;120(23):2367–2376.
628. Sonnylal S, Denton CP, Zheng B, et al. Postnatal induction of transforming growth factor beta signaling in fibroblasts of mice recapitulates clinical, histologic, and biochemical features of scleroderma. *Arthritis Rheum* 2007;56(1):334–344.
629. Nguyen VA, Sgonc R, Dietrich H, et al. Endothelial injury in internal organs of University of California at Davis line 200 (UCD 200) chickens, an animal model for systemic sclerosis (scleroderma). *J Autoimmun* 2000;14(2):143–149.
630. Wagner EF, Eferl R. Fos/AP-1 proteins in bone and the immune system. *Immunol Rev* 2005;208:126–140.
631a. Redd BL, Jr. Radiation nephritis. Review, case report and animal study. *Am J Roentgenol Radium Ther Nucl Med* 1960;83:88–106.
631b. Mostofi FK. Radiation effects on the kidney. In: Mostofi FK, Smith DE, eds. *The kidney*. Baltimore, MD: Williams & Wilkins, p. 338. (International Academy of Pathology monograph no. 6.), 1966.
631c. Domagk G. Die Röntgenstrahlenwirkung auf das Gewebe, im besonderen betrachtet an den Nieren: Morphologische und funktionelle Veränderungen *Beitr Pathol Anat* 1927;77:525.
632. Zuelzer WW, Palmer HD, Newton WA, Jr. Unusual glomerulonephritis in young children probably radiation nephritis. *Am J Pathol* 1950;26(6):1019–1039.
633a. Luxton RW. Radiation nephritis. *Q J Med* 1953;22(86):215–242.
633b. Russell H. Renal sclerosis: "Postradiation nephritis" following upon irradiation of the upper abdomen. *Edinb Med J* 1953;60:474.
634. Luxton RW. Radiation nephritis. A long-term study of 54 patients. *Lancet* 1961;2(7214):1221–1224.
635. Cohen EP, Robbins ME. Radiation nephropathy. *Semin Nephrol* 2003;23(5):486–499.
636. Krochak RJ, Baker DG. Radiation nephritis. Clinical manifestations and pathophysiologic mechanisms. *Urology* 1986;27(5):389–393.
637. Glatstein E, Fajardo LF, Brown JM. Radiation injury in the mouse kidney—I. Sequential light microscopic study. *Int J Radiat Oncol Biol Phys* 1977;2(9–10):933–943.
638. Bergstein J, Andreoli SP, Provisor AJ, et al. Radiation nephritis following total-body irradiation and cyclophosphamide in preparation for bone marrow transplantation. *Transplantation* 1986;41(1):63–66.
639. Cogan MG, Arieff AI. Radiation nephritis and intravascular coagulation. *Clin Nephrol* 1978;10(2):74–78.
640. Steele BT, Lirenman DS. Acute radiation nephritis and the hemolytic uremic syndrome. *Clin Nephrol* 1979;11(5):272–274.
641. Jennette JC, Ordonez NG. Radiation nephritis causing nephrotic syndrome. *Urology* 1983;22(6):631–634.
642. Arneil GC, Harris F, Emmanuel IG, et al. Nephritis in two children after irradiation and chemotherapy for nephroblastoma. *Lancet* 1974;1(7864):960–963.
643. Stoffel MP, Pollok M, Fries J, et al. Radiation nephropathy after radiotherapy in metastatic medullary thyroid carcinoma. *Nephrol Dial Transplant* 2001;16(5):1082–1083.
644. Churchill DN, Hong K, Gault MH. Radiation nephritis following combined abdominal radiation and chemotherapy (bleomycin-vinblastine). *Cancer* 1978;41(6):2162–2164.
645. Dhaliwal RS, Adelman RD, Turner E, et al. Radiation nephritis with hypertension and hyperreninemia following chemotherapy: cure by nephrectomy. *J Pediatr* 1980;96(1):68–70.
646. Keane WF, Crosson JT, Staley NA, et al. Radiation-induced renal disease. A clinicopathologic study. *Am J Med* 1976;60(1):127–137.
647. Russell H. Renal sclerosis: "Postradiation nephritis" following upon irradiation of the upper abdomen. *Edinb Med J* 1953;60:474.
648. Bolliger A, Laidley JWS. Experimental renal disease produced by x-rays: Histological changes in the kidney exposed to a measured amount of unfiltered rays of medium wave length. *Med J Aust* 1930;1:136.
649. Madrazo A, Suzuki Y, Churg J. Radiation nephritis: acute changes following high dose of radiation. *Am J Pathol* 1969;54(3):507–527.
650. Madrazo A, Suzuki Y, Churg J. Radiation nephritis. II. Chronic changes after high doses of radiation. *Am J Pathol* 1970;61(1):37–56.
651. Madrazo A, Schwarz G, Churg J. Radiation nephritis: A review. *J Urol* 1975;114(6):822–827.
652. Moulder JE, Fish BL. Late toxicity of total body irradiation with bone marrow transplantation in a rat model. *Int J Radiat Oncol Biol Phys* 1989;16(6):1501–1509.
653. Cohen EP, Fish BL, Moulder JE. The renin-angiotensin system in experimental radiation nephropathy. *J Lab Clin Med* 2002;139(4):251–257.
654. Jaenke RS, Robbins ME, Bywaters T, et al. Capillary endothelium. Target site of renal radiation injury. *Lab Invest* 1993;68(4):396–405.
655. Robbins ME, Stephens LC, Thames HD, et al. Radiation response of the monkey kidney following contralateral nephrectomy. *Int J Radiat Oncol Biol Phys* 1994;30(2):347–354.

656. Stephens LC, Robbins ME, Johnston DA, et al. Radiation nephropathy in the rhesus monkey: Morphometric analysis of glomerular and tubular alterations. *Int J Radiat Oncol Biol Phys* 1995;31(4):865–873.
657. Cohen EP, Bonsib SA, Whitehouse E, et al. Mediators and mechanisms of radiation nephropathy. *Proc Soc Exp Biol Med* 2000;223(2): 218–225.
658. Madrazo AA, Churg J. Radiation nephritis. Chronic changes following moderate doses of radiation. *Lab Invest* 1976;34(3):283–290.
659. Rosen VJ, Cole LJ, Wachiel LW, et al. Ultrastructural studies of x-ray induced glomerular disease in rats subjected to uninephrectomy and food restriction. *Lab Invest* 1968;18(3):260–268.
660. Teixeira Vde P, Boim MA, Segreto HR, et al. Acute, subacute, and chronic x-ray effects on glomerular hemodynamics in rats. *Ren Fail* 1994;16(4):457–470.
661. Mostofi FK, Pani KC, Ericsson J. Effects of irradiation on canine kidney. *Am J Pathol* 1964;44:707–725.
662. Eisenbrandt DL, Phemister RD. Radiation injury in the neonatal canine kidney. I. Pathogenesis. *Lab Invest* 1977;37(5):437–446.
663. Eisenbrandt DL, Phemister RD. Radiation injury in the neonatal canine kidney. II. Quantitative morphology. *Lab Invest* 1978;38(3):225–231.
664. Jaenke RS, Phemister RD, Norrdin RW. Progressive glomerulosclerosis and renal failure following perinatal gamma radiation in the beagle. *Lab Invest* 1980;42(6):643–655.
665. Juncos LI, Cornejo JC, Gomes J, et al. Abnormal endothelium-dependent responses in early radiation nephropathy. *Hypertension* 1997;30(3 Pt 2):672–676.
666. Withers HR, Mason KA, Thames HD Jr. Late radiation response of kidney assayed by tubule-cell survival. *Br J Radiol* 1986;59(702):587–595.
667. Robbins ME, Soranson JA, Wilson GD, et al. Radiation-induced changes in the kinetics of glomerular and tubular cells in the pig kidney. *Radiat Res* 1994;138(1):107–113.
668. Robbins ME, Bonsib SM, Soranson JA, et al. Radiation-induced changes in glomerular and tubular cell kinetics and morphology following irradiation of a single kidney in the pig. *Int J Radiat Oncol Biol Phys* 1995;32(4):1071–1081.
669. Rossini M, Naito T, Yang H, et al. Sulodexide ameliorates early but not late kidney disease in models of radiation nephropathy and diabetic nephropathy. *Nephrol Dial Transplant* 2010;25(6):1803–1810.
670. Scharpfenecker M, Floot B, Russell NS, et al. The TGF-beta co-receptor endoglin regulates macrophage infiltration and cytokine production in the irradiated mouse kidney. *Radiother Oncol* 2012;105(3):313–320.
671. Kruse JJ, Floot BG, te Poele JA, et al. Radiation-induced activation of TGF-beta signaling pathways in relation to vascular damage in mouse kidneys. *Radiat Res* 2009;171(2):188–197.
672. Liu DG, Wang TM. Role of connective tissue growth factor in experimental radiation nephropathy in rats. *Chin Med J* 2008;121(19):1925–1931.
673. Zhao W, Robbins ME. Inflammation and chronic oxidative stress in radiation-induced late normal tissue injury: therapeutic implications. *Curr Med Chem* 2009;16(2):130–143.
674. Zhao W, O'Malley Y, Wei S, et al. Irradiation of rat tubule epithelial cells alters the expression of gene products associated with the synthesis and degradation of extracellular matrix. *Int J Radiat Biol* 2000;76(3):391–402.
675. Zhao W, Spitz DR, Oberley LW, et al. Redox modulation of the profibrogenic mediator plasminogen activator inhibitor-1 following ionizing radiation. *Cancer Res* 2001;61(14):5537–5543.
676. Robbins ME, O'Malley Y, Zhao W, et al. The role of the tubulointerstitium in radiation-induced renal fibrosis. *Radiat Res* 2001;155(3):481–489.
677. Robbins ME, Zhao W, Davis CS, et al. Radiation-induced kidney injury: A role for chronic oxidative stress? *Micron* 2002;33(2):133–141.
678. Lenarczyk M, Cohen EP, Fish BL, et al. Chronic oxidative stress as a mechanism for radiation nephropathy. *Radiat Res* 2009;171(2):164–172.
679. Sieber F, Muir SA, Cohen EP, et al. High-dose selenium for the mitigation of radiation injury: a pilot study in a rat model. *Radiat Res* 2009;171(3):368–373.
680. Sieber F, Muir SA, Cohen EP, et al. Dietary selenium for the mitigation of radiation injury: effects of selenium dose escalation and timing of supplementation. *Radiat Res* 2011;176(3):366–374.
681. Adams MJ, Grant EJ, Kodama K, et al. Radiation dose associated with renal failure mortality: a potential pathway to partially explain increased cardiovascular disease mortality observed after whole-body irradiation. *Radiat Res* 2012;177(2):220–228.
682. Hoffmeister PA, Hingorani SR, Storer BE, et al. Hypertension in long-term survivors of pediatric hematopoietic cell transplantation. *Biol Blood Marrow Transplant* 2010;16(4):515–524.
683. Kal HB, van Kempen-Harteveld ML. Renal dysfunction after total body irradiation: Dose-effect relationship. *Int J Radiat Oncol Biol Phys* 2006;65(4):1228–1232.
684. Dean AL, Abels JC. Study by the newer renal function tests of an unusual case of hypertension following irradiation of one kidney and the relief of the patient by nephrectomy. *J Urol* 1944;52:497.
685. Shearer DH, Hendee WR. Roentgenography. In: Putnam CE, Ravin CE, eds. *Textbook of Diagnostic Imaging*. Philadelphia: WB Saunders, 1988:24.
686. Cogan SR, Ritter, II. Radiation nephritis; a clinicopathologic correlation of three surviving cases. *Am J Med* 1958;24(4):530–534.
687. Bloomfield DK, Schneider DH, Vertes V. Renin and angiotensin II studies in malignant hypertension after x-irradiation for seminoma. *Ann Intern Med* 1968;68(1):146–151.
688. Kapur S, Chandra R, Antonovych T. Acute radiation nephritis. Light and electron microscopic observations. *Arch Pathol Lab Med* 1977;101(9):469–473.
689. Colquhoun J. Hypoplasia of the abdominal aorta following therapeutic irradiation in infancy. *Radiology* 1966;86(3):454–456.
690. Gerlock AJ, Jr., Goncharenko VA, Ekelund L. Radiation-induced stenosis of the renal artery causing hypertension: case report. *J Urol* 1977;118(6):1064–1065.
691. McGill CW, Holder TM, Smith TH, et al. Postradiation renovascular hypertension. *J Pediatr Surg* 1979;14(6):831–833.
692. Staab GE, Tegtmeyer CJ, Constable WC. Radiation-induced renovascular hypertension. *AJR Am J Roentgenol* 1976;126(3):634–637.
693. Wilson C, Ledingham JM, Cohen M. Hypertension following x-irradiation of the kidneys. *Lancet* 1958;1(7010):9–16.
694. Fisher ER, Hellstrom HR. Pathogenesis of hypertension and pathologic changes in experimental renal irradiation. *Lab Invest* 1968;19(5):530–538.
695. Asscher AW, Wilson C, Anson SG. Sensitisation of blood-vessels to hypertensive damage by x-irradiation. *Lancet* 1961;1(7177):580–583.
696. Byrom FB. The pathogenesis of hypertensive encephalopathy and its relation to the malignant phase of hypertension; experimental evidence from the hypertensive rat. *Lancet* 1954;267(6831):201–211.
697. Moulder JE, Cohen EP, Fish BL. Captopril and losartan for mitigation of renal injury caused by single-dose total-body irradiation. *Radiat Res* 2011;175(1):29–36.
698. Cohen EP, Fish BL, Moulder JE. Mitigation of radiation injuries via suppression of the renin-angiotensin system: emphasis on radiation nephropathy. *Curr Drug Targets* 2010;11(11):1423–1429.
699. Cohen EP, Irving AA, Drobyski WR, et al. Captopril to mitigate chronic renal failure after hematopoietic stem cell transplantation: a randomized controlled trial. *Int J Radiat Oncol Biol Phys* 2008;70(5):1546–1551.
700. Moulder JE, Fish BL, Cohen EP. Treatment of radiation nephropathy with ACE inhibitors and AII type-1 and type-2 receptor antagonists. *Curr Pharm Des* 2007;13(13):1317–1325.
701. Juncos LI, Carrasco Duenas S, Cornejo JC, et al. Long-term enalapril and hydrochlorothiazide in radiation nephritis. *Nephron* 1993;64(2):249–255.
702. Juncos L, Cornejo JC, Cejas H, et al. Mechanisms of hypertension in renal radiation. *Hypertension* 1990;15(2 Suppl):I132-I136.
703. Shapiro AP, Cavallo T, Cooper W, et al. Hypertension in radiation nephritis. Report of a patient with unilateral disease, elevated renin activity levels, and reversal after unilateral nephrectomy. *Arch Intern Med* 1977;137(7):848–851.
704. Verheij M, Dewit LG, Valdes Olmos RA, et al. Evidence for a renovascular component in hypertensive patients with late radiation nephropathy. *Int J Radiat Oncol Biol Phys* 1994;30(3):677–683.
705. Paterson RR. Renal damage from radiation during treatment of seminoma testis. *J Fac Radiol* 1952;3:270.
706. Kunkler PB, Farr RF, Luxton RW. The limit of renal tolerance to x-rays; an investigation into renal damage occurring following the treatment of tumours of the testis by abdominal baths. *Br J Radiol* 1952;25(292):192–201.
707. Dawson LA, Kavanagh BD, Paulino AC, et al. Radiation-associated kidney injury. *Int J Radiat Oncol Biol Phys* 2010;76(3 Suppl):S108-S115.

708. Cheng JC, Schultheiss TE, Wong JY. Impact of drug therapy, radiation dose, and dose rate on renal toxicity following bone marrow transplantation. *Int J Radiat Oncol Biol Phys* 2008;71(5):1436–1443.
709. Emami B, Lyman J, Brown A, et al. Tolerance of normal tissue to therapeutic irradiation. *Int J Radiat Oncol Biol Phys* 1991;21(1):109–122.
710. Cassady JR. Clinical radiation nephropathy. *Int J Radiat Oncol Biol Phys* 1995;31(5):1249–1256.
711. Jansen EP, Saunders MP, Boot H, et al. Prospective study on late renal toxicity following postoperative chemoradiotherapy in gastric cancer. *Int J Radiat Oncol Biol Phys* 2007;67(3):781–785.
712. Peschel RE, Chen M, Seashore J. The treatment of massive hepatomegaly in stage IV-S neuroblastoma. *Int J Radiat Oncol Biol Phys* 1981;7(4):549–553.
713. Esiashvili N, Chiang KY, Hasselle MD, et al. Renal toxicity in children undergoing total body irradiation for bone marrow transplant. *Radiother Oncol* 2009;90(2):242–246.
714. Mitus A, Tefft M, Fellers FX. Long-term follow-up of renal functions of 108 children who underwent nephrectomy for malignant disease. *Pediatrics* 1969;44(6):912–921.
715. Bolling T, Ernst I, Pape H, et al. Dose-volume analysis of radiation nephropathy in children: Preliminary report of the risk consortium. *Int J Radiat Oncol Biol Phys* 2011;80(3):840–844.
716. Dewit L, Anninga JK, Hoefnagel CA, et al. Radiation injury in the human kidney: A prospective analysis using specific scintigraphic and biochemical endpoints. *Int J Radiat Oncol Biol Phys* 1990;19(4):977–983.
717. Thompson PL, Mackay IR, Robson GS, et al. Late radiation nephritis after gastric x-irradiation for peptic ulcer. *Q J Med* 1971;40(157):145–157.
718. Lawton CA, Fish BL, Moulder JE. Effect of nephrotoxic drugs on the development of radiation nephropathy after bone marrow transplantation. *Int J Radiat Oncol Biol Phys* 1994;28(4):883–889.
719. Moulder JE, Fish BL. Influence of nephrotoxic drugs on the late renal toxicity associated with bone marrow transplant conditioning regimens. *Int J Radiat Oncol Biol Phys* 1991;20(2):333–337.
720. Sullivan MP, Takahashi Y. Incidence of abnormal urinary findings in children exposed to the atomic bomb in Hiroshima. *Pediatrics* 1957;19(4 Part 1):607–613.
721. Ozasa K, Shimizu Y, Suyama A, et al. Studies of the mortality of atomic bomb survivors, Report 14, 1950–2003: an overview of cancer and noncancer diseases. *Radiat Res* 2012;177(3):229–243.
722. Konchalovsky MV, Baranov AE, Kolganov AV. Multiple organ involvement and failure: selected Russian radiation accident cases re-visited. *Br J Radiol Suppl* 2005;27:26–29.
723. Gruss E, Bernis C, Tomas JF, et al. Acute renal failure in patients following bone marrow transplantation: prevalence, risk factors and outcome. *Am J Nephrol* 1995;15(6):473–479.
724. Zager RA. Acute renal failure in the setting of bone marrow transplantation. *Kidney Int* 1994;46(5):1443–1458.
725. Chappell ME, Keeling DM, Prentice HG, et al. Haemolytic uraemic syndrome after bone marrow transplantation: an adverse effect of total body irradiation? *Bone Marrow Transplant* 1988;3(4):339–347.
726. Cohen EP, Lawton CA, Moulder JE. Bone marrow transplant nephropathy: radiation nephritis revisited. *Nephron* 1995;70(2):217–222.
727. Cohen EP, Lawton CA, Moulder JE, et al. Clinical course of late-onset bone marrow transplant nephropathy. *Nephron* 1993;64(4):626–635.
728. George JN, Li X, McMinn JR, et al. Thrombotic thrombocytopenic purpura-hemolytic uremic syndrome following allogeneic HPC transplantation: A diagnostic dilemma. *Transfusion* 2004;44(2):294–304.
729. Lawton CA, Cohen EP, Barber-Derus SW, et al. Late renal dysfunction in adult survivors of bone marrow transplantation. *Cancer* 1991;67(11):2795–2800.
730. Leblond V, Sutton L, Jacquiaud C, et al. Evaluation of renal function in 60 long-term survivors of bone marrow transplantation. *J Am Soc Nephrol* 1995;6(6):1661–1665.
731. Verburgh CA, Vermeij CG, Zijlmans JM, et al. Haemolytic uraemic syndrome following bone marrow transplantation: case report and review of the literature. *Nephrol Dial Transplant* 1996;11(7):1332–1337.
732. Hingorani S. Chronic kidney disease in long-term survivors of hematopoietic cell transplantation: epidemiology, pathogenesis, and treatment. *J Am Soc Nephrol* 2006;17(7):1995–2005.
733. Ho VT, Cutler C, Carter S, et al. Blood and marrow transplant clinical trials network toxicity committee consensus summary: thrombotic microangiopathy after hematopoietic stem cell transplantation. *Biol Blood Marrow Transplant* 2005;11(8):571–575.
734. Ruutu T, Barosi G, Benjamin RJ, et al. Diagnostic criteria for hematopoietic stem cell transplant-associated microangiopathy: results of a consensus process by an International Working Group. *Haematologica* 2007;92(1):95–100.
735. Changsirikulchai S, Myerson D, Guthrie KA, et al. Renal thrombotic microangiopathy after hematopoietic cell transplant: role of GVHD in pathogenesis. *Clin J Am Soc Nephrol* 2009;4(2):345–353.
736. Raife TJ, Lager DJ. Chronic thrombotic microangiopathy associated with antineoplastic therapy with minimal hematologic effects. *Mayo Clin Proc* 2002;77(4):323–328.
737. Troxell ML, Pilapil M, Miklos DB, et al. Renal pathology in hematopoietic cell transplantation recipients. *Mod Pathol* 2008;21(4):396–406.
738. Siami K, Kojouri K, Swisher KK, et al. Thrombotic microangiopathy after allogeneic hematopoietic stem cell transplantation: an autopsy study. *Transplantation* 2008;85(1):22–28.
739. Chang A, Hingorani S, Kowalewska J, et al. Spectrum of renal pathology in hematopoietic cell transplantation: A series of 20 patients and review of the literature. *Clin J Am Soc Nephrol* 2007;2(5):1014–1023.
740. El-Seisi S, Gupta R, Clase CM, et al. Renal pathology at autopsy in patients who died after hematopoietic stem cell transplantation. *Biol Blood Marrow Transplant* 2003;9(11):683–688.
741. Iskandar SS, Browning MC, Lorentz WB. Mesangiolytic glomerulopathy in a bone marrow allograft recipient. *Hum Pathol* 1989;20(3):290–292.
742. Antignac C, Gubler MC, Leverger G, et al. Delayed renal failure with extensive mesangiolysis following bone marrow transplantation. *Kidney Int* 1989;35(6):1336–1344.
743. Laskin BL, Goebel J, Davies SM, et al. Small vessels, big trouble in the kidneys and beyond: Hematopoietic stem cell transplantation-associated thrombotic microangiopathy. *Blood* 2011;118(6):1452–1462.
744. Troxell ML, Norman D, Mittalhenkle A. Glomerular fibrin thrombi in ABO and crossmatch compatible renal allograft biopsies. *Pathol Res Pract* 2011;207(1):15–23.
745. Glezerman IG, Jhaveri KD, Watson TH, et al. Chronic kidney disease, thrombotic microangiopathy, and hypertension following T cell-depleted hematopoietic stem cell transplantation. *Biol Blood Marrow Transplant* 2010;16(7):976–984.
746. Igaki H, Karasawa K, Sakamaki H, et al. Renal dysfunction after total-body irradiation. Significance of selective renal shielding blocks. *Strahlenther Onkol* 2005;181(11):704–708.
747. Lawton CA, Barber-Derus SW, Murray KJ, et al. Influence of renal shielding on the incidence of late renal dysfunction associated with T-lymphocyte deplete bone marrow transplantation in adult patients. *Int J Radiat Oncol Biol Phys* 1992;23(3):681–686.
748. Borg M, Hughes T, Horvath N, et al. Renal toxicity after total body irradiation. *Int J Radiat Oncol Biol Phys* 2002;54(4):1165–1173.
749. Kal HB, VAN Kempen-Harteveld ML. Induction of severe cataract and late renal dysfunction following total body irradiation: Dose-effect relationships. *Anticancer Res* 2009;29(8):3305–3309.
750. Elliott MA, Nichols WL, Jr, Plumhoff EA, et al. Posttransplantation thrombotic thrombocytopenic purpura: A single-center experience and a contemporary review. *Mayo Clin Proc* 2003;78(4):421–430.
751. Zeigler ZR, Rosenfeld CS, Andrews DF III, et al. Plasma von Willebrand factor antigen (vWF:AG) and thrombomodulin (TM) levels in adult thrombotic thrombocytopenic purpura/hemolytic uremic syndromes (TTP/HUS) and bone marrow transplant-associated thrombotic microangiopathy (BMT-TM). *Am J Hematol* 1996;53(4):213–220.
752. Daly AS, Xenocostas A, Lipton JH. Transplantation-associated thrombotic microangiopathy: twenty-two years later. *Bone Marrow Transplant* 2002;30(11):709–715.
753. Cutler C, Henry NL, Magee C, et al. Sirolimus and thrombotic microangiopathy after allogeneic hematopoietic stem cell transplantation. *Biol Blood Marrow Transplant* 2005;11(7):551–557.
754. Mii A, Shimizu A, Masuda Y, et al. Renal thrombotic microangiopathy associated with chronic humoral graft versus host disease after hematopoietic stem cell transplantation. *Pathol Int* 2011;61(1):34–41.

755. Mii A, Shimizu A, Kaneko T, et al. Renal thrombotic microangiopathy associated with chronic graft-versus-host disease after allogeneic hematopoietic stem cell transplantation. *Pathol Int* 2011;61(9):518–527.
756. Min CK, Kim SY, Lee MJ, et al. Vascular endothelial growth factor (VEGF) is associated with reduced severity of acute graft-versus-host disease and nonrelapse mortality after allogeneic stem cell transplantation. *Bone Marrow Transplant* 2006;38(2):149–156.
757. Down JD, Berman AJ, Warhol M, et al. Late complications following total-body irradiation and bone marrow rescue in mice: Predominance of glomerular nephropathy and hemolytic anemia. *Int J Radiat Biol* 1990;57(3):551–565.
758. Safwat A, Nielsen OS, el-Badawy S, et al. Late renal damage after total body irradiation and bone marrow transplantation in a mouse model: effect of radiation fractionation. *Eur J Cancer* 1995;31A(6):987–992.
759. Sarode R, McFarland JG, Flomenberg N, et al. Therapeutic plasma exchange does not appear to be effective in the management of thrombotic thrombocytopenic purpura/hemolytic uremic syndrome following bone marrow transplantation. *Bone Marrow Transplant* 1995;16(2): 271–275.
760. van der Plas RM, Schiphorst ME, Huizinga EG, et al. von Willebrand factor proteolysis is deficient in classic, but not in bone marrow transplantation-associated, thrombotic thrombocytopenic purpura. *Blood* 1999;93(11):3798–3802.
761. Moulder JE, Fish BL, Cohen EP. ACE inhibitors and AII receptor antagonists in the treatment and prevention of bone marrow transplant nephropathy. *Curr Pharm Des* 2003;9(9):737–749.
762. Cohen EP, Bedi M, Irving AA, et al. Mitigation of late renal and pulmonary injury after hematopoietic stem cell transplantation. *Int J Radiat Oncol Biol Phys* 2012;83(1):292–296.
763. Robbins ME, Diz DI. Pathogenic role of the renin-angiotensin system in modulating radiation-induced late effects. *Int J Radiat Oncol Biol Phys* 2006;64(1):6–12.
764. Cosar R, Yurut-Caloglu V, Eskiocak S, et al. Radiation-induced chronic oxidative renal damage can be reduced by amifostine. *Med Oncol* 2012;29(2):768–775.
765. Geraci JP, Sun MC, Mariano MS. Amelioration of radiation nephropathy in rats by postirradiation treatment with dexamethasone and/or captopril. *Radiat Res* 1995;143(1):58–68.
766. Lambert B, Cybulla M, Weiner SM, et al. Renal toxicity after radionuclide therapy. *Radiat Res* 2004;161(5):607–611.
767. Vegt E, de Jong M, Wetzels JF, et al. Renal toxicity of radiolabeled peptides and antibody fragments: mechanisms, impact on radionuclide therapy, and strategies for prevention. *J Nucl Med* 2010;51(7):1049–1058.
768. van Essen M, Krenning EP, Kam BL, et al. Peptide-receptor radionuclide therapy for endocrine tumors. *Nat Rev Endocrinol* 2009;5(7):382–393.
769. de Jong M, Kwekkeboom D, Valkema R, et al. Radiolabelled peptides for tumour therapy: current status and future directions. Plenary lecture at the EANM 2002. *Eur J Nucl Med Mol Imaging* 2003;30(3):463–469.
770. Reubi JC, Schar JC, Waser B, et al. Affinity profiles for human somatostatin receptor subtypes SST1-SST5 of somatostatin radiotracers selected for scintigraphic and radiotherapeutic use. *Eur J Nucl Med* 2000;27(3):273–282.
771. Kwekkeboom DJ, Bakker WH, Kooij PP, et al. [177Lu-DOTAOTyr3] octreotate: Comparison with [111In-DTPAo]octreotide in patients. *Eur J Nucl Med* 2001;28(9):1319–1325.
772. Moll S, Nickeleit V, Mueller-Brand J, et al. A new cause of renal thrombotic microangiopathy: Yttrium 90-DOTATOC internal radiotherapy. *Am J Kidney Dis* 2001;37(4):847–851.
773. Otte A, Herrmann R, Heppeler A, et al. Yttrium-90 DOTATOC: First clinical results. *Eur J Nucl Med* 1999;26(11):1439–1447.
774. Barone R, Borson-Chazot F, Valkema R, et al. Patient-specific dosimetry in predicting renal toxicity with (90)Y-DOTATOC: relevance of kidney volume and dose rate in finding a dose-effect relationship. *J Nucl Med* 2005;46(Suppl 1):99S–106S.
775. Virgolini I, Britton K, Buscombe J, et al. In- and Y-DOTA-lanreotide: Results and implications of the MAURITIUS trial. *Semin Nucl Med* 2002;32(2):148–155.
776. Bodei L, Cremonesi M, Zoboli S, et al. Receptor-mediated radionuclide therapy with 90Y-DOTATOC in association with amino acid infusion: A phase I study. *Eur J Nucl Med Mol Imaging* 2003;30(2):207–216.
777. Valkema R, De Jong M, Bakker WH, et al. Phase I study of peptide receptor radionuclide therapy with [In-DTPA]octreotide: the Rotterdam experience. *Semin Nucl Med* 2002;32(2):110–122.
778. Goldenberg DM. Targeted therapy of cancer with radiolabeled antibodies. *J Nucl Med* 2002;43(5):693–713.
779. Tempero M, Leichner P, Baranowska-Kortylewicz J, et al. High-dose therapy with 90Yttrium-labeled monoclonal antibody CC49: a phase I trial. *Clin Cancer Res* 2000;6(8):3095–3102.
780. Witzig TE, White CA, Gordon LI, et al. Safety of yttrium-90 ibritumomab tiuxetan radioimmunotherapy for relapsed low-grade, follicular, or transformed non-hodgkin's lymphoma. *J Clin Oncol* 2003;21(7):1263–1270.
781. Bunjes D, Buchmann I, Duncker C, et al. Rhenium 188-labeled anti-CD66 (a, b, c, e) monoclonal antibody to intensify the conditioning regimen prior to stem cell transplantation for patients with high-risk acute myeloid leukemia or myelodysplastic syndrome: results of a phase I-II study. *Blood* 2001;98(3):565–572.
782. Maack T, Johnson V, Kau ST, et al. Renal filtration, transport, and metabolism of low-molecular-weight proteins: a review. *Kidney Int* 1979;16(3):251–270.
783. Trejtnar F, Novy Z, Petrik M, et al. In vitro comparison of renal handling and uptake of two somatostatin receptor-specific peptides labeled with indium-111. *Ann Nucl Med* 2008;22(10):859–867.
784. Van Binnebeek S, Baete K, Terwinghe C, et al. Significant impact of transient deterioration of renal function on dosimetry in PRRT. *Ann Nucl Med* 2013;27:74–77.
785. Kurtzman SH, Russo A, Mitchell JB, et al. 212Bismuth linked to an antipancreatic carcinoma antibody: Model for alpha-particle-emitter radioimmunotherapy. *J Natl Cancer Inst* 1988;80(6):449–452.
786. Akizawa H, Arano Y, Mifune M, et al. Effect of molecular charges on renal uptake of 111In-DTPA-conjugated peptides. *Nucl Med Biol* 2001;28(7):761–768.
787. Ekblad T, Tran T, Orlova A, et al. Development and preclinical characterisation of 99mTc-labelled Affibody molecules with reduced renal uptake. *Eur J Nucl Med Mol Imaging* 2008;35(12):2245–2255.
788. Garcia Garayoa E, Schweinsberg C, Maes V, et al. Influence of the molecular charge on the biodistribution of bombesin analogues labeled with the [99mTc(CO)3]-core. *Bioconjug Chem* 2008;19(12):2409–2416.
789. Chen X, Park R, Shahinian AH, et al. Pharmacokinetics and tumor retention of 125I-labeled RGD peptide are improved by PEGylation. *Nucl Med Biol* 2004;31(1):11–19.
790. Chinol M, Bodei L, Cremonesi M, et al. Receptor-mediated radiotherapy with Y-DOTA-DPhe-Tyr-octreotide: the experience of the European Institute of Oncology Group. *Semin Nucl Med* 2002;32(2):141–147.
791. Kwekkeboom DJ, Bakker WH, Kam BL, et al. Treatment of patients with gastro-entero-pancreatic (GEP) tumours with the novel radiolabelled somatostatin analogue [177Lu-DOTA(0),Tyr3]octreotate. *Eur J Nucl Med Mol Imaging* 2003;30(3):417–422.
792. Behr TM, Goldenberg DM, Becker W. Reducing the renal uptake of radiolabeled antibody fragments and peptides for diagnosis and therapy: Present status, future prospects and limitations. *Eur J Nucl Med* 1998;25(2):201–212.
793. de Jong M, Rolleman EJ, Bernard BF, et al. Inhibition of renal uptake of indium-111-DTPA-octreotide in vivo. *J Nucl Med* 1996;37(8):1388–1392.
794. Rolleman EJ, Valkema R, de Jong M, et al. Safe and effective inhibition of renal uptake of radiolabelled octreotide by a combination of lysine and arginine. *Eur J Nucl Med Mol Imaging* 2003;30(1):9–15.
795. Waldherr C, Pless M, Maecke HR, et al. Tumor response and clinical benefit in neuroendocrine tumors after 7.4 GBq (90)Y-DOTATOC. *J Nucl Med* 2002;43(5):610–616.
796. Valkema R, Pauwels SA, Kvols LK, et al. Long-term follow-up of renal function after peptide receptor radiation therapy with (90)Y-DOTA(0),Tyr(3)-octreotide and (177)Lu-DOTA(0), Tyr(3)-octreotate. *J Nucl Med* 2005; 46 Suppl 1:83S-91S.
797. Stahl AR, Wagner B, Poethko T, et al. Renal accumulation of [111In] DOTATOC in rats: influence of inhibitors of the organic ion transport and diuretics. *Eur J Nucl Med Mol Imaging* 2007;34(12):2129–2134.
798. Rolleman EJ, Krenning EP, Bernard BF, et al. Long-term toxicity of [(177)Lu-DOTA (0),Tyr (3)]octreotate in rats. *Eur J Nucl Med Mol Imaging* 2007;34(2):219–227.
799. Gotthardt M, van Eerd-Vismale J, Oyen WJ, et al. Indication for different mechanisms of kidney uptake of radiolabeled peptides. *J Nucl Med* 2007;48(4):596–601.
800. Krenning EP, Kwekkeboom DJ, Valkema R, et al. Peptide receptor radionuclide therapy. *Ann N Y Acad Sci* 2004;1014:234–245.