SECTION 3

The patient with glomerular disease

CHAPTER 42

The glomerulus and the concept of glomerulonephritis

Alexander Woywodt and Diana Chiu

Introduction

Glomerular diseases are the pathological processes most often found on native renal biopsy (Jennette et al., 2007). Their clinical presentation is varied, ranging from mild proteinuria detected as a chance finding in an entirely asymptomatic patient to rapidly progressive renal failure in the context of life-threatening systemic disease. Some patterns of glomerular disease on biopsy are characteristically associated with, but not specific to, each of these clinical presentations. These six clinical syndromes and common correlates on renal biopsy provide a convenient and appropriate first approach to glomerular disease (Table 42.1).

The evolution of the concept of glomerular disease

The concept that the kidneys produce urine emerged relatively late in antiquity. Egyptian medicine around 2000 BC first toyed with the idea that urine must be produced somewhere in the bladder area (Stratta et al., 1999). It took as long as another one and a half millennia until Hippocrates of Kos (460–377 BC) observed urine abnormalities, such has floating bubbles, in patients of whom we must assume had some form of glomerular disease (Eknoyan, 1988). Only much later did Pliny (23–79 AD) and Galen (130–200 AD) recognize and state much more explicitly the role of the kidneys in producing urine (Stratta et al., 1999). It is clear from his books that Pliny saw and recognized patients with blood in the urine, with peripheral oedema and also advocated various potions for treatment (De Santo et al., 1989). Rufus of Ephesus, in the first century AD, also described haematuria in the first ever textbook on diseases of the kidneys (Eknoyan, 2002) and we have to assume that at least some of the cases described had glomerular disease.

Not much progress was made in Europe in subsequent centuries and the medieval understanding of the kidney and urine essentially relied on concepts and ideas developed in antiquity. What progress there was relied very much on the contribution of Arabic physicians, such as Rhazes (865–925 AD) (Changizi Ashtiyani and Cyrus, 2010), who described haematuria that we have to assume was glomerular in origin (Changizi Ashtiyani and Cyrus, 2010). Similarly, Avicenna (980–1037 AD) had a keen interest in the kidney and possessed a good concept of the role of the kidneys, akin to the ideas of Pliny and Galen, although much of his interest was on urological problems (Changizi Ashtiyani et al., 2011). We must also credit these Arabic physician scholars for the taking their interest in urine analysis into clinical practice, as is demonstrated by textbook illustrations from the period (Fig. 42.1) (Eknoyan, 1994).

This focus on urine analysis continued to be a main theme in medieval medicine and made its way from the consulting rooms of Arabic physicians to clinical practice all over Europe. Driven in particular by the Salernitan School of Medicine (800–1400 AD), the visual inspection of urine (uroscopy) thus gained popularity throughout medieval Europe as evidenced by the widespread use of urine charts (Fig. 42.2). It is quite remarkable just how detailed these urine charts were in terms of different shades of colour and their presumed diagnostic significance (Diskin, 2008). It must be said, however, that uroscopy was viewed largely as a way to deduce changes in the composition of body fluids (*humores*), not as a diagnostic tool in kidney disease. Therefore, although some urine samples shown in Fig. 42.2 are suggestive of glomerulonephritis (GN), the link was clearly not understood. It has also been suggested that uroscopy was both over-used and also abused by charlatans and impostors (Stratta et al., 1999). Interestingly, the technique of urine collection was also thought to be important: Ismail of Jurjani, an eleventh-century Persian physician, first recommended the 24-hour urine collection we request today (Armstrong, 2006). Oedema was also occasionally observed and the term 'dropsy' was in widespread use as an umbrella term for an assortment of underlying diseases of the heart, liver, and kidney (Stratta et al., 1999).

A more detailed concept of glomerular disease (George, 2003) now required both substantial progress in terms of anatomy and histology of the kidney and a more detailed way of investigating abnormal urine. In 1664, Dekkers in Leiden first added acetic acid to urine and observed a milk-like precipitant (Cameron, 2003), although he failed to relate his observation to the peripheral oedema of the patient. Two years later, made possible by Galileo Galilei's invention of the lens, Marcello Malpighi (1628–1694) published his discovery of the glomerulus (Mezzogiorno, 1997; George, 2006). However, it was not until 1764, when Domenico Cotugno (1736–1822) (Fig. 42.3) in Naples demonstrated 'albumin' in the urine of a 28-year-old soldier with oedema and gave us the term albuminuria (Schena, 1994a). His report was clearly noted by fellow scholars (Schena, 1994b) although it did not lead to an understanding of the link between oedema and proteinuria. Indeed, well into the nineteenth century John Blackall (Jennette, 2007), physician to the Devon and Exeter Hospital, published a comprehensive work on dropsy (Blackall, 1814; Fine and English, 1994), which failed to grasp the connection.

Table 42.1 Clinical glomerular syndromes and some, but not all, histologic patterns of glomerular injury that can cause each syndrome. See Chapter 45 for a pathophysiological approach and diagram

Clinical presentation	Key features	Common patterns of histologic injury and diagnoses on renal biopsy
Asymptomatic proteinuria and haematuria and chronic glomerulonephritis	Hypertension, invisible glomerular haematuria with proteinuria and mild renal impairment, dense renal parenchyma on ultrasound	Alport syndrome and thin basement membrane disease, IgA nephropathy
Macroscopic haematuria	Visible, painless, glomerular haematuria, often coinciding with upper respiratory tract infection, invisible haematuria and proteinuria in between the attacks with or without mild renal impairment	IgA nephropathy
Nephritic syndrome	Abrupt onset of oedema and hypertension, glomerular haematuria and red cell casts, proteinuria (usually < 1.5 g/day)	Endocapillary-proliferative GN, IgA nephropathy
Nephrotic syndrome	Oedema, proteinuria (adult) > 3.5 g/day, hypoalbuminuria, hyperlipidaemia, with or without renal impairment	Amyloidosis, diabetic nephropathy, focal segmental glomerulosclerosis (FSGS), membranous GN, minimal change disease and its variants, membranoproliferative GN, pre-eclampsia, IgA nephropathy
Rapidly progressive glomerulonephritis	May or may not have extrarenal symptoms (skin, lung, joints); rapidly declining renal function (days, weeks), glomerular haematuria with red cell casts and proteinuria.	Focal necrotizing and crescentic glomerulonephritis, endocapillary proliferative glomerulonephritis, fibrillary glomerulonephritis, membranoproliferative GN

Fig. 42.1 Doctor performing urine analysis. There is a group of patients holding their little baskets, each containing a *matula* (the vessel in which urine is collected), awaiting their turns with the physician. The *matula* became a symbol of medical powers in general and physicians would make a ritual of holding it to the light before giving a diagnosis. It was also used as a billboard in some European cities (Armstrong, 2006).
From Avicenna (?980–1037), Canon (US National Library of Medicine Images from the History of Medicine Collection, image in the public domain <http://ihm.nlm.nih.gov/luna/servlet/detail/NLMNLM~1~1~101407164~151440:-Avicenna-s-Canon-?printerFriendly=1>).

However, these observations set the scene for Richard Bright when he became interested in the subject in 1816 (Cameron, 2003). His crucial role was perhaps not so much to make a singular pioneering discovery, but rather to put previous and scattered observations together into a unifying concept that included clinical, biochemical, and morphological characteristics. It was through his work that the term 'nephritis' came to us, although clearly Bright's generic term encompassed a much wider spectrum of renal diseases that we would associate with the term today (Stratta et al., 1999). Bright's ideas rapidly gained popularity (Stratta et al., 1999) and for almost a

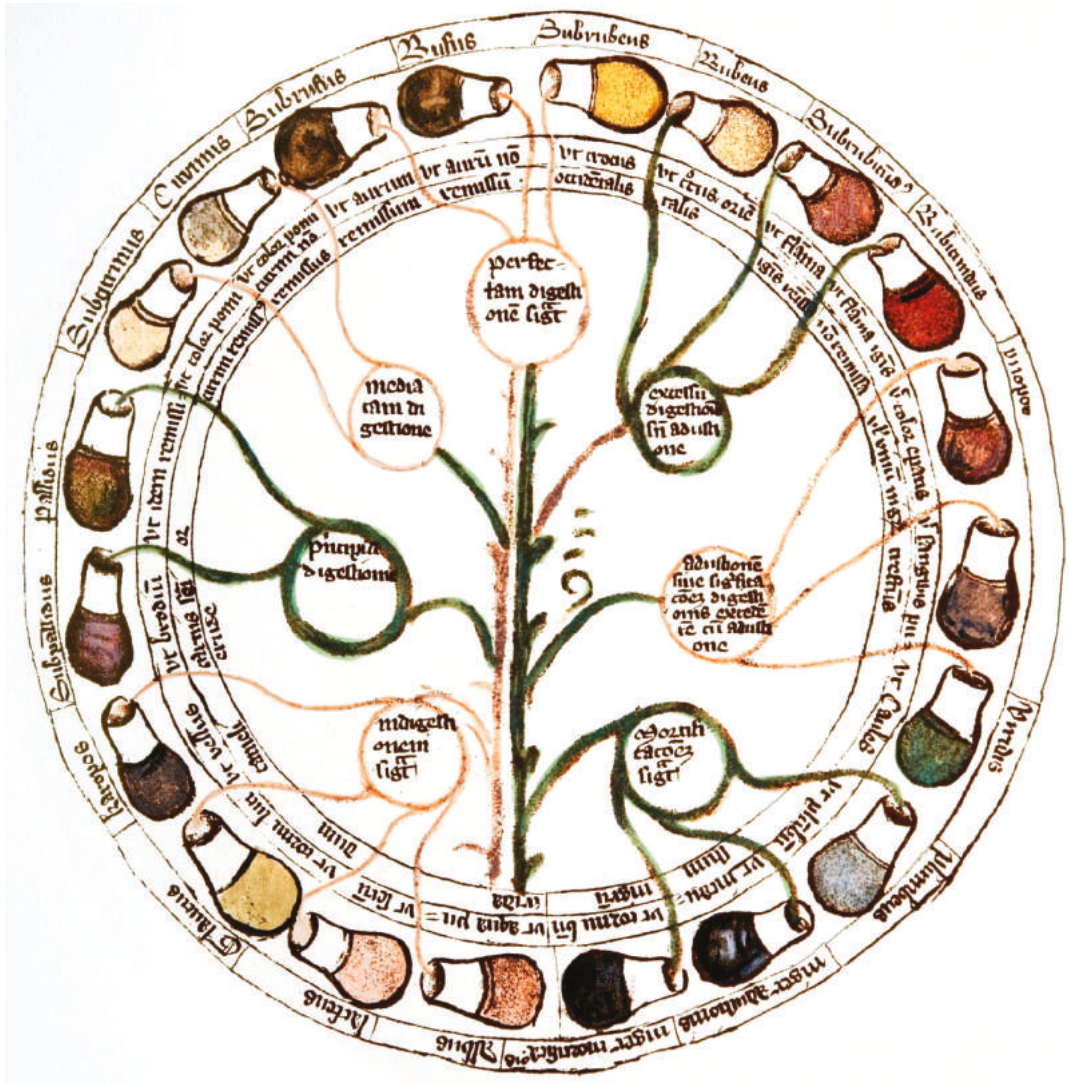

Fig. 42.2 Medieval urine wheel from Fasciculus Medicinae, a fifteenth-century collection of medical texts owned by Johannes de Ketham.
From Sciencephoto Ltd, London, with permission.

century nephritis was to bear the eponym 'Bright's disease'. A reassessment of his original specimens (Weller and Nester, 1972) demonstrated just how incredibly accurate his descriptions of kidney disease actually were. Bright also showed that low protein levels in serum were associated with urine protein leak and understood that kidney damage leads to retention of urea (Stratta et al., 1999).

The following years saw the crucial transition from vitalism to experimental physiology, pioneered by Claude Bernard (Arunachalam and Woywodt, 2010) and with it the discovery, by Carl Ludwig (1816–1895), of glomerular filtration and tubular re-absorption (Davis et al., 1996). Subsequently, Frerichs in his monograph on Bright's disease published in 1851, was the first to emphasize the importance of 'anatomical lesions', from which one could deduce the underlying pathological processes (Schwarz and Ritz, 1997). Remarkably, Frerichs also predicted some degree of interplay between interstitial scarring and glomerulosclerosis, a concept that rings very familiar today (Schwarz and Ritz, 1997). The second half of the nineteenth century also saw the term 'glomerulonephritis' emerge, coined by Swiss-German pathologist Edwin Klebs in his textbook of pathology (Ritz et al., 1994). Distinct entities of GN were then described from the beginning of the twentieth century (Stratta et al., 1999): Heinrich Reichel (1876–1943) in Vienna (Reichel, 1905) and later British nephrologist Arthur Osman (1893–1972) (Cameron, 1997) accurately described post-streptococcal GN (Rodríguez-Iturbe and Batsford, 2007) although William Charles Wells (1757–1817). had, a century earlier, already observed that haematuria follows scarlet fever in some cases (Wells, 1812).

The next important milestone towards a modern understanding of GN was reached when physician Franz Volhard (1872–1950) (Fig. 42.4) and pathologist Karl Theodor Fahr (1877–1945) published, in 1914, a new classification for glomerular disease (Fogazzi and Ritz, 1998; and see Fig. 70.1). Their seminal contribution was firstly the use of a clinico-pathological approach as we know it today, which is also reflected in the accurate and at the same time astonishingly beautiful illustrations in their textbook (Fogazzi and Ritz, 1998). In addition, they were the first to distinguish nephrotic and nephritic syndrome in the post-Bright, but still very much pre-biopsy, era of nephrology (Luft and Dietz, 1993).

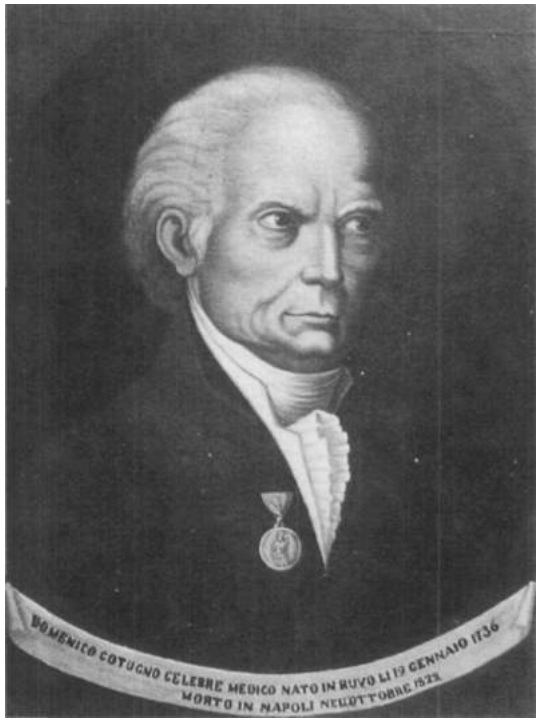

Fig. 42.3 Domenico Cotugno (1736–1822). Apart from his role in discovering albuminuria, Cotugno is also notable for having been appointed as Professor of Medicine at the age of only 19 at the famous Medical School of Salerno in Italy.
From Schena (1994), with permission.

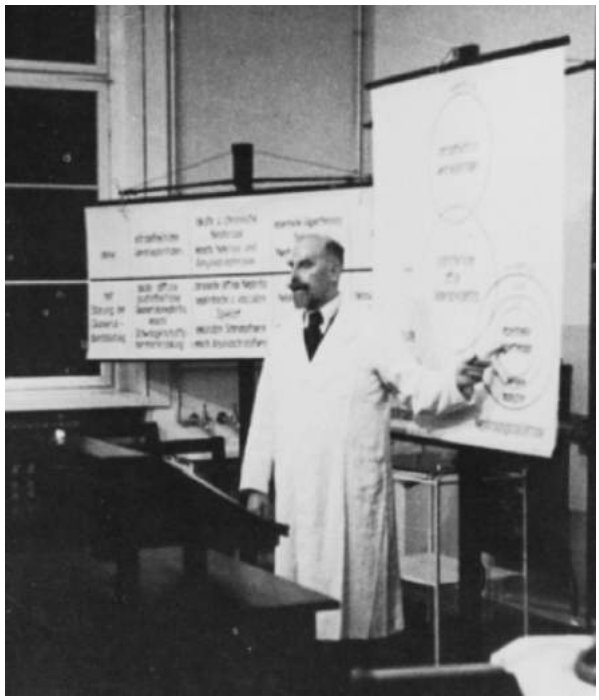

Fig. 42.4 Franz Volhard lecturing to students. On the board are Volhard's famous circle diagrams separating the various forms of Bright's disease, with and without hypertension. Volhard points to the group with essential 'red' hypertension.
From Wolf (2000), with permission.

Around the same time of Volhard and Fahr's textbook, examination of the urine sediment saw a revival, pioneered by Thomas Addis (1881–1949) in Stanford, originally a Scottish haematologist (Blagg, 2009). He also standardized the technique for quantitative evaluation of the urine sediment—the 'Addis count'. Remarkably, the origin of proteinuria remained to some degree controversial until well into the 1930s (Waldherr and Ritz, 1999). It took until the 1940s when Edmund Randerath (1881–1949) in Germany deduced, from elegant experiments in salamanders, that the glomerulus was indeed the source of proteinuria (Waldherr and Ritz, 1999). Important clinical observations were also made during that time, such as that of a peculiar syndrome of pulmonary haemorrhage and GN by Ernest Goodpasture (1886–1960) in 1919 (Goodpasture, 2009), followed by the first reports of renal involvement in what we now know as small vessel vasculitis by Wohlwill in 1923 (Matteson, 2002), Klinger in 1931 (Klinger, 1931), and Wegener in 1936 (Woywodt et al., 2006). However, in general the understanding and classification of GN remained patchy and essentially confined the ideas of Volhard and Fahr well into the 1950s, not least because histological diagnoses could only be made at autopsy.

Further progress towards our current understanding of glomerular disease now required the introduction, in the 1950s, of percutaneous renal biopsy (Cameron and Hicks, 1997). This milestone development is usually associated with the names of Poul Iversen (1889–1966) and Claus Brun (b. 1910) in Copenhagen (Cameron and Hicks, 1997). However, Nils Alwall (1904–1986), who is more commonly known for his seminal contribution to dialysis, performed renal biopsies as early as 1944 (Alwall, 1952). However he did not pursue this approach further, chiefly due to an early patient death (Cameron and Hicks, 1997). Crucial advances in laboratory medicine then allowed for further progress in the analysis of biopsy material and a more and more diverse nomenclature of what had until recently carried the umbrella term of Bright's disease. Immunofluorescence became available in the 1960s, leading to the discovery of immunoglobulin (Ig)-A nephropathy by Jean Berger (1930–2011) and Nicole Hinglais at Hôpital Necker in Paris (Berger and Hinglais, 1968; Woo et al., 2009). By 1963, antibodies directed against class-specific epitopes of the Ig light chains were available commercially. Finally, electron microscopy appeared in the 1950s and was increasingly used by the mid 1960s (Carlson, 1961).

These developments culminated in the 1961 Ciba Symposium on Renal Biopsy in London. It is probably fair to say that this event was in itself a milestone towards our current understanding of GN. In the spirit of Volhard and Fahr it was also a celebration of a very successful collaboration between physician and pathologist as narrated elsewhere by Robert Heptinstall, himself one of the doyens of renal pathology in the twentieth century (Heptinstall, 1990). By 1961, this collaboration had established as a standard the diagnosis of GN with renal biopsy, and of the interpretation of biopsy specimens with light microscopy, immunofluorescence, and electron microscopy. These developments also nurtured the establishment of nephrology as a specialty in its own right, with, among others, the first European Congress of Nephrology being held in Geneva in 1960. From here on, developments in our understanding of GN are beyond the scope of this chapter, since they are part of our present concepts of the disease, rather than its history.

Classification of glomerular disease

The overview presented here may be considered alongside Chapter 18 describing the appearances of the renal biopsy, and Chapter 45 describing major pathophysiological mechanisms behind the major presentations. For didactic reasons, we will differentiate between primarily non-inflammatory and inflammatory glomerular disease, that is, GN. However, this scheme is to some degree arbitrary and artificial. Many non-inflammatory glomerular diseases, while not primarily mediated by cells and effectors of the immune system, are still propagated and have their progression determined by secondary inflammatory processes (Abbate et al., 1998). Alpha-1-antitrypsin deficiency, for example, although primarily an inherited disease not characterized by immune activation, features deposition of immunoglobulins and complement (Heidet and Gubler, 2009). Similarly, diabetic nephropathy, although primarily very clearly a metabolic non-inflammatory disorder, is characterized by abnormal cytokine profiles (Navarro-Gonzalez and Mora-Fernandez, 2008). Moreover, immunosuppression has been used successfully in animal models of the disease (Utimura et al., 2003).

Classification of non-inflammatory glomerular disease

Inherited non-inflammatory glomerular disease

The group of inherited glomerular diseases includes a long list of categories, individual diseases, and syndromes, some of whom are very rare (Kashtan and Gubler, 2009). Table 42.2 provides an overview. Many other inherited diseases of the kidney, such as the nephronophthisis group (see Chapter 316), spare the glomerulus. When observed in these disorders, glomerular changes and proteinuria are usually regarded as secondary to scarring and loss of nephrons. Some knowledge even of the rare inherited glomerular diseases is important, as this will often enable the clinician to make a clinical diagnosis and also guide genetic testing. Crucially, a spot diagnosis will usually be facilitated not by the renal features

Table 42.2 Examples of inherited non immune-mediated glomerular disease (See also Section 15 and Kashtan and Gubler, 2009)

Category	Main site of injury	Genetics and pathogenetic mechanisms	Associated extrarenal disease	Typical clinical presentation
Disorders of GBM collagen and its transcription (Kashtan and Gubler, 2009) (see Chapter 30)				
Alport syndrome (type IV collagen) (Kashtan, 1999) (Chapter 322)	Glomerular basement membrane	*COL4A1* and *COL4A2* at 13q34 *COL4A3* and *COL4A4* at 2q35–37 *COL4A5* and *COL4A6* on chromosome X.	Sensorineural deafness and anterior lenticonus; leiomyomas (Kashtan, 1999)	Microscopic haematuria, followed by proteinuria, hypertension and slow progression to ESRD
Nail patella syndrome (Kashtan and Gubler, 2009; Lemley, 2009) (Chapter 326)	Glomerular basement membrane	Mutation in *LMX1B* transcription factor at 9q34 (autosomal dominant) (Bongers et al., 2005); this seems to affect *COL4A4* and *COL4A3* (Morello et al., 2001; Lemley, 2009)	Sensorineural deafness, dystrophic nails, hypoplasia of patellae/elbows	Microscopic haematuria, proteinuria and renal impairment, progressive
Thin basement membrane disease (TBMN) (Tryggvason and Patrakka, 2006) (Chapter 325)	Glomerular basement membrane	Disorder of collagen IV trimer α3:α4:α5; autosomal-dominant with mutations in *COL4A5*, *COL4A3*, or *COL4A4* (Tryggvason and Patrakka, 2006)	None	Microscopic haematuria, minimal proteinuria and normal renal function.
Podocytopathies (see Chapter 327)				
Denys–Drash/Frasier syndrome (Chapter 329)	Podocyte	WT1 gene at 11p13 (Baird et al., 1992)	Wilms tumour, pseudohermaphroditism	Nephrotic syndrome, progressing to ESRD
Laminin deficiency (Pierson syndrome) (Chapter 320)	Podocyte	Mutation in *LAMB2*, the gene encoding the β2 chain of laminin, at 3p14–p22 (Zenker et al., 2005)	Cataract, microcoria and other eye abnormalities (Choi et al., 2008)	Congenital nephrotic syndrome (Kashtan and Gubler, 2009)
Steroid-resistant nephrotic syndrome (SRNS) (Caridi et al., 2010; Hildebrandt, 2010) (Chapter 327)	Podocyte	SRNS1 (Finnish type and adult variants): Nephrin at 19q13.1 (Godefroid and Dahan, 2010) SRNS2: (recessive): podocin at 1q25–q31 (Boute et al., 2000) SRNS3: *PLCE1* gene at 10q23 (Boyer et al., 2010) SRNS4: *CD2AP* gene at 6p12 (Kim et al., 2003) (and others, see Chapter 327)	*NPHS1*: pyloric stenosis in some families	Severe steroid-resistant nephrotic syndrome

(*continued*)

Table 42.2 Continued

Category	Main site of injury	Genetics and pathogenetic mechanisms	Associated extrarenal disease	Typical clinical presentation
Storage and deposition diseases				
Hereditary amyloidosis (Kashtan and Gubler, 2009) (Chapter 152)	Glomerulus, blood vessels	Protein mutations: transthyretin, gelsolin, the apolipoproteins, lysozyme, fibrinogen Periodic fever syndromes: familial Mediterranean fever (FMF), Muckle–Wells syndrome, hyper IgD syndrome (Kashtan and Gubler, 2009)	Highly variable, including vascular involvement, ocular amyloid; neuropathy and autonomic failure	Nephrotic syndrome with renal impairment and often progression to ESRD
Fabry disease (Muckle Wells syndrome, 2007) (Chapter 335)	Glomerulus, vascular endothelium, tubulointerstitial cells	X-linked lysosomal α-galactosidase deficiency	Neuropathy, cardiovascular disease, angiokeratoma of the skin, corneal opacities	Progressive CKD with vascular/cardiac disease and severe neuropathy (Clarke, 2007)
Other storage and deposition diseases: alpha-1-antitrypsin deficiency, Alagille syndrome (Krantz et al., 1987), and others.	Glomerulus, vascular endothelium, tubulointerstitial cells	For review see Kashtan and Gubler (2009)	Often multisystem disease (Kashtan and Gubler, 2009)	Proteinuria, renal impairment and occasionally ESRD (Alagille syndrome) (Kashtan and Gubler, 2009)

CD2AP = CD2-associated protein; CKD = chronic kidney disease; ESRD = end-stage renal disease; IgD = immunoglobulin D; LAMB-2 = laminin beta2 chain; *LMX1B* = LIM homeobox transcription factor 1 beta; OMIM = Online Mendelian Inheritance in Man; *PLCE-1* = 1-phosphatidylinositol-4,5-bisphosphate phosphodiesterase epsilon-1; *TRPC-6* = transient receptor potential cation channel, subfamily C, member 6; WT-1 = Wilms tumour 1.

of the disease, but by its typical extrarenal manifestations. Alport syndrome (see Chapter 322), the nail patella syndrome (see Chapter 326), and Fabry disease (see Chapter 336) serve as good examples.

The topic is also of importance because the number of young patients with inherited disorders graduating from paediatric to adult renal care continues to increase, not least due to improved management and better survival (Watson et al., 2011) (see Chapter 322).

Among the inherited glomerular diseases, the disorders of glomerular type IV collagen (Table 42.2), that is, Alport syndrome (see Chapter 322) and the thin basement membrane disease (see Chapter 325), are most commonly encountered in adult nephrology. Their genetic basis, clinical manifestations, and diagnosis have been well understood for quite some time although progress has now been made with regard to mutation analysis. Other diseases have been added to the spectrum, such as the nail–patella syndrome (caused by mutations in the gene encoding the LMX1B transcription factor, see Chapter 326) (Table 42.2). They feature significant, and characteristic, extrarenal manifestations, namely hearing impairment in Alport syndrome, and hypoplasia of patellae and elbows in the nail–patella syndrome (Table 42.2).

Exciting progress has also occurred regarding a growing group of genetic defects of the podocyte and the term 'podocytopathies' has been coined (Barisoni et al., 2007) (see Chapter 327). These disorders, while mostly rare, are nonetheless of importance, particularly in paediatric and adolescent nephrology. Their importance stems from the fact that they usually present with severe nephrotic syndrome that is resistant to steroids and other immunosuppressant drugs (Wiggins, 2007). These disorders have in common a genetic defect of one of the proteins of the podocyte's ultrastructure, resulting in proteinuria, nephrotic syndrome, and progressive renal impairment, often in childhood or adolescence (Wiggins, 2007). Taxonomy and nomenclature of these diseases are still very much in the making (Barisoni et al., 2007) but their characterization has already improved our understanding of the glomerular slit membrane (Welsh and Saleem, 2010) with many of the mutated proteins playing a crucial role in its structural integrity and function (see Chapter 327). These disorders also serve as an example of a disease in which mutations determine age of onset and treatment response (Hildebrandt, 2010). Some of the proteins that are implicated, such as nephrin and podocin, are well conserved in evolution, which underscores their pivotal role in glomerular filtration (Weavers et al., 2009). Nephrin appears to have a particularly central role in the slit diaphragm and interactions with many other proteins have now been described (Welsh and Saleem, 2010). It is highly likely that some more podocytopathies are going to emerge in the near future, together with more crucial proteins of the slit diaphragm (Michaud and Kennedy, 2007).

The next subcategory of inherited glomerular diseases is that of the storage and deposition disorders, which includes hereditary amyloidosis (see Chapter 152), Fabry disease (see Chapter 335), and finally some even rarer syndromes, such as the Alagille syndrome (Kashtan and Gubler, 2009). Some authors differentiate, within this group, between diseases with primary glomerular involvement, such as Fabry disease, and others with secondary glomerular involvement, such as hereditary amyloidosis (see Chapter 152). Notable progress has occurred within the group of hereditary amyloidosis and a number of individual disease entities are now well characterized (Table 42.2). Much progress has also been made with regard to Fabry disease, and particularly its treatment with enzyme replacement (see Chapter 338).

Acquired non-inflammatory glomerular disease

This group encompasses glomerular diseases due to drugs and medication, metabolic diseases, deposition disorders, and those due to vascular disease (Table 42.3). Differentiating these from the

Table 42.3 Acquired non-immune-mediated glomerular disease

Acquired non-immune-mediated glomerular disease	Main site of injury	Pathogenetic mechanisms	Typical clinical presentation
Drug-induced (Izzedine et al., 2006) (Chapter 82 and Chapter 362)	Endothelial cell, podocyte, GBM	Mostly unclear and idiosyncratic although some concept are beginning to emerge, e.g. proteinuria due to mTOR inhibitors (Inoki et al., 2011) and VEGF inhibitors (George et al., 2007)	New-onset proteinuria with or without renal impairment in conjunction with drug treatment
Metabolic/deposition diseases			
AA amyloid (Chapter 152)	Diffuse deposition in glomerulus, interstitium, blood vessels	Extracellular tissue deposition of fibrils that are composed of fragments of serum amyloid A (SAA) protein in conjunction with an chronic inflammatory condition (Gillmore et al., 2001)	Nephrotic syndrome with renal impairment and the presence of a predisposing rheumatic or a chronic inflammatory disease extrarenal abnormalities (hepatosplenomegaly, macroglossia, restrictive cardiomyopathy) (Gertz and Kyle, 1991)
AL amyloid (Chapter 152)	Diffuse deposition in glomerulus, interstitium, blood vessels	Clonal proliferation of plasma cells, leading to production of monoclonal immuno-globulins and tissue deposition of organized immunoglobulin fibrils (Sanchorawala, 2006)	Nephrotic syndrome with renal impairment in conjunction with a serum/urinary paraprotein; extrarenal abnormalities (hepatosplenomegaly, macroglossia, restrictive cardiomyopathy) (Kyle and Greipp, 1983)
Diabetic nephropathy (Chapter 149)	Endothelium, mesangial cell, podocyte	Multifactorial, including glomerular hyperfiltration, direct effects of hyperglycaemia and advanced glycation end products, vascular injury and podocyte changes	Proteinuria or nephrotic syndrome with progressive renal impairment in a patient with long-standing diabetes, usually with concurrent evidence of other end-organ damage
Monoclonal immunoglobulin deposition diseases (MIDD): light chain deposition disease, heavy chain deposition disease (Chapter 150)	Diffuse deposition in glomerulus, interstitium, blood vessels	Clonal proliferation of plasma cells, leading to production of monoclonal immunoglobulins and tissue deposition of non-organized immunoglobulins(Ronco et al., 2006)	Proteinuria/nephrotic syndrome with renal impairment in a patient with monoclonal Ig in serum and/or urine and evidence of plasma cell proliferation on bone marrow biopsy
Radiation nephritis (Luxton, 1961) (Chapter 91)	Endothelium, glomerulus	Cellular injury due to irradiation	Proteinuria (Luxton, 1961) (occasionally nephrotic syndrome (Jennette and Ordonez, 1983)) and renal impairment (can be progressive)
Vascular disorders			
Hypertensive nephropathy (Chapter 211)	Endothelial cell	Ischaemic injury, leading to glomerulosclerosis, with nephron loss, hyperfiltration, and further injury (Harvey et al., 1992)	Slowly progressive renal impairment and mild-to-moderate proteinuria in a patient with long-standing hypertension (Fogo et al., 1997)
Pre-eclampsia and HELLP syndrome (Chapter 296)	Endothelial cell	Imbalance between pro- and anti-angiogenic factors (Maynard and Karumanchi, 2011), leading to microangiopathy	New onset of hypertension and proteinuria after 20 weeks of gestation (Sibai, 2003)
Thrombotic microangiopathy (Chapter 174)	Endothelial cell	Glomerular platelet-rich microthrombi, fibrin deposition, and ischaemia (Benz and Amann, 2010)	AKI, laboratory evidence of MAHA, and dysfunction of other organ systems (Forzley and Clark, 2009)
Others (antiphospholipid syndrome, embolism and cholesterol embolism (Chapters 164, 212))	Endothelial cell	Macro- and microthrombi and ischaemia	AKI with (embolism) or without (cholesterol embolism) flank pain; typical laboratory features

AKI = acute kidney injury; GBM = glomerular basement membrane; HELLP syndrome = haemolytic anaemia; elevated liver enzymes, low platelets syndrome; Ig = immunoglobulin; MAHA = microangiopathic haemolytic anaemia; mTOR = mammalian target of rapamycin; VEGF = vascular endothelial growth factor.

inflammatory glomerulonephritides is in some cases arbitrary. The monoclonal immunoglobulin deposition diseases (MIDDs) and AL amyloidosis, for example, are regarded as deposition diseases by some authors while others view them as glomerulonephritides. The taxonomy of immunotactoid and fibrillary glomerulopathy is similarly controversial.

Drug-induced glomerular disease is comparatively rare and much less well appreciated than nephrotoxicity occurring in the tubulointerstitium (see Chapter 84). A long list of drugs has been implicated, although causality is often difficult to ascertain. Diabetic nephropathy (see Chapter 149) is the prime example of a metabolic disorder causing glomerular disease. Diabetic nephropathy is now the leading cause of end-stage renal failure in many developed countries and its early recognition and effective management determines the fate of a large proportion of patients seen in adult nephrology. The remainder of the acquired metabolic and

deposition diseases and AL amyloid (see Chapter 152) have some clinical importance, although this is perhaps still surpassed by their scientific relevance (Table 42.3). Nephrologists and haematologists have made some progress in the management of AL amyloid and the MIDDs, although their management remains challenging for the clinician. In comparison, radiation nephritis (see Chapter 91) is exceedingly rare and poorly understood. Finally, a number of vascular disorders (Table 42.3) are clinically important and some of them are of substantial scientific interest as well. In particular, pre-eclampsia (see Chapter 296) and thrombotic microangiopathy (see Chapter 174) come to mind (Table 42.3), with interesting new insight into the pathogenesis occurring during the last decade.

Classification of inflammatory glomerular diseases (glomerulonephritis)

Attempts to classify this group of diseases have been made ever since German-Swiss pathologist Edwin Klebs first coined the term 'glomerulonephritis' in his textbook published in 1870 (Klebs, 1870). Between 1881 and 1893, American physician Francis Delafield (1841–1915) worked on a complex classification of 'Bright's disease' that differentiated between acute and chronic forms, and subdivided each into different subcategories (Campbell et al., 2003). Osler also emphasized the course of the disease over time as a criterion for classification and, making reference to Delafield, used the terms acute and chronic nephritis (Stratta et al., 1999). Volhard and Fahr, in their landmark textbook published in 1914, made another attempt at dividing the umbrella term Bright's disease into different clinical patterns of glomerular disease, namely 'degenerative/nephrotic', 'focal/diffuse', and 'atherosclerotic' (Luft and Dietz, 1993). Thomas Addis (1881–1949) in 1925 also used the term 'chronic latent nephritis' (Addis, 1925). In 1929, W. T. Longcope (1877–1953) (Longcope, 1929) proposed two types of GN, namely 'focal glomerular nephritis' and 'diffuse glomerular nephritis'. Calvin Ellis (1826–1883) in 1942 also differentiated between two types of GN, one with preceding infection; type 1 featured haematuria, and a high percentage of recovery while type 2 was 'lipoid nephrosis' with a more insidious onset and infrequent recovery (Ellis, 1942).

The widespread introduction of renal biopsy in the 1950s and 1960s led to more patterns of histologic injury (Table 42.1) being defined from the combined use of light and electron microscopy, and immunofluorescence microscopy. Immunological terms, referring to patterns of complement and immunoglobulin deposition, thus made their appearance in the classification in the 1960, such

Table 42.4 Patterns of glomerular injury observed by light microscopy and examples of glomerulonephritides and other glomerular disorders that can cause each pattern of injury Patterns are described in more detail in Chapter 18

Pattern of glomerular injury	Glomerulonephritides that cause this pattern of injury	Other glomerular diseases that can cause this pattern of injury
No abnormality by light microscopy	Minimal change disease (Chapter 55) and variants, mild/early glomerular disease (e.g. IgA nephropathy)	Thin basement membrane disease (Chapter 325) and early Alport syndrome (Chapter 321) Early/mild amyloidosis (Chapter 152)
Thick capillary walls without hypercellularity or mesangial expansion	Membranous GN (with thickened glomerular basement membrane) (Chapter 60) Fibrillary GN (with predominant capillary wall deposits) (Chapter 81)	Pre-eclampsia (with endothelial swelling) (Chapter 296) Thrombotic microangiopathy (with expanded sub-endothelial zone) (Chapter 174)
Thick capillary walls with mesangial expansion but little or no hypercellularity	Secondary membranous GN with mesangial deposits (Chapter 63) Fibrillary GN (Chapter 81) Dense deposit disease (type II membranoproliferative GN) (Chapter 80)	Diabetic nephropathy (Chapter 149) Amyloidosis (Chapter 152) Monoclonal immunoglobulin deposition disease (Chapter 150)
Focal segmental glomerulosclerosis (FSGS) without hypercellularity	FSGS (primary or secondary) (Chapter 57) Chronic sclerotic disease of focal GN; secondary FSGS (Box 57.1)	Alport syndrome (Chapter 321)
Mesangial or endocapillary hypercellularity	Mesangioproliferative GN; IgA nephropathy (Chapter 65) and others Endocapillary proliferative/post-infectious GN (Chapter 623) Membranoproliferative GN type I–III (Chapter 80)	
Extracapillary hypercellularity	Crescentic GN (Chapter 70) Collapsing variant FSGS (Chapter 57) and HIV nephropathy (Chapter 186)	Drug-induced collapsing variant FSGS (bisphosphonates) (Chapter 82)
Membranoproliferative lobular or nodular pattern	Membranoproliferative GN type I–III (Chapter 80) Fibrillary or Immunotactoid GN (Chapter 81)	Diabetic nephropathy (Chapter 149) Monoclonal immunoglobulin deposition disease (Chapter 150) Thrombotic microangiopathy (particularly healing) (Chapter 174)
Diffuse global glomerular sclerosis	End-stage glomerular disease	End-stage vascular disease End-stage tubulointerstitial disease

Adapted from Johnson et al. (2000).

as that of IgA nephropathy (Berger and Hinglais, 1968) in 1968. In some conditions, additional criteria for classification emerged on the basis of serological markers and extrarenal disease as with the discovery of antibodies against the cytoplasm of neutrophils in 1982 (Davies et al., 1982). In parallel, individual diseases were named on the basis of their aetiology, such as post-streptococcal GN (Rodríguez-Iturbe and Batsford, 2007). It is worthwhile to remember that this was a long, and often chaotic, process, essentially reflecting the tortuous development of nephrology and renal pathology in the nineteenth and twentieth century, and not conscious process of layering.

As of today, a universally accepted and unifying diagnostic classification of GN that integrates clinical features, morphology on renal biopsy, and other immunological findings, does not exist. To the physician, the clinical syndromes associated with GN (Table 42.1) remain a good starting point to narrow the differential diagnosis, to guide investigations and diagnostic thinking, and to begin a process that will eventually lead to a specific diagnosis (Jennette et al., 2007). In comparison to the clinical syndromes associated with tubulointerstitial and vascular lesions, these syndromes are quite specific and predict a lesion of the glomerular capillary wall with reasonable certainty (Jennette et al., 2007).

Of note, even the nomenclature of glomerular disease lacks consensus. Some authors, for example, have referred to minimal change disease (see Chapter 53) as a GN while others prefer the term glomerulopathy, on the account that inflammatory lesions are lacking. Another good example is the term of membranoproliferative GN (MPGN) (Appel et al., 2005) (see Chapter 80), which contrasts with that of mesangiocapillary GN preferred by others (D'Amico and Ferrario, 1992).

In the absence of a unifying classification and nomenclature of GN, this book relies on a morphological categorization based on patterns on renal biopsy, except where aetiological factors are clearly identified (e.g. HIV-associated nephropathy), an associated multisystem disease is defined (e.g. lupus nephritis), or the immunopathogenesis is well characterized (e.g. antiglomerular basement membrane (anti-GBM) disease). Table 42.4 lists common patterns of glomerular injury by light microscopy and the glomerulonephritides that can cause these patterns. For the sake of completeness, glomerular diseases other than GN are also listed as a differential diagnosis. This is, of course, not a classification by aetiology as many histological patterns have a variety of aetiologies. Some have therefore emphasized that renal biopsy yields a histological pattern, not a distinct diagnosis (Johnson et al., 2000 and see Chapter 18). In MPGN (see Chapter 61), for example, further investigations and tests, such as obtaining a family history, hepatitis C serology, alpha-1-antitrypsin levels, etc., will allow for a diagnosis based on aetiology. Another good example is focal necrotizing/crescentic GN (see Chapter 70) where only comprehensive testing for antineutrophil cytoplasmic antibodies, anti-GBM, etc. allows for a clinically useful diagnosis. Conversely, one aetiology may produce different patterns of histological damage: hepatitis B, for example, is capable of causing as diverse patterns of injury as membranous GN, MPGN, and even polyarteritis nodosa (see Chapter 185) (Johnson and Couser, 1990).

References

Abbate, M., Zoja, C., Corna, D., *et al.* (1998). In progressive nephropathies, overload of tubular cells with filtered proteins translates glomerular permeability dysfunction into cellular signals of interstitial inflammation. *J Am Soc Nephrol*, 9, 1213–24.

Addis, T. (1925). A clinical classification of Bright's diseases. *J Am Med Assoc*, 85, 163–7.

Alwall, N. (1952). Aspiration biopsy of the kidney, including i.a. a report of a case of amyloidosis diagnosed through aspiration biopsy of the kidney in 1944 and investigated at an autopsy in 1950. *Acta Med Scand*, 143, 430–5.

Appel, G. B., Cook, H. T., Hageman, G., *et al.* (2005). Membranoproliferative glomerulonephritis type II (dense deposit disease): an update. *J Am Soc Nephrol*, 16, 1392–403.

Armstrong, J. A. (2006). Urinalysis in Western culture: a brief history. *Kidney Int*, 71, 384–7.

Arunachalam, C. and Woywodt, A. (2010). Turbid urine and beef-eating rabbits: Claude Bernard (1813–78)—a founder of modern physiology. *NDT Plus*, 3, 335–7.

Baird, P. N., Santos, A., Groves, N., *et al.* (1992). Constitutional mutations in the WT1 gene in patients with Denys-Drash syndrome. *Hum Mol Genet*, 1, 301–5.

Barisoni, L., Schnaper, H. W., and Kopp, J. B. (2007). A proposed taxonomy for the podocytopathies: a reassessment of the primary nephrotic diseases. *Clin J Am Soc Nephrol*, 2, 529–42.

Barisoni, L., Schnaper, H. W., and Kopp, J. B. (2007). A proposed taxonomy for the podocytopathies: a reassessment of the primary nephrotic diseases. *Clin J Am Soc Nephrol*, 2, 529–42.

Benz, K. and Amann, K. (2010). Thrombotic microangiopathy: new insights. *Curr Opin Nephrol Hypertens*, 19, 242–7.

Berger, J. and Hinglais, N. (1968). [Intercapillary deposits of IgA-IgG]. *J Urol Nephrol* (Paris), 74, 694–5.

Blackall, J. (1814). *Observations on the Nature and Cure of Dropsies etc.etc.* London: Longman, Hurst, Rees, Orme and Brown.

Blagg, C. R. (2009). Thomas Addis, 1881–1949, clinical scientist, hematologist and pioneering nephrologist: a brief biography. *J Nephrol*, 22 Suppl 14, 115–19.

Bongers, E. M. H. F., Huysmans, F. T., Levtchenko, E., *et al.* (2005). Genotype-phenotype studies in nail-patella syndrome show that LMX1B mutation location is involved in the risk of developing nephropathy. *Eur J Hum Genet*, 13, 935–46.

Boute, N., Gribouval, O., Roselli, S., *et al.* (2000). NPHS2, encoding the glomerular protein podocin, is mutated in autosomal recessive steroid-resistant nephrotic syndrome. *Nat Genet*, 24, 349–54.

Boyer, O., Benoit, G., Gribouval, O., *et al.* (2010). Mutational analysis of the PLCE1 gene in steroid resistant nephrotic syndrome. *J Med Genet*, 47, 445–52.

Cameron, J. S. (1997). Arthur Arnold Osman (1893-1972): a forgotten pioneer of nephrology. *Nephrol Dial Transplant*, 12, 1526–30.

Cameron, J. S. (2003). Milk or albumin? The history of proteinuria before Richard Bright. *Nephrol Dial Transplant*, 18, 1281–5.

Cameron, J. S. and Hicks, J. (1997). The introduction of renal biopsy into nephrology from 1901 to 1961: a paradigm of the forming of nephrology by technology. *Am J Nephrol*, 17, 347–58.

Campbell, J. L. and Eknoyan, G. (2003). Francis Delafield (1841–1915): the original contributions of an American investigator to diseases of the kidney. *J Nephrol*, 16, 779–84.

Caridi, G., Trivelli, A., Sanna-Cherchi, S., *et al.* (2010). Familial forms of nephrotic syndrome. *Pediatr Nephrol*, 25, 241–52.

Carlson, H. E. (1961). Electron microscopy of the kidney in health and disease. *South Med J*, 54, 985–92.

Changizi Ashtiyani, S. and Cyrus, A. (2010). Rhazes, a genius physician in diagnosis and treatment of kidney calculi in medical history. *Iran J Kidney Dis*, 4, 106–10.

Changizi Ashtiyani, S., Shamsi, M., Cyrus, A., *et al.* (2011). A critical review of the works of pioneer physicians on kidney diseases in ancient Iran: Avicenna, Rhazes, Al-akhawayni, and Jorjani. *Iran J Kidney Dis*, 5, 300–8.

Choi, H. J., Lee, B. H., Kang, J. H., *et al.* (2008). Variable phenotype of Pierson syndrome. *Pediatr Nephrol*, 23, 995–1000.

Clarke, J. T. (2007). Narrative review: Fabry disease. *Ann Intern Med*, 146, 425–33.
D'Amico, G. and Ferrario, F. (1992). Mesangiocapillary glomerulonephritis. *J Am Soc Nephrol*, 2, S159–66.
Davies, D. J., Moran, J. E., Niall, J. F., *et al.* (1982). Segmental necrotising glomerulonephritis with antineutrophil antibody: possible arbovirus aetiology? *Br Med J (Clin Res Ed)*, 285, 606.
Davis, J. M., Thurau, K., and Häberle, D. (1996). Carl Ludwig: the discoverer of glomerular filtration. *Nephrol Dial Transplant*, 11, 717–20.
De Santo, N. G., Capasso, G., Giordano, D. R., *et al.* (1989). Nephrology in the natural history of Pliny the Elder (23–79 A.D.). *Am J Nephrol*, 9, 252–60.
Diskin, C. J. (2008). de Ketham revisited: a modern-day urine wheel. *Med J Aust*, 189, 658–9.
Eknoyan, G. (1988). Origins of nephrology: Hippocrates, the father of clinical nephrology. *Am J Nephrol*, 8, 498–507.
Eknoyan, G. (1994). Arabic medicine and nephrology. *Am J Nephrol*, 14, 270–8.
Eknoyan, G. (2002). Rufus of Ephesus and his 'Diseases of the Kidneys'. *Nephron*, 91, 383–90.
Ellis, A. (1942). Natural history of Bright's disease. *Lancet*, I, 1–7.
Fine, L. G. and English, J. A. (1994). John Blackall (1771–1860): failure to see the obvious in dropsical patients with coagulable urine? *Am J Nephrol*, 14, 371–6.
Floege, J., Burns, M. W., Alpers, C. E., *et al.* (1992). Glomerular cell proliferation and PDGF expression precede glomerulosclerosis in the remnant kidney model. *Kidney Int*, 41, 297–309.
Fogazzi, G. B. and Ritz, E. (1998). Novel classification of glomerulonephritis in the monograph of Franz Volhard and Theodor Fahr. *Nephrol Dial Transplant*, 13, 2965–7.
Fogo, A., Breyer, J. A., Smith, M. C., *et al.* (1997). Accuracy of the diagnosis of hypertensive nephrosclerosis in African Americans: a report from the African American Study of Kidney Disease (AASK) Trial. AASK Pilot Study Investigators. *Kidney Int*, 51, 244–52.
Forzley, B. R. and Clark, W. F. (2009). TTP/HUS and prognosis: the syndrome and the disease(s). *Kidney Int Suppl*, S59–61.
George, B. A., Zhou, X. J., and Toto, R. (2007). Nephrotic syndrome after bevacizumab: case report and literature review. *Am J Kidney Dis*, 49, e23–9.
George, C. R. (2003). The cellular history of the glomerulus. *J Nephrol*, 16, 949–57.
Gertz, M. A. and Kyle, R. A. (1991). Secondary systemic amyloidosis: response and survival in 64 patients. *Medicine*, 70, 246–56.
Gillmore, J. D., Lovat, L. B., Persey, M. R., *et al.* (2001). Amyloid load and clinical outcome in AA amyloidosis in relation to circulating concentration of serum amyloid A protein. *Lancet*, 358, 24–9.
Godefroid, N. and Dahan, K. (2010). Expanding the clinical spectrum of congenital nephrotic syndrome caused by NPHS1 mutations. *Nephrol Dial Transplant*, 25, 2837–9.
Goodpasture, E. W. (2009). Landmark publication from The American Journal of the Medical Sciences: the significance of certain pulmonary lesions in relation to the etiology of influenza. *Am J Med Sci*, 338, 148–51.
Harvey, J. M., Howie, A. J., Lee, S. J., *et al.* (1992). Renal biopsy findings in hypertensive patients with proteinuria. *Lancet*, 340, 1435–6.
Heidet, L. and Gubler, M. -C. (2009). The renal lesions of Alport syndrome. *J Am Soc Nephrol*, 20, 1210–15.
Heptinstall, R. H. (1990). The development of renal pathology. *Am J Kidney Dis*, 16, 568–73.
Hildebrandt, F. (2010). Genetic kidney diseases. *Lancet*, 375, 1287–95.
Hirooka, M., Hirota, M., and Kamada, M. (1988). Renal lesions in Cockayne syndrome. *Pediatr Nephrol*, 2, 239–43.
Inoki, K., Mori, H., Wang, J., *et al.* (2011). mTORC1 activation in podocytes is a critical step in the development of diabetic nephropathy in mice. *J Clin Invest*, 121, 2181–96.
Izzedine, H., Launay-Vacher, V., Bourry, E., *et al.* (2006). Drug-induced glomerulopathies. *Expert Opin Drug Saf*, 5, 95–106.
Jennette, J. C., Olson, J. L., Schwartz, M. M., *et al.* (2007). Primer on the pathologic diagnosis of renal disease. In J. C. Jennette, J. L. Olson, M. M. Schwartz *et al.* (eds.) *Heptinstall's Pathology of the Kidney*, pp. 97–123. Philadelphia, PA: Lippincott.
Jennette, J. C. and Ordonez, N. G. (1983). Radiation nephritis causing nephrotic syndrome. *Urology*, 22, 631–4.
Johnson, R. J. and Couser, W. G. (1990). Hepatitis B infection and renal disease: clinical, immunopathogenetic and therapeutic considerations. *Kidney Int*, 37, 663–76.
Johnson, R. J., Rennke, H., and Feehally, J. (2000). Introduction to glomerular disease: pathogenesis and classification. In R. J. Johnson and J. Feehally (eds.) *Comprehensive Clinical Nephrology*, pp. 1263–70. Edinburgh: Mosby.
Kashtan, C. and Gubler, M. C. (2009). Inherited glomerular disease. In E. D. Avner, W. E. Harmon, P. Niaudet, *et al.* (eds.) *Pediatric Nephrology*, pp. 621–41. Berlin: Springer.
Kashtan, C. E. (1999). Alport syndrome. An inherited disorder of renal, ocular, and cochlear basement membranes. *Medicine*, 78, 338–60.
Kim, J. M., Wu, H., Green, G., *et al.* (2003). CD2-associated protein haploinsufficiency is linked to glomerular disease susceptibility. *Science*, 300, 1298–300.
Klebs, T. A. E. (1870). *Handbuch der pathologischen Anatomie*. Berlin: Hirschwald.
Klinger, H. (1931). Grenzformen der periarteriitis nodosa. *Frankfurt Pathol*, 42, 455.
Krantz, I. D., Piccoli, D. A., and Spinner, N. B. (1997). Alagille syndrome. *J Med Genet*, 34, 152–7.
Kyle, R. A. and Greipp, P. R. (1983). Amyloidosis (AL). Clinical and laboratory features in 229 cases. *Mayo Clin Proc*, 58, 665–83.
Longcope, W. T. (1929). The pathogenesis of glomerular nephritis. *Bull Johns Hopkins Hosp*, 45, 335–60.
Luft, F. and Dietz, R. (1993). Franz Volhard in historical perspective. *Hypertension*, 22, 253–6.
Luxton, R. W. (1961). Radiation nephritis. A long-term study of 54 patients. *Lancet*, 2, 1221–4.
Malpass, K. (2011). Peripheral neuropathies: new pathogenetic insights into Charcot-Marie-Tooth disease. *Nat Rev Neurol*, 7(9), 476.
Matteson, E. (2002). Historical perspective of vasculitis: polyarteritis nodosa and microscopic polyangiitis. *Curr Rheumatol Rep*, 4, 67–74.
Maynard, S. E. and Karumanchi, S. A. (2011). Angiogenic factors and preeclampsia. *Semin Nephrol*, 31, 33–46.
Mezzogiorno, A. and Mezzogiorno, V. (1997). Marcello Malpighi (1628–1694). *Am J Nephrol*, 17, 269–73.
Michaud, J. -L. R. and Kennedy, C. R. J. (2007). The podocyte in health and disease: insights from the mouse. *Clin Sci*, 112, 325–35.
Navarro-Gonzalez, J. F. and Mora-Fernandez, C. (2008). The role of inflammatory cytokines in diabetic nephropathy. *J Am Soc Nephrol*, 19, 433–42.
Paul, M. D., Fernandez, D., Pryse-Phillips, W., *et al.* (1990). Charcot-Marie-Tooth disease and nephropathy in a mother and daughter with a review of the literature. *Nephron*, 54, 80–5.
Reichel, H. (1905). *Ueber Nephritis bei Scharlach* [About nephritis in scarlet fever]. *Z Heil*, 6.
Ritz, E., Kuster, S., and Zeier, M. (1994). Clinical nephrology in 19th century Germany. *Am J Nephrol*, 14, 443–7.
Rodríguez-Iturbe, B. and Batsford, S. (2007). Pathogenesis of poststreptococcal glomerulonephritis a century after Clemens von Pirquet. *Kidney Int*, 71, 1094–104.
Ronco, P., Plaisier, E., Mougenot, B., *et al.* (2006). Immunoglobulin light (Heavy)-chain deposition disease: from molecular medicine to pathophysiology-driven therapy. *Clin J Am Soc Nephrol*, 1, 1342–50.
Rovin, B. H., Roncone, D., McKinley, A., *et al.* (2007). APOE Kyoto mutation in European Americans with lipoprotein glomerulopathy. *N Engl J Med*, 357, 2522–4.
Sanchorawala, V. (2006). Light-chain (AL) amyloidosis: diagnosis and treatment. *Clin J Am Soc Nephrol*, 1, 1331–41.

Schena, F. P. (1994a). Domenico Cotugno and his interest in proteinuria. *Am J Nephrol*, 14, 325–9.
Schena, F. P. (1994b). The role of Domenico Cotugno in the history of proteinuria. *Nephrol Dial Transplant*, 9, 1344–5.
Schwarz, U. and Ritz, E. (1997). Glomerulonephritis and progression—Friedrich Theodor von Frerichs, a forgotten pioneer. *Nephrol Dial Transplant*, 12, 2776–8.
Schwimmer, J. A., Markowitz, G. S., Valeri, A. M., *et al.* (2003). Secondary focal segmental glomerulosclerosis in non-obese patients with increased muscle mass. *Clin Nephrol*, 60, 233–41.
Sethi, S. (2008). Renal failure with intracapillary thrombi. Lipoprotein glomerulopathy. *Kidney Int*, 73, 1097–8.
Sibai, B. M. (2003). Diagnosis and management of gestational hypertension and preeclampsia. *Obstet Gynecol*, 102, 181–92.
Stratta, P., Canavese, C., Sandri, L., *et al.* (1999). The concept of 'glomerulonephritis'. The fascinating history of evolution and emergence of a specialist's nosology focus on Italy and Torino. *Am J Nephrol*, 19, 83–91.
Strom, E. H., Banfi, G., Krapf, R., *et al.* (1995). Glomerulopathy associated with predominant fibronectin deposits: a newly recognized hereditary disease. *Kidney Int*, 48, 163–70.
Strom, E. H., Sund, S., Reier-Nilsen, M., *et al.* (2011). Lecithin: cholesterol acyltransferase (LCAT) deficiency: renal lesions with early graft recurrence. *Ultrastruct Pathol*, 35, 139–45.
Tryggvason, K. and Patrakka, J. (2006). Thin basement membrane nephropathy. *J Am Soc Nephrol*, 17, 813–22.
Utimura, R., Fujihara, C. K., Mattar, A. L., *et al.* (2003). Mycophenolate mofetil prevents the development of glomerular injury in experimental diabetes. *Kidney Int*, 63, 209–16.
Waldherr, R. and Ritz, E. (1999). Edmund Randerath (1899–1961): experimental proof for the glomerular origin of proteinuria. *Kidney Int*, 56, 1591–6.
Watson, A. R., Harden, P., Ferris, M., *et al.* (2011). Transition from pediatric to adult renal services: a consensus statement by the International Society of Nephrology (ISN) and the International Pediatric Nephrology Association (IPNA). *Pediatr Nephrol*, 26(10), 1753–7.
Weavers, H., Prieto-Sanchez, S., Grawe, F., *et al.* (2009). The insect nephrocyte is a podocyte-like cell with a filtration slit diaphragm. *Nature*, 457, 322–6.
Weller, R. O. and Nester, B. (1972). Histological reassessment of three kidneys originally described by Richard Bright in 1827–36. *Br Med J*, 2, 761–3.
Wells, W. C. (1812). Observations on the dropsy that succeeds scarlet fever. *Trans Soc Imp Med Cir Knowledge*, 3, 167–86.
Welsh, G. I. and Saleem, M. A. (2010). Nephrin-signature molecule of the glomerular podocyte? *J Pathol*, 220, 328–37.
Wiggins, R. C. (2007). The spectrum of podocytopathies: a unifying view of glomerular diseases. *Kidney Int*, 71, 1205–14.
Wolf, G. (2000). Franz Volhard and his students' tortuous road to renovascular hypertension. *Kidney Int*, 57, 2156–66.
Woo, K.T., Glassock, R. J., and Lai, K. N. (2009). IgA nephropathy: discovery of a distinct glomerular disorder. In K. N. Lai (ed.) *Recent Advances in IgA Nephropathy*, pp. 1–7. Hong Kong: World Scientific Ebooks.
Woywodt, A., Haubitz, M., Haller, H., *et al.* (2006). Wegener's granulomatosis. *Lancet*, 367, 1362–6.
Zenker, M., Pierson, M., Jonveaux, P., *et al.* (2005). Demonstration of two novel LAMB2 mutations in the original Pierson syndrome family reported 42 years ago. *Am J Med Genet A*, 138, 73–4.
Zucchelli, P., Cagnoli, L., Casanova, S., *et al.* (1983). Focal glomerulosclerosis in patients with unilateral nephrectomy. *Kidney Int*, 24, 649–55.

CHAPTER 43

The renal glomerulus: the structural basis of ultrafiltration

Marlies Elger and Wilhelm Kriz

Introduction

The correct name for the structure to be described is 'renal corpuscle'; 'glomerulus" strictly refers only to the tuft of glomerular capillaries (glomerular tuft). However, the use of the term glomerulus for the entire corpuscle is widely accepted.

A renal corpuscle is made up of a tuft of specialized capillaries supplied by an afferent arteriole, drained by an efferent arteriole, and enclosed in Bowman's capsule (Figs 43.1 and 43.2). The entire tuft of capillaries is covered by the epithelial cells (podocytes), representing the visceral layer of Bowman's capsule. At the vascular pole, the visceral layer of Bowman's capsule transforms into the parietal layer, which is a simple squamous epithelium. At the urinary pole the parietal epithelium abruptly changes into the epithelium of the proximal tubule. The space between both layers of Bowman's capsule is called the urinary space that opens into the tubule lumen. The glomerular basement membrane (GBM) lies at the interface between the glomerular capillaries and the mesangium at one side and the podocytes at the other. At the vascular pole the GBM transforms into the multilayered basement membrane of the parietal epithelium of Bowman's capsule (parietal basement membrane (PBM)).

Renal corpuscles (glomeruli) are the first components of the nephrons. Thus, the number of nephrons exactly correlates with the number of renal corpuscles—in man, this is about 1 million in each kidney, in rat about 30,000 per kidney. Renal corpuscles are roughly spherical in shape, with a diameter of approximately 200 µm in man, and about 120 µm in rat. In rodents, juxtamedullary renal corpuscles are generally somewhat larger (by about 20%) than midcortical and superficial corpuscles (Tisher and Brenner, 1989; Kriz et al., 1992); however, this does not apply to the human kidney.

The glomerular tuft—a 'wonder net'

At the entrance to Bowman's capsule the afferent arteriole divides into several (three to five) primary capillary branches (Figs 43.1 and 43.3) (Yang and Morrison, 1980). Each of these branches gives rise to a capillary network (glomerular lobule) which runs towards the urinary pole, turns back towards the vascular pole, and unites with tributaries form the other lobules to form the efferent arteriole. The separation into lobules is not strict; near the vascular pole anastomoses are found interconnecting the lobules, but they are not formed between the afferent and efferent portions of the same lobule (Winkler et al., 1991).The efferent arteriole develops deep within the centre of the glomerular tuft from the confluence of tributaries from all lobules. Thus, in contrast to the afferent arteriole, the efferent arteriole has an intraglomerular segment. Upon leaving the glomerulus, the efferent arteriole is closely associated with the extraglomerular mesangium. The intraglomerular segment is completely surrounded by mesangium; a smooth muscle layer is gradually established until the efferent arteriole leaves the extraglomerular mesangium.

Topography of a glomerular lobule—glomerular capillaries are unique

The capillary network, together with the mesangium, is surrounded by the GBM followed by the visceral epithelium (podocytes). Deep invaginations of the GBM separate the tuft into lobules, less deep invaginations separate individual capillaries. Since the GBM does not completely encircle the capillary tube, a small portion of the endothelium is directly attached to the mesangium. The mesangial–endothelial interface (juxtamesangial portion) comprises only a small portion of the capillary circumference. The major part of the endothelial tube is in close contact with the GBM (pericapillary or peripheral portion) and the layer of interdigitating foot processes of the visceral epithelium. This part of the capillary wall represents the actual filtering surface (Fig. 43.4).

At the points where the capillaries come into contact with the mesangium, the GBM and the podocyte layer deviate from a pericapillary course and cover the mesangium; these points have been called mesangial angles. Therefore, two parts of the GBM and the visceral epithelium can be distinguished: a pericapillary part and a perimesangial part. The pericapillary part of the GBM is smooth and follows the outline of the capillary, whereas the perimesangial part is irregular in thickness and is frequently wrinkled.

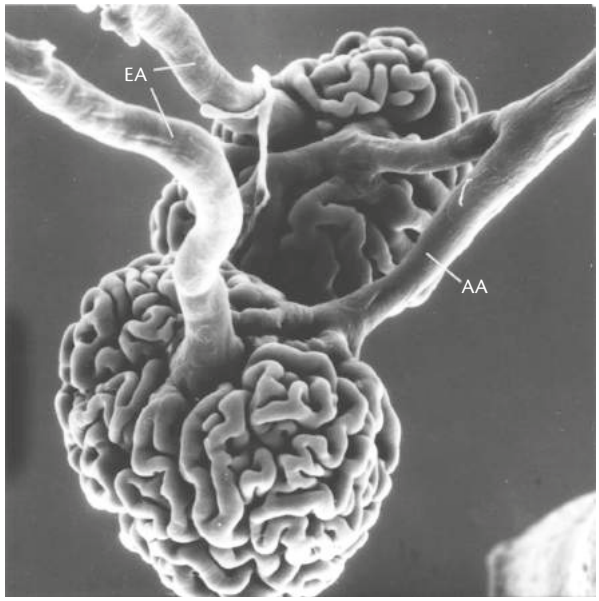

Fig. 43.1 Scanning electron micrograph (400×) showing a vascular cast of two juxtamedullary glomeruli (rat). Each capillary tuft is supplied by an afferent arteriole (AA) which, on the surface of the tuft, immediately divides into several branches. Efferent arterioles (EA) emerge out of the centre of the tuft.

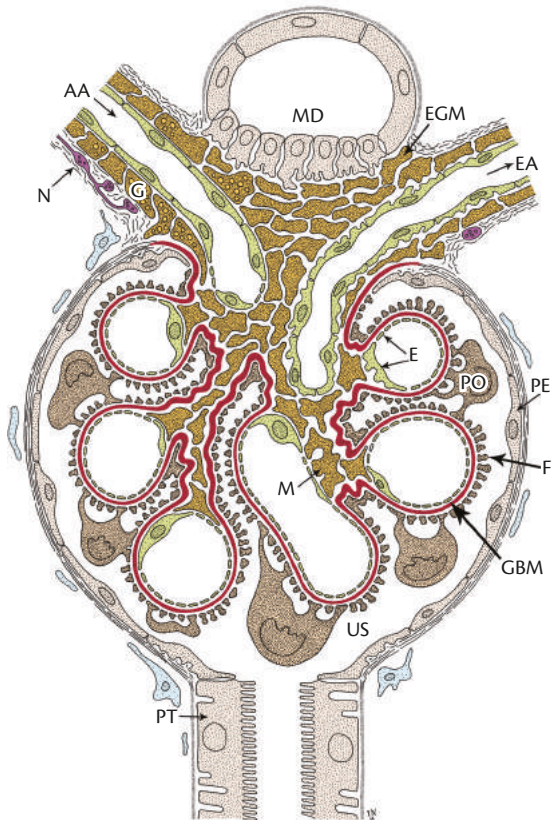

Fig. 43.2 Diagram of a longitudinal section through a renal corpuscle and the juxtaglomerular apparatus (JGA). The capillary tuft consists of a network of specialized capillaries, which are outlined by a fenestrated endothelium (E). At the vascular pole an afferent arteriole (AA) enters and an efferent arteriole (EA) leaves the tuft. The capillary network is surrounded by Bowman's capsule, comprising two different epithelia: the visceral and the parietal epithelium. The visceral epithelium consisting of highly branched podocytes (PO) directly follows—together with the glomerular basement membrane (GBM)—the surface of the capillaries and the mesangium (M). At the vascular pole, the visceral epithelium and the GBM are reflected into the parietal epithelium (PE) of Bowman's capsule (and its basement membrane), which passes over into the epithelium of the proximal tubule (PT) at the urinary pole. Mesangial cells (M) are situated in the axes of glomerular lobules. At the vascular pole the glomerular mesangium is continuous with the extraglomerular mesangium (EGM), consisting of cells and matrix. The EGM, together with the terminal portion of the afferent arteriole (containing the granular cells, G), the efferent arteriole, and the macula densa (MD), establish the JGA. All cells that are suggested to be of smooth muscle origin are shown in a dark colour. F = foot processes; N = sympathetic nerve terminals; US = urinary space.

Capillary endothelium—a perforated, highly charged structure

The capillary tube is made up of a particular kind of fenestrated endothelial cells (Fig. 43.4). The 'fenestrae' actually represent round to oval pores of varying in size from 50 to 100 nm in diameter (Fig. 43.5). Unlike endothelia with diaphragm-bridged fenestrae at other sites of the body (e.g. peritubular capillaries) the endothelial pores in mature glomerular capillaries lack a diaphragm corroborated by the lack of immunreactivity to the glycoprotein PV-1 (Stan et al., 2004). In the glomerulus an endothelium with diaphragm-bridged fenestrae is only found in the direct tributaries to the efferent arteriole. For details, see the work by Elger and colleagues (Elger et al., 1991; Elger et al., 1998).

PV-1 is considered an essential molecule to the formation of both stomatal (caveolar) and fenestrial diaphragms (Stan, 2005). Both of them as well as PV-1-positive endothelial cells are abundant during development of glomerular endothelia (Ichimura et al., 2008). Thus, the formation of transendothelial pores lacking diaphragms is preceded by the formation of fenestrae with diaphragms (Satchell et al., 2009). The same is true during the recapillarization of the glomerular tuft, for example, in Thy-1 nephritis. Of note, the formation of glomerular endothelial fenestrae is independent of caveolin-1 (a caveolar protein) indicating that fenestrae do not develop from caveolae (Drab et al., 2001; Sörensson et al., 2002).

The cell bodies of endothelial cells are generally located adjacent to the mesangial interface. The peripheral flat 'porous' parts of the cells comprise about 60% of the capillary surface. Individual pores are encircled by a network of microfilaments (Vasmant et al., 1984). Clusters of pores are separated by ridges of cytoplasm, containing intermediate filaments and microtubules. Pores occupy about 13% of the capillary surface in the rat (Bulger et al., 1983); in absolute terms, the total area of all pores in a rat glomerulus amounts to about 22 mm^2 (Larsson et al., 1980). Under clinical settings, loss of endothelial pores correlates with decrease of glomerular filtration rate, as was shown in diabetes type 1 and type 2 and in pre-eclampsia (Lafayette et al., 1998; Toyoda et al., 2007; Weil et al., 2011; Salmon and Satchell, 2012; Satchell, 2012).

A layer of membrane-bound and loosely attached molecules covers the capillary endothelium on its *luminal* side. Depending on the visualizing technique it amounts to a thickness of about 300–500 nm and covers the entire surface including the endothelial pores (Haraldsson et al., 2009). This entire layer may be called

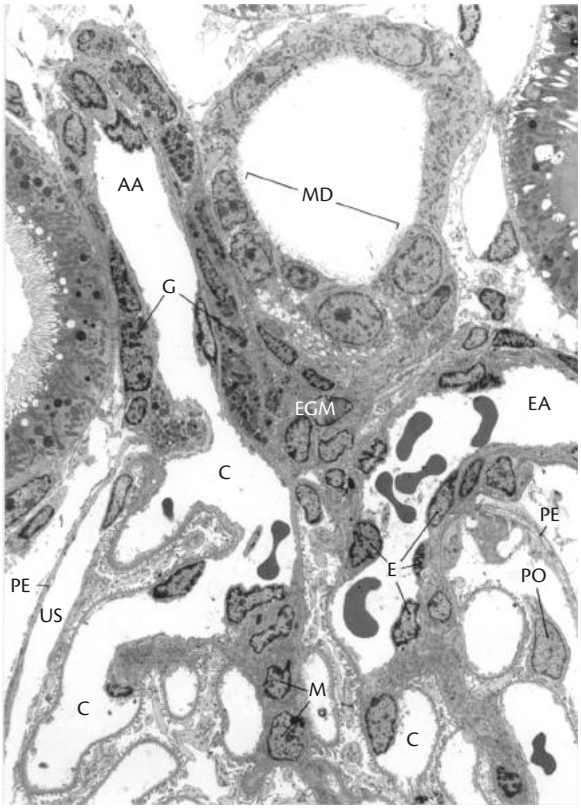

Fig. 43.3 Longitudinal section through the glomerular vascular pole showing the juxtaglomerular apparatus with both arterioles (rat). At the entrance into the glomerulus, the afferent arteriole (AA) immediately branches into capillaries (C). The efferent arteriole (EA) usually arises deeper in the tuft and can be identified by the high number of endothelial cells (E) at the exit from the glomerulus. The macula densa (MD) of the thick ascending limb is in contact with the extraglomerular mesangium (EGM) and the glomerular arterioles. The media of the AA contains granular cells (G). M, mesangial cells; PE, parietal epithelium; PO, podocytes; US, urinary space. Transmission electron micrograph (1300×).

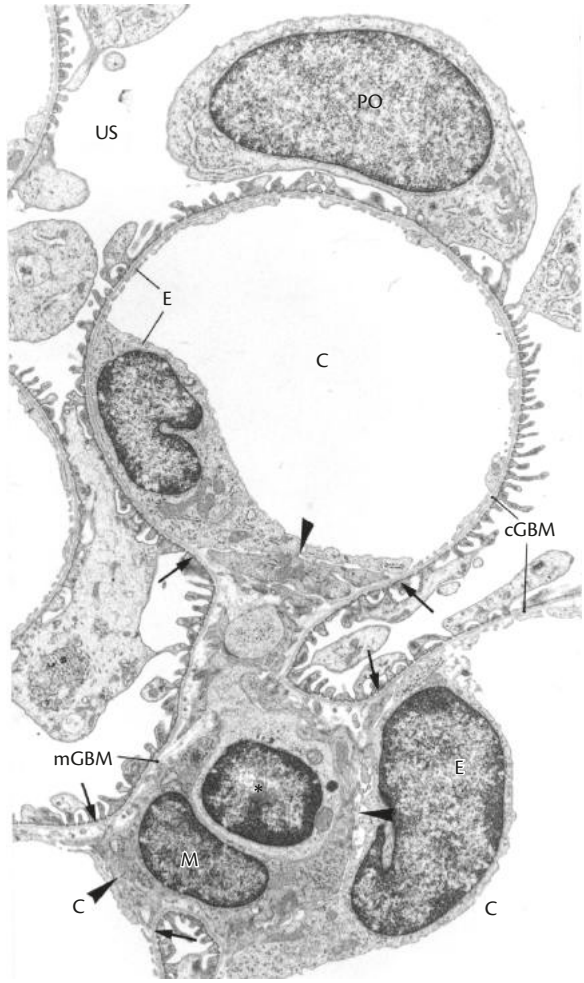

Fig. 43.4 Part of a glomerular lobule (rat), showing the arrangement of structures in the glomerular tuft. The capillary (C) is outlined by a flat fenestrated endothelium (E). The podocyte layer (PO) and the glomerular basement membrane (GBM) do not encircle the individual capillary completely, they form a common surface cover around the lobule. In the peripheral portion of the capillary the filtration barrier is formed (see also Fig. 43.5). Two subdomains of the GBM are delineated from each other by mesangial angles (arrows): the pericapillary GBM (cGBM) faced by the podocyte layer and the endothelial layer, and the perimesangial GBM (mGBM) bordered by the podocyte layer and the mesangium. Within the mesangium two types of cells are shown: contractile mesangial cells (M) and a cell (*) which is probably a macrophage that has invaded the mesangium. Note the intimate relationships between the endothelium and the mesangium (arrowheads). US = urinary space. 6100×.

glycocalyx. Due to polyanionic glycoproteins it is negatively charged (Horvat et al., 1986; Sawada et al., 1986).

The components of the glycocalyx proper (Pries et al., 2000) are covalently bound to the endothelial cell membrane. The thickness of this sublayer amounts to about 50–100 nm and is composed of the membrane-bound proteoglycans such as glypican (with heparan sulphate side chains) and syndecan (with chondroitin and heparan sulphate side chains) (Haraldsson et al., 2008). Attached to components of the glycocalyx proper are secreted proteoglycans, for example, versican and perlecan, hyaluronan (a non-sulphated glycosaminoglycan), as well as adsorbed plasma proteins (including orosomucoid and albumin) (Haraldsson et al., 2008). This layer of more loosely attached components is vulnerable, for example, by haemodynamic factors (Ryan et al., 1976; Fridén et al., 2011; Haraldsson et al., 2012) it can be visualized by intravital (Desjardins et al., 1990) and electron microscopic techniques (Rostgaard et al., 2002; Hjalmarsson et al., 2004).

Damage to the glomerular endothelial glycocalyx is clinically apparent as albuminuria (Obeidat et al., 2012; Salmon and Satchell, 2012) (see below). Experimentally, degradation of glycosaminoglycans in glomerular capillaries increases the clearance of albumin (Jeansson et al., 2006), and administration of hyaluronidase causes proteinuria (Meuwese et al., 2010). Removal of sialic acid residues results in albuminuria (Gelberg et al., 1996; Bakker et al., 2005). Elution of non-covalently bound components of the glomerular endothelial glycocalyx caused a 12-fold increase in the fractional clearance of albumin (Fridén et al., 2011). Thus, both soluble and bound components of the endothelial glycocalyx determine permeability of the glomerular filtration barrier.

Endothelial cells are active participants in processes controlling coagulation, inflammation, and immune processes, and an aberration in the controlling mechanisms may contribute to the development of disease within the glomerulus (Savage, 1998). Renal endothelial cells share an antigen system with cells of the monocyte/macrophage lineage; they express surface antigens of the

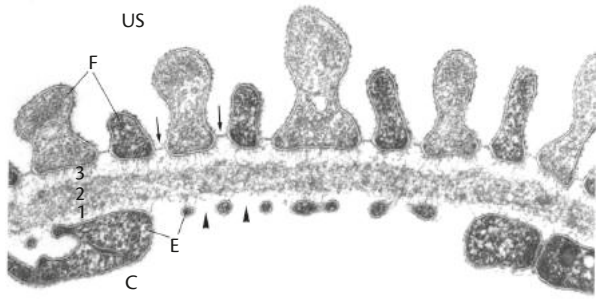

Fig. 43.5 Filtration barrier. The peripheral part of the glomerular capillary wall comprises the fenestrated endothelial layer (E), the glomerular basement membrane, and the interdigitating foot processes (F). The filtration slits between the foot processes are bridged by thin diaphragms (long arrows). Arrowheads point to the endothelial pores. The glomerular basement membrane shows a lamina densa (2) bounded by the lamina rara interna (l) and the lamina rara externa (3). In this picture, tannic acid staining allows discrimination between the alternating foot processes of two neighbouring podocytes: the more densely stained processes belong to one cell, and the others to the neighbouring cell. C = capillary lumen. 60,000×.

class II histocompatibility antigens. Similar to platelets, glomerular endothelial cells contain components of the coagulation pathway and are capable of binding factors IXa and Xa, and of synthesizing, releasing, and binding von Willebrand factor (factor VIII) (Wiggins et al., 1989). Glomerular endothelial cells synthesize and release endothelin-1 and endothelium-derived relaxing factor (EDRF) (Ott et al., 1993; Herman et al., 1998).

Visceral epithelium—filtration slits are formed by interdigitating foot processes

The visceral epithelial layer of Bowman's capsule consists of highly differentiated cells, the podocytes (Kriz et al., 2013a). The podocytes are attached to the outer surface of glomerular capillaries, that is, to the GBM only by their processes, their cell bodies float within the filtrate in Bowman's space. In the developing glomerulus, the visceral epithelium consists of simple polygonal cells. In rat, mitotic activity of these cells is completed soon after birth, along with the cessation of the formation of new nephron anlagen (Nagata et al., 1993), and the final number of podocytes is determined. In humans this point is reached during prenatal life. Differentiated podocytes are unable to replicate (Fries et al., 1989; Nagata and Kriz, 1992; Kriz, 2002), thus, in the adult degenerated podocytes cannot be replaced. In response to an extreme stimulation (long-term treatment with FGF-2) the nucleus may enter into mitosis; however, the cells are not able to complete cell division (cytokinesis), usually resulting in mitotic catastrophe or, at best, in binucleated cells (Kriz et al., 1995b). Recent evidence that there may be podocyte progenitors at the junction of the parietal stem cells with proximal tubular cells (see Chapter 344) notwithstanding, there is little evidence for significant replacement in adults.

Podocytes have a voluminous cell body, which bulges into the urinary space (Figs 43.6 and 43.7). Long primary processes emerge from the cell body and extend towards the capillaries, to which they affix by their distal portions and their final ramifications the so-called foot processes. The foot processes of neighbouring podocytes regularly interdigitate with each other, leaving between them

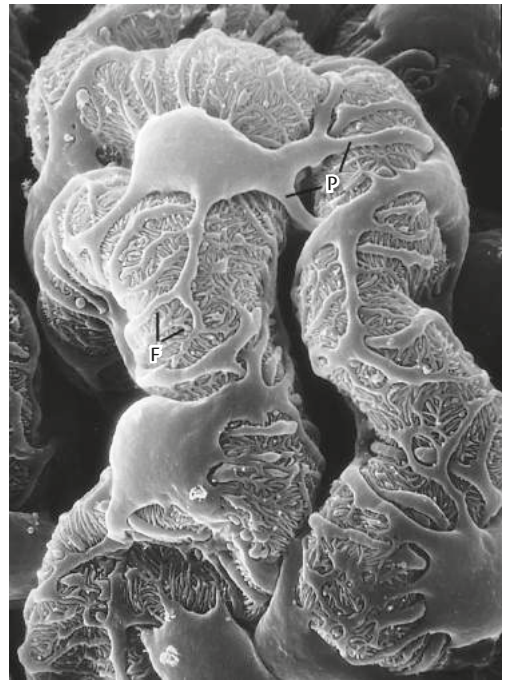

Fig. 43.6 Scanning electron micrograph (3300×) of rat glomerular capillaries. The urinary side of the capillary is covered by the highly branched podocytes. The interdigitating system of primary (P) and secondary (F) processes lines the entire surface of the glomerular basement membrane and proceeds also beneath the cell bodies (see Fig. 43.7). In between the interdigitating foot processes (F) of neighbouring cells the filtration slits are spared.

meandering slits (filtration slits), which are bridged by a thin proteinaceous membrane (slit membrane or slit diaphragm).

Podocytes are polarized epithelial cells with a luminal (apical) and an abluminal (basal) cell membrane. The basal domain corresponds to the sole plates of the foot processes, which are embedded into the GBM up to approximately 60 nm. The border between basal and luminal membranes is represented by the slit diaphragm.

The *luminal membrane*, including the slit diaphragm, is covered by a thick surface coat, which is rich in sialoglycoproteins that are responsible for the high negative surface charge of podocytes. They include podoendin (Huang and Langlois, 1985), SGP115/107 (Mendrick and Rennke, 1988), and podocalyxin (Kerjaschki et al., 1984; Sawada et al., 1986), which via the linker protein NHERF2 (Na^+/H^+-exchanger regulatory factor 2) and ezrin is attached to the actin cytoskeleton. The surface charge of podocytes contributes to the maintenance of the interdigitating pattern of the foot processes. In response to neutralization of the surface charge by cationic substances (e.g. protamine sulphate, poly-l-lysin), the glomerular epithelium undergoes a series of changes, including retraction of foot processes and formation of adhesive junctions between adjacent foot processes (Seiler et al., 1977).

Podocytes display many surface receptors and ion channels that are generally found on the entire surface of podocytes, many of them accumulate close to the slit membrane; the schematic in Fig. 43.9 shows some of them. They include receptors for angiotensin II (AT1 and AT2; Nitschke et al., 2000; Sharma et al., 2001), noradrenaline (α1; Huber et al., 1998), acetylcholine (M5; Nitschke et al., 2001), prostaglandin (Bek et al., 1999), ATP (Fischer et al., 2001), endothelin (ETA; Rebibou et al.,

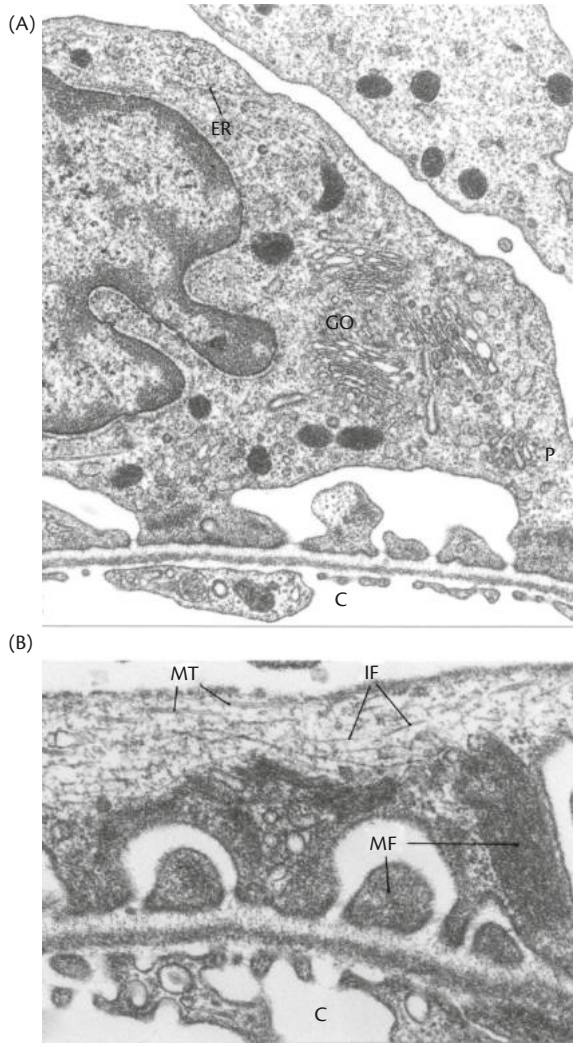

Fig. 43.7 (A) Electron micrograph (25,000×) showing part of a podocyte cell body anchored via processes to the glomerular basement membrane. The majority of these processes are foot processes. The others are ridge-like bases of the cell body and distal portions of primary processes (P). Note the prominent Golgi apparatus (GO); also rough endoplasmic reticulum (ER) is fairly abundant. C = capillary lumen. (B) Primary and secondary processes of podocytes showing cytoskeletal elements. Intermediate filaments (IF) and microtubules (MT) are abundant in the primary processes; thick bundles of microfilaments (MF) are located in the foot processes. C = capillary lumen. 46,000×.

1992; Spath et al., 1995), ANP (Zhao et al., 1994), PTH/PTHrP (Jorgensen, 1966; Endlich et al., 2001b), TGFβ (Yamamoto et al., 1998), IL-4/IL-13 (van den Berg et al., 2000), FGF-2 (Ford et al., 1997), C3b-receptor (Kazatchkine et al., 1982), and gp330/megalin (Kerjaschki and Farquharl, 1983). Megalin, a glycoprotein of 330 kDa (Saito et al., 1994) is the major podocyte antigen of rat Heymann nephritis; in man megalin is lacking.

In addition to non-specific cation channels (NSCC), the specific Ca^{2+} channels TRPC5 and TRPC6 (Greka et al., 2012) are currently in discussion as important regulators of foot process dynamics.

The cell body contains a prominent nucleus, a well-developed Golgi apparatus, abundant rough and smooth endoplasmic reticulum, prominent lysosomes, and mitochondria (Fig. 43.7A). In contrast to the cell body, the cell processes contain only a few organelles. The organelles in the cell body indicate a high level of anabolic as well as catabolic activity. In addition to the work necessary to sustain structural integrity of these specialized cells, most components of the GBM are synthesized by the podocytes (Abrahamson, 1987, 2012). The cell contains a well-developed cytoskeleton. In the cell body and the primary processes, microtubules and intermediate filaments (vimentin, desmin) dominate (Fig. 43.7B). Microfilament bundles containing actin, myosin, and α-actinin are found in a highly organized pattern in the foot processes. In addition, in the cell body and the primary processes, microfilaments are seen as a thin layer underlying the cell membrane (Bachmann et al., 1983; Vasmant et al., 1984; Drenckhahn et al., 1988; Kriz et al., 1995a).

Bundles of microtubules and intermediate filaments extend from the cell body to the distal end of the primary processes. The microtubules are of mixed polarity, which appears to be essential for process formation of podocytes (Kobayashi et al., 1998). As intracellular transport systems the microtubules are probably involved in the transport of substances (e.g. for the GBM) to the peripheral parts of the cell. Another important function of the microtubules and intermediate filaments may be found in their cytoskeletal properties in relation to mechanical forces. Microtubules resist compression of their long axis and, as bundles interconnected by microtubule-associated proteins, they withstand bending forces. Intermediate filaments, on the other hand, are able to resist tensile forces (Wang et al., 1993).

In the foot processes, a prominent actin based contractile apparatus is present consisting of bundles of microfilaments running longitudinally through the processes. At the transition to the primary processes, the microfilament bundles form loops that are connected to the microtubules of the primary processes. Peripherally, the microfilament bundles anchor in the dense cytoplasm associated with the inner aspect of the basal cell membrane (Fig. 43.8).

The bases of the foot processes are firmly attached to the GBM, mediated by integrins and dystroglycans (Fig. 43.9). The integrin complex is comprised mainly of α3β1 integrin dimers, which are connected inside the cell to the complex of vinculin, paxillin, and talin, and outside the cell to collagen IV α3, α4, and α5 chains as well as to laminin α5/β2/γ1 (Miner, 1999; Kreidberg and Symons, 2000). In addition, further integrin dimers have been found (Schordan et al., 2010). The dystroglycan complex (Durbeej et al., 1998; Raats et al., 2000; Regele et al., 2000) consists of the cytoplasmic adaptor protein utrophin, of the transmembranous β-dystroglycan, and of the extracellular matrix-binding α-dystroglycan which is a receptor for agrin and laminin α5 chains. In addition to the important mechanical relevance of these connections (which is insufficiently understood), outside-in signalling via these systems quite obviously influences the function of the cytoskeleton which, in cases of any stress to the podocytes, may lead to foot process effacement (FPE) (see below).

The filtration slits are the site of convective fluid flow through the visceral epithelium. The total length of the filtration slit in a rat glomerulus amounts to about 50 cm (Kriz et al., 1995a; Mundel and Kriz, 1995). Since the slit has a rather constant width of 30–40 nm, the total area of the slit membrane approximates 20 $\mu m^2 \times 10^3$, comprising about 10–13% of the peripheral capillary surface.

The structure and biochemical composition of the slit membrane are still incompletely understood. Chemically fixed and tannic acid treated tissue reveals a zipper-like structure with a row of 'pores' (approximately 4 × 14 nm in size on either side of a central bar

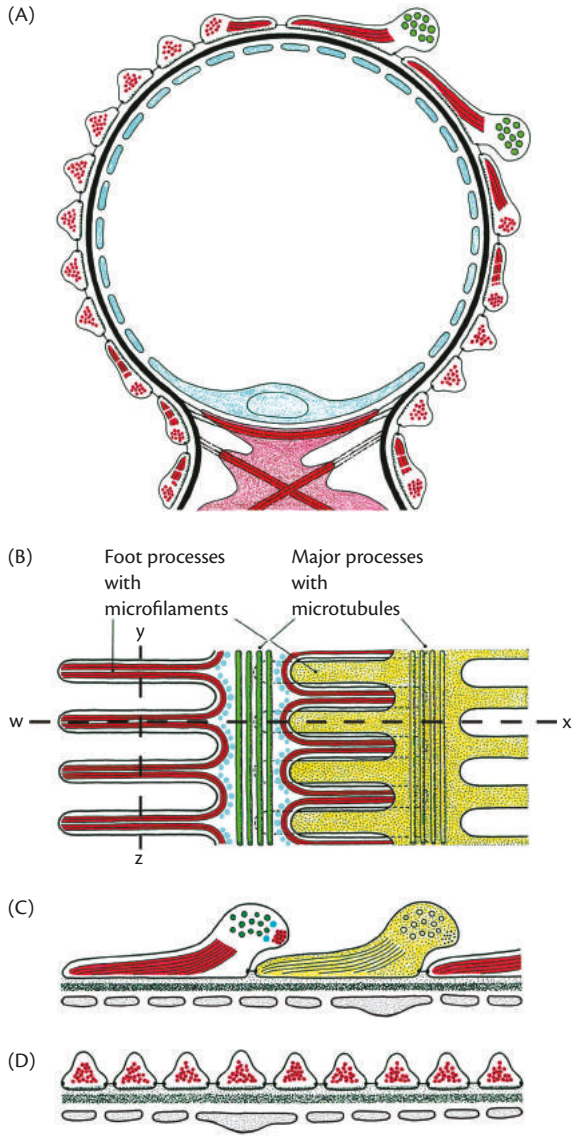

Fig. 43.8 Arrangement of cytoskeletal elements in podocyte processes: (A) as seen in a cross section through a capillary (B) view from above; (C) section of foot processes parallel (along the line w to x) and (D) perpendicular (along the line y to z) to the longitudinal axis of foot processes. Two major processes (one in white, one in yellow) with their foot processes are shown. The actin filaments (red) of foot processes form continuous loops which terminate in the foot process sole plates. At their bends they are in close association with microtubules (green) that run longitudinally in the major processes.
After Mundel et al. (1995).

(Rodewald et al., 1974). In quick-frozen tissue, a more homogeneous structure with only a central bar is apparent (Hora et al., 1990). Proteins that establish the slit membrane include nephrin, Neph1, 2, and 3, p-cadherin, and FAT. Presently, it is not clear how these proteins participate in the molecular organization of this structure. The cytoplasmic tails of these proteins allow a dynamic connection of the slit membrane to the cytoskeleton. Proteins that mediate and/or regulate this connection include ZO1, α-, ß-, γ-catenins, podocin, CD2AP, and α-actinin 4. Fig. 43.9 summarizes some main features of the molecular organization of podocyte foot processes (modified after Endlich et al., 2001a).

Relevance of podocytes

It is difficult to precisely define the function of podocytes; they are still a mysterious cell type. Generally, they have been considered as a modified type of a pericyte with specialized intercellular junctions, that is, the slit diaphragm; thus, as a supportive cell that, in addition, controls paracellular permeability. The pericyte function, counteracting the distension of the capillary wall, has been seriously challenged in recent time (Kriz et al., 2013b; see below). The most important function is provided by the slit diaphragm that essentially contributes to the barrier function of the glomerular filter. We will discuss this function below together with the possible relevance of the other layers of the glomerular barrier.

As seen during glomerular development podocytes have the exclusive commandership in building a glomerulus. In the adult, they are responsible for the maintenance of the complex structure of the glomerular tuft, with vascular endothelial growth factor A (VEGF-A) playing the major role in the regulatory processes (Kriz, 2007). As shown recently in a transgenic model with deletion of β-catenin (Grouls et al., 2012) podocytes develop within the parietal epithelium of Bowman's capsule followed by formation of a small but complete tuft-like structure consisting of a proper capillary supported by a mesangium, a GBM and covered by an interdigitating foot process layer.

Relevance of podocytes in glomerular pathology

Podocytes are terminally differentiated cells incapable of replicating. Thus, lost podocytes cannot be replaced by proliferation of neighbouring undamaged cells. We are born with a certain number of podocytes, roughly 800 per glomerulus in the 2 million nephrons of the two kidneys. So far there is little evidence for much replenishment of this population from progenitors in adults (see Chapter 344). The only way to immediately compensate for lost podocytes consists of cell hypertrophy in order to cover the glomerular tuft with a smaller number of podocytes. This mechanism, however, increases their vulnerability to any challenge.

Moreover, podocytes live in a precarious situation, being fixed to the outer aspect of glomerular capillaries only by their processes, their cell bodies float in the filtrate in Bowman's capsule. This exposes podocytes to the danger of being lost by detachment. Indeed, podocytes are continually excreted as viable cells in the urine, and the rate of excretion dramatically increases in glomerular diseases (Hara et al., 1995, 1998; Nakamura et al., 2000; Vogelmann et al., 2003; Petermann et al., 2004; Yu et al., 2005; Weil et al., 2011). This fully agrees with structural findings in many models of glomerular disease showing that podocytes detach as viable cells from the GBM (Kriz et al., 2013b).

This precarious situation leads to a very specific set of reactions of podocytes to challenges that may be interpreted as to serve for a stronger adhesion to the GBM, thus counteracting detachment and loss into the urine. FPE, that is, the retraction of the foot processes into the primary processes, finally into the cell bodies, can be seen as a mechanism to promote the adherence of podocytes to the GBM. Since FPE seems to be inevitably associated with proteinuria we come to the conclusion that changes in the slit diaphragm are secondary to FPE, and that the associated defect in the filtration

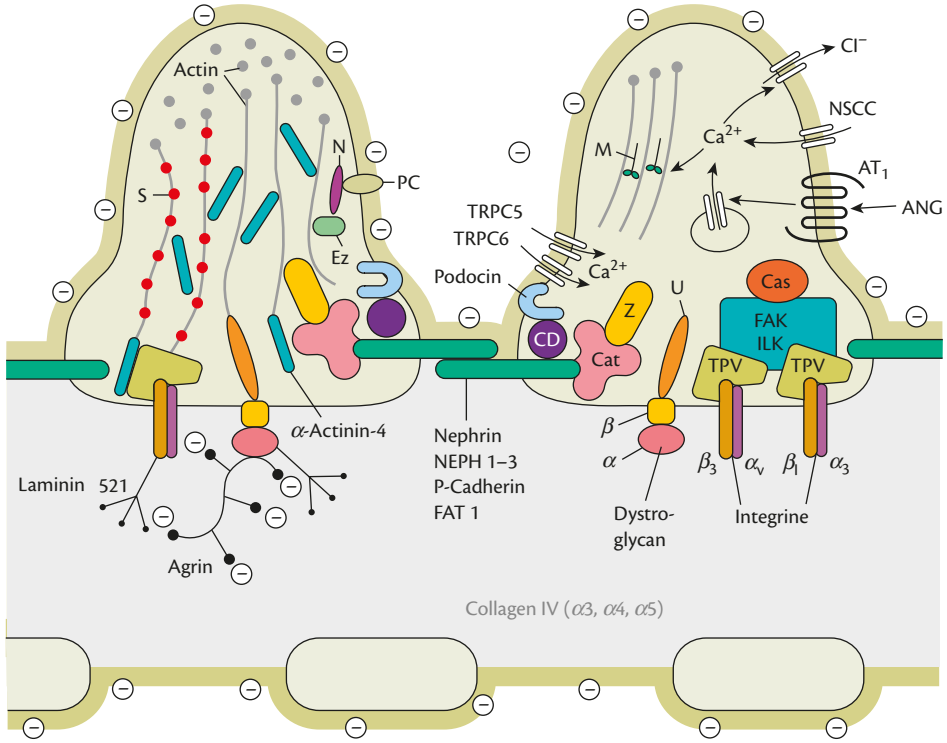

Fig. 43.9 Glomerular filtration barrier. Two podocyte foot processes bridged by the slit membrane, the GBM, and the porous capillary endothelium are shown. The surfaces of podocytes and of the endothelium are covered by a negatively-charged glycocalyx containing the sialoprotein podocalyxin (PC). The GBM is mainly composed of collagen IV (α3, α4, and α5), of laminin 11 (α5, β2, and γ1 chains) and the heparan sulphate proteoglycan agrin. The slit membrane represents a porous proteinaceous membrane composed of (as far as is known) Nephrin, Neph1, 2, and 3, P-cadherin, and FAT1. The actin-based cytoskeleton of the foot processes connects to both the GBM and the slit membrane. With regard to the GBM, β1/α3 integrin dimers specifically interconnect the TVP complex (talin, paxillin, vinculin) to laminin 11; the β and α dystroglycans interconnect utrophin to agrin. The slit membrane proteins are joined to the cytoskeleton via various adaptor proteins, including Podocin, Zonula occludens protein 1 (ZO-1; Z), CD2 associated protein (CD), and catenins (Cat). The Ca^{2+} channels TRPC6 and TRPC5 are concentrated at the slit membrane. Among the many surface receptors only the angiotensin II (ANG II) type 1 receptor (AT1) is shown. Cas= P130Cas; Ez = ezrin; FAK = focal adhesion kinase; ILK = integrin-linked kinase; M = myosin; N = NHERF2 (Na^+/H^+-exchanger regulatory factor); NSCC = non-selective cation channel; S = synaptopodin.
Modified from Endlich et al. (2001a).

barrier leading to albumin leakage is, under stressful situations, not a top priority for the podocytes. The survival of the podocyte is more important and protein leakage is, at least in part, merely an epiphenomenon that occurs in many situations when podocytes under stress make adaptations to resist detachment.

These two weaknesses of podocytes (inability to replicate and the danger of being lost by detachment) are responsible that loss of podocytes occupies centre stage in glomerular pathology. The progressing loss of podocytes is essentially responsible for the progression of chronic renal disease to end stage (see Chapter 139).

Glomerular basement membrane—the backbone of the glomerular tuft

The GBM represents the skeletal backbone of the glomerular tuft (Fig. 43.4). In transmission electron micrographs of traditionally fixed tissue, the GBM appears as a trilaminar structure made up of a lamina densa bounded by two less dense layers—the lamina rara interna and externa (Fig. 43.5). In humans, the thickness of the GBM ranges from 300 to 370 nm (Steffes et al., 1983); in children it may be considerably thinner (Morita et al., 1988). In rats and other experimental animals, the thickness is between 110 and 190 nm (Rasch, 1979; Bulger et al., 1988).

In accordance with basement membranes at other sites, the major components of the GBM include type IV collagen, heparan sulphate proteoglycans, and laminin (Mohan and Spiro, 1986; Timpl and Dziadek, 1986). Types V and VI collagen, and entactin/nidogen have also been demonstrated. On the other hand, the GBM has many unique properties, notably a distinct spectrum of type IV collagen and laminin isoforms (Couchman et al., 1994; Miner, 1999; Abrahamson, 2012), described in Chapter 320.

At early stages of glomerulogenesis, only α1 and α2 chains are detected, but as the glomerular capillaries begin to mature, there is a gradual increase in α3, α4, and α5 chains (Miner et al., 1994). Consistent with the distribution of the isoforms, podocytes—but not endothelial cells—synthesize the α3α4α5 (345) network (St John and Abrahamson, 2001; Abrahamson et al., 2009), whereas the α1 and α2 chains (112 network) likely derive from the glomerular endothelial cells (Miner, 2011)

At early stages of glomerulogenesis, Laminin LM-111 and LM-511 are the major laminin components (Miner et al., 1997). However, as the capillaries begin to mature, LM-521 begins to be deposited by the podocytes and endothelial cells, and LM-111 and -511 are eventually eliminated (Miner et al., 1994; Miner et al., 1997). Mice lacking β2 develop massive proteinuria and FPE, and die during early postnatal life, known in humans as

Pierson syndrome (Hansen and Abrass, 1999; Miner, 1999; Zenker et al., 2004).

The major role in mediating the interconnection of the various components of the GBM (as well as the connection to the surface receptors of podocytes and endothelial cells) is played by laminin (and entactin/nidogen).

The relative amount of components varies between basement membranes of different sources. The GBM contains more type IV collagen and less laminin than does the renal tubular basement membrane (Brees et al., 1995). Because of covalent cross-linking, the type IV collagen network provides a stronger scaffolding to render the basement membrane more resilient and permanent than a laminin polymer (Yurchenco and Cheng, 1994). The higher content of type IV collagen in the GBM than in the tubular basement membrane may be indicative of a greater tensile strength of the GBM adapted to the high transmural pressure differences at this site.

Filtration barrier—filtration occurs along an extracellular pathway

The filtration barrier is composed of (a) the endothelium with large open pores, (b) the dense matrix network of the GBM, and (c) the slit diaphragms between the podocyte foot processes.

Compared with the barrier established in capillaries elsewhere in the body, there are at least two outstanding characteristics of the filtration barrier in the glomerulus: the permeability for water, small solutes, and ions is extremely high, while the permeability for plasma proteins the size of albumin and larger is very low.

The high hydraulic permeability is easily explained by the fact that filtration occurs along extracellular routes. All components of this route, the endothelial pores (including the glycocalyx), the highly hydrated GBM, and the slit membrane can be expected to be quite permeable for water and small solutes. The hydraulic conductance of the individual layers of the filtration barrier is difficult to examine. In a mathematical model of glomerular filtration the hydraulic resistance of the endothelium was predicted to be small, whereas the GBM and filtration slits each contributed roughly one-half of the total hydraulic resistance of the capillary wall (Drumond and Deen, 1994).

Harper argues that the existence of a sub-podocyte space should alter our views on free filtration beyond the slit diaphragm (Salmon et al. 2009).

In pathological models as well as in human glomerulopathies such as membranous nephropathy or minimal change nephropathy, FPE leads to a drastic reduction of the overall filtration slit length. This decrease in slit length (or slit frequency) is correlated with a decrease in the ultrafiltration coefficient, K_f, (Kiberd, 1992; Guasch and Myers, 1994). The decrease in total slit membrane area also causes an increase in the average path length for the filtrate through the GBM, thereby explaining the decreased hydraulic permeability in these nephropathies (Drumond et al., 1994).

The barrier function for macromolecules is based on the size, shape, and charge of the respective molecule (reviewed in Daniels et al., 1993; Deen et al., 2001); the relevance of each of these parameters is in debate.

Our interpretation of the available data is that a size/shape barrier for very large molecules (effective radii of > 4.0 nm) is provided by the slit membrane (Deen et al., 2001). Since most of plasma proteins, including albumin, are negatively charged, their repulsion is mainly charge dependent.

The size/shape selectivity for macromolecules of the filtration barrier seems to be established by the slit membrane (Deen et al., 2001). Uncharged macromolecules up to an effective radius of 1.8 nm pass freely through the filter. Larger compounds are more and more restricted (indicated by their fractional clearances which progressively decrease), and are totally restricted at effective radii of > 4.0 nm. The term 'effective radius' is an empirical value, measured in artificial membranes, which takes into account the shape of macromolecules and attributes a radius to non-spherical molecules. Plasma albumin has an effective radius of 3.6 nm; without the repulsion due to the negative charge, plasma albumin would pass through the filter in considerable amounts (see below) (Deen et al., 1979). The importance of the slit diaphragm for size selectivity is evidenced by experiments with ferritin (radius 6.1 nm). Whereas anionic ferritin particles accumulate at the level of endothelial fenestrae and the subendothelial space, cationized ferritin penetrates the lamina densa and accumulates beneath the slit diaphragm.

The charge barrier may be seen to consist of two components. First, negatively charged molecules are accumulated throughout the entire depth of the filtration barrier, including the surface coats of endothelial and epithelial cells, and the high content of negatively charged heparan sulphate proteoglycans in the GBM. The glycocalyx/surface coat of the endothelium seems to be most effective, establishing an electronegative shield at the entry side (discussed in detail above in conjunction with the endothelium). Polyanionic macromolecules in the plasma, such as albumin, are repelled by these assemblies of negatively charged molecules, but the effect is not complete. A minor portion of such molecules penetrates into the filter and the mechanism how it is prevented from being excreted into the urine is under debate.

A recent study by the group of Marcus Moeller (Hausmann et al., 2010) proposes an electrophoretic mechanism for the repulsion of this fraction of negatively charged macromolecules. According to their hypothesis (based on direct measurements in the Necturus kidney) the convective flow of the filtrate through the filter creates a potential difference that increases the negativity of the urinary side of the glomerular filter compared to the capillary side by up to −0.05 mV. Thus, albumin molecules that enter the filter will, on their way through the filter, be exposed to increasingly negatively charged surroundings. Thereby, they will be repelled at various depths in the filter and forced to diffuse back into the capillary. The charm of this hypothesis consists of being independent of any structural pore size. The barrier consist of a strictly filtration dependent potential difference. Thus, without sufficient convective flow of filtrate no barrier will be created. Among other attractive features this might help explain the so-called orthostatic proteinuria that is observed in patients only in upright position but not when supine (Chapter 51): a low perfusion pressure does not generate sufficient flow through the filter to create a charge potential.

Mesangium—maintenance of the structural integrity of the tuft

The mesangium occupies the axial region of a glomerular lobule and consists of mesangial cells and the surrounding mesangial matrix (Figs 43.4 and 43.10), first described by (Zimmermann,

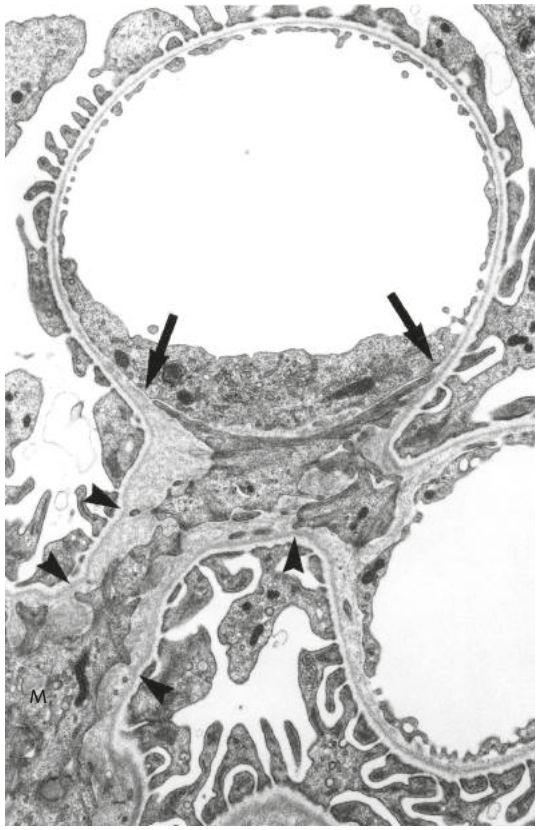

Fig. 43.10 Glomerular capillary and mesangium. In the juxtacapillary region, long mesangial cell processes extend between opposite mesangial angles, where they are fixed to the GBM (arrows). In the axial region, finger-like processes connect the mesangial cells to the perimesangial glomerular basement membrane (arrowheads). Note bundles of microfilaments in the cell processes. M = mesangial cell. 13,000×.

1929). Since the ultrastructural characterization of the mesangium in the early sixties (Latta et al., 1960; Farquhar et al., 1962), mesangial cells have been in the forefront of glomerular research. They are generally believed to form a supporting framework that maintains the structural integrity of the glomerular tuft.

The mesangial matrix consists mostly of basement membrane components (Karkavelas et al., 1988). At variance to the GBM it contains the α1 and α2 chains of type IV collagen, the β1 chain of laminin, considerable amounts of fibronectin, chondroitin sulphate proteoglycan, the small leucine-rich proteoglycans biglycan and decorin and the heparan sulphate proteoglycans perlecan, bamacan, and collagen type XVIII (Border et al., 1989; Couchman et al., 1994; Miner, 1999); in addition, microfibrillar proteins are abundant (Gibson et al., 1989; Sterzel et al., 2000).

Mesangial cells are considered to be contractile cells that have a common origin with smooth muscle cells. They are irregular in shape, with numerous cytoplasmic processes filled with prominent assemblies of microfilaments (Fig. 43.10) that contain actin, myosin, and α-actinin (Kreisberg et al., 1985; Drenckhahn et al., 1988). Moreover, the mesangial cells are electrically coupled by gap junctions (Pricam et al., 1974). Mesangial cells possess a great variety of receptors including for angiotensin II, vasopressin, atrial natriuretic factor, prostaglandins, TGFβ, PDGF-B, EGF, and CTGF (Dworkin et al., 1983; Stockand and Sansom, 1998). A more complete list is found in a review by Schlöndorff and Banas (2009).

The GBM is the primary effector site of mesangial cell contraction (Sakai and Kriz, 1987; Kriz et al., 1990). Mesangial cells are connected extensively with the GBM, either by direct adherence of mesangial cell processes (focal adhesions) or by microfibrils (Fig. 43.10). These connections, which appear to be mechanically strong, are found throughout the mesangial region. Microfibrils are unbranched non-collagenous, tubular structures about 15 nm thick. They are a major component of the mesangial matrix, as has been shown by transmission electron microscopy after tannic acid staining (Mundel et al., 1988) and by immunocytochemistry using antibodies against several microfibrillar proteins (Gibson et al., 1989). Microfibrils are generally coated by fibronectin (Schwartz et al., 1985).

The high content of fibronectin within the mesangium (Madri et al., 1980) may be related to the need for firm connections between the different mesangial components: cells to matrix and among the various components of the matrix itself. Fibronectin mediates the connection between actin and extracellular matrix components, including type IV collagen (Burridge et al., 1988). Important in these connections are α3β1 dimers of integrins, which bind fibronectin to the termini of actin filaments (Cosio et al., 1990). As a whole, these abundant interconnections between the cells and matrix as well as between the various matrix components establish a strong mechanical cohesion to counteract the expansion of the mesangium.

Parietal epithelium

The parietal layer of Bowman's capsule consists of squamous epithelial cells resting on a basement membrane (Figs 43.2 and 43.3). The flat cells are polygonal, with a central cilium and few microvilli. Parietal cells are filled with bundles of actin filaments running in all directions (Pease, 1968). Within the cells surrounding the vascular pole, the actin filaments are very dense and located within cytoplasmic ridges that run in a circular fashion around the glomerular entrance. The finding of muscarinic receptors on the parietal epithelium (Lebrun et al., 1992) indicates that the contractile tone of these cells is subject to regulation.

Recent observations suggest that a niche of glomerular epithelial stem cells resides within the parietal epithelium at the transition to the proximal tubule (Sagrinati et al., 2006; Ronconi et al., 2009). It is an intriguing hypothesis that proliferating stem cells from this locus may transform into podocytes and may reach the tuft by migration via the transition at glomerular vascular pole. Migration of parietal cells onto the tuft via the vascular pole and subsequent transition into podocytes have been shown to occur in the new-born mouse (Appel et al., 2008). However, evidence that such a process may be of any relevance in the adult has so far not been presented (Appel et al., 2008).

The PBM is, in contrast to the GBM, composed of several dense layers separated by translucent layers and contains bundles of fibrils ('microligaments'; Mbassa et al., 1988). Collagen α1, α2, α5 and α6 (type IV) chains prevail in this basement membrane (Miner, 1999). In addition collagen type XIV was found (Lethias et al., 1994).

In contrast to the GBM, the predominant proteoglycan of this basement membrane is a chondroitin sulphate proteoglycan, and the laminin isoforms 1 and 10 prevail (Couchman et al., 1994; Miner, 1999). The transition from the GBM to the basement membrane of Bowman's capsule borders the glomerular entrance. This

transitional region is mechanically connected to the smooth muscle cells of the afferent and efferent arterioles as well as to extraglomerular mesangial cells (see below).

Extraglomerular mesangium—a closure device of the glomerular entrance

At the vascular pole of the glomerulus the mesangium passes through the opening of Bowman's capsule and continues into the extraglomerular mesangium (Barajas et al., 1989; Barajas, 1997) (Figs 43.2 and 43.3). The extraglomerular mesangium represents a solid complex of cells and matrix that is neither penetrated by blood vessels nor lymphatic capillaries.

The extraglomerular mesangium is located in the cone-shaped space between the two glomerular arterioles and the macula densa cells of the thick ascending limb and, laterally, faces the renal interstitium. Extraglomerular mesangial cells are flat and elongated, separating into bunches of long cell processes at their poles (Spanidis and Wunsch, 1979). They are arranged in several layers, parallel to the base of the macula densa. The cells are embedded in a matrix similar in composition as the mesangial matrix; however, microfibrils are comparably rarely found. Affixation of macula densa cells to the extraglomerular mesangium appears to be mediated by β6-integrin, which is known to associate with αv-integrin to form the fibronectin binding heterodimer αvβ6 (Breuss et al., 1993).

Although direct evidence is lacking, extraglomerular mesangial cells can be expected to be contractile for several reasons. First, they contain prominent bundles of microfilaments containing F-actin in their processes. Second, like intraglomerular mesangial cells, they have strong structural similarities with arteriolar smooth muscle cells and granular cells, suggesting that they are all of the same origin. Third, these cells are all extensively coupled by gap junctions (Pricam et al., 1974; Taugner et al., 1978). Fourth, high amounts of heat shock protein 25 (HSP25) are present in glomerular and, especially, in extraglomerular mesangial cells. HSP25/27, which is believed to be a mediator of sustained smooth muscle cell contraction, might be a component of the contraction machinery in the glomerular and extraglomerular cells (Müller et al., 1999). The contractile processes of extraglomerular mesangial cells are connected to the basement membrane of Bowman's capsule and to the walls of both glomerular arterioles. As a whole, the extraglomerular mesangium interconnects all structures of the glomerular entrance. The extraglomerular mesangium can be regarded as a closure device of the glomerular entrance, maintaining its structural integrity against the distending forces exerted on the entrance by the high intra-arteriolar and intraglomerular pressure (Elger et al., 1998). A function of the extraglomerular mesangium for the recruitment of mesangial cells has been proposed. In anti-Thy-1 glomerulonephritis the cellular re- population of the mesangium apparently occurs from the extraglomerular mesangium (Hugo et al., 1997).

Juxtaglomerular apparatus—intersection of local and systemic regulation

The juxtaglomerular apparatus (JGA) is situated at the vascular pole of the glomerulus. It comprises: (a) the macula densa, (b) the extraglomerular mesangium (described above), and (c) the terminal portion of the afferent arteriole with its renin-producing granular cells, as well as the beginning of the efferent arteriole (Fig. 43.3).

The macula densa is a plaque of specialized cells within the thick ascending limb at the site where the latter is affixed to the extraglomerular mesangium of the parent glomerulus. The most obvious structural features are the large, narrowly packed, cell nuclei, which account for the name 'macula densa' (Zimmermann, 1933).

In contrast to other parts of the thick ascending limb, the cells of the macula densa do not interdigitate with each other but have a polygonal outline. The luminal cell membrane is densely studded by stubby microvilli and bears one cilium. At their bases, the cells display numerous infoldings and folds of the plasma membrane; the latter are anchored to the underlying basement membrane, blending with the matrix of the extraglomerular mesangium (Kriz et al., 1992). The lateral membrane of macula densa cells bears folds and finger-like villi which are frequently connected to those of neighbouring cells by desmosomes. Near the apex, the cells are joined by tight junctions consisting of several parallel junctional strands similar to those in the thick ascending limb throughout. The cells contain the usual cytoplasmic organelles, comprising some small mitochondria, Golgi apparatus, and smooth endoplasmic reticulum; free ribosomes are abundant but rough endoplasmic reticulum is rare.

The lateral intercellular spaces are a prominent feature of the macula densa. Electron microscopic studies, and studies on isolated macula densa segments *in vitro*, have shown that the width of the lateral intercellular spaces varies under different functional conditions (Kaissling et al., 1982; Kirk et al., 1985). In agreement with the suggestion that water flow through the macula densa epithelium is secondary to active sodium reabsorption, compounds such as furosemide, that block sodium transport, as well as high osmolalities of impermeable solutes such as mannitol, are associated with narrowing of the intercellular spaces (Kaissling et al., 1982; Alcorn et al., 1986). The spaces are apparently dilated under most physiological conditions, usually regarded as normal control conditions.

The most conspicuous difference in the protein inventory between macula densa cells and any other epithelial cell of the nephron is the high content of nitric oxide synthase I (Mundel et al., 1992; Persson and Bachmann, 2000) and of cyclooxygenase-2 (Schnermann, 2001) in macula densa cells.

The granular cells are assembled in clusters within the terminal portion of the afferent arteriole, replacing smooth muscle cells (Fig. 43.3). Their name refers to the cytoplasmic granules, which are dark, membrane-bound, and irregular in size and shape. Renin, the major secretion product, is stored in these granules. Small granules with crystalline substructure represent protogranules containing both renin prosegment and mature renin. Renin release occurs by exocytosis into the surrounding interstitium (Taugner and Hackenthal, 1989).

Granular cells are modified smooth muscle cells. Under conditions requiring enhanced renin synthesis (e.g. volume depletion or stenosis of the renal artery) additional smooth muscle cells located upstream in the wall of the afferent arteriole transform into granular cells. Granular cells are connected to the extraglomerular mesangial cells, to adjacent smooth muscle cells, and to endothelial cells by gap junctions, and are densely innervated by sympathetic nerve terminals (Taugner and Hackenthal, 1989).

The structural organization of the JGA suggests a regulatory function. Goormaghtigh (1937) was the first to propose that some component of the distal urine is sensed by the macula densa and this information is used to adjust the tonus of the glomerular

arterioles, thereby producing a change in glomerular blood flow and filtration rate. Moreover, since the JGA is the major site of renin secretion, the function of the JGA is of great systemic relevance. These two functions (regulation of the vascular tone of glomerular arterioles and regulation of renin release from granular cells) seem to be strictly separated from each other. For both mechanisms, it is well established that changes in the chloride concentration of the tubular fluid at the macula densa cause graded releases of mediators that reach their target by diffusion, thus acting in a paracrine fashion (Kurtz, 2011). Note that the extraglomerular mesangium that mediates the contact between the macula densa and the effector cells is not vascularized, so that the build-up of any paracrine agent would not be perturbed by blood flow.

With respect to renin release, the most likely paracrine mediators of this process are prostaglandin E_2 and nitric oxide (Wilcox et al., 1992; Peti-Peterdi et al., 2003; Schweda and Kurtz, 2004). With respect to the vasoconstrictor response purinergic mediators, either ATP or adenosine, as first suggested by Oswald and colleagues in 1980 (Osswald et al., 1980), appear to play the major role (Schnermann and Levine, 2003; Castrop et al., 2004; Thomson et al., 2000). For an up-to-date discussion of the function of the JGA, see the reviews by Schnermann and Levine (2003), Persson et al. (2004), Komlosi et al. (2004), Kurtz (2011), and Schnermann and Briggs (2013).

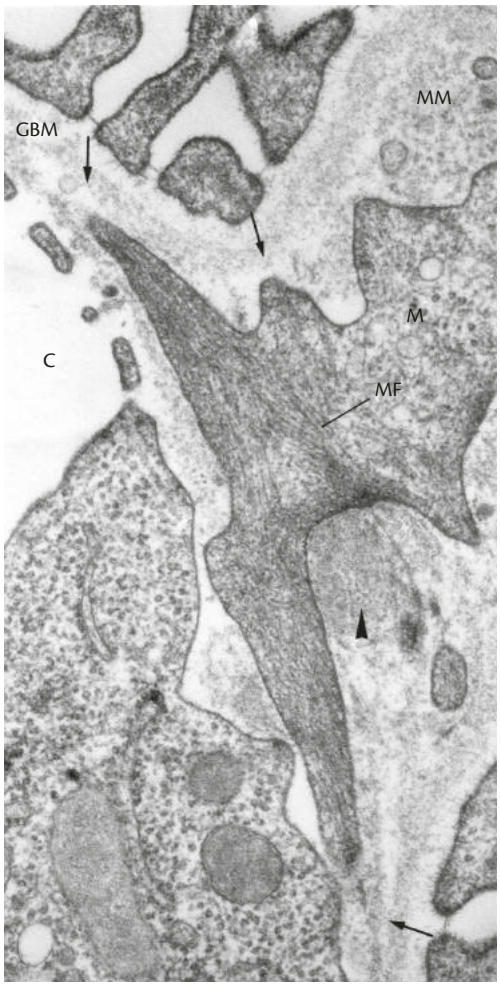

Fig. 43.11 Juxtacapillary portion of mesangium showing tongue-like mesangial processes fixed to the glomerular basement membrane (GBM) at the mesangial angles (arrows). Note the rich equipment of mesangial processes with bundles of microfilaments (MF) which are attached to the cell membrane. The mesangial matrix (MM) contains abundant microfibrils (arrowhead). C = capillary lumen. 61,200×.

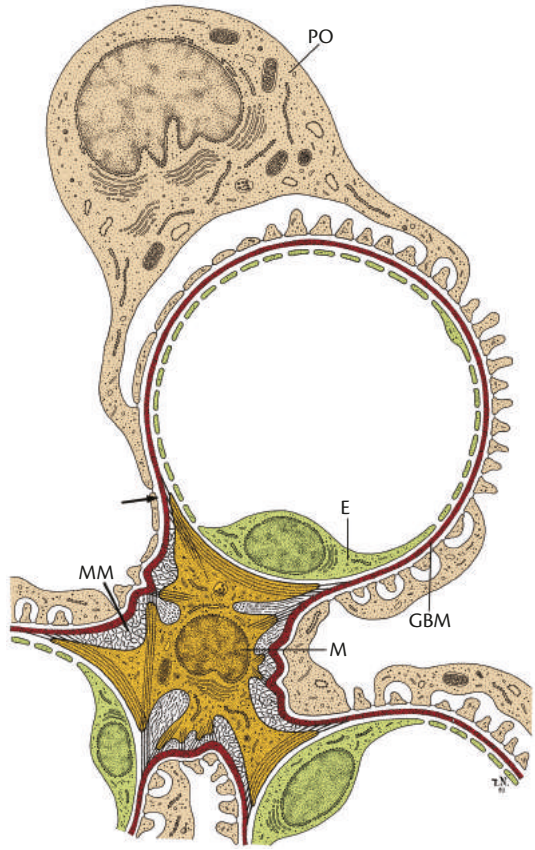

Fig. 43.12 Schematic showing the filtration barrier as well as the centrolobular position of a mesangial cell (M) and its relationships to the glomerular capillaries and to the glomerular basement membrane (GBM). The glomerular capillary consists of a fenestrated endothelium (E). The peripheral portion of the capillary is surrounded by the GBM which, at the mesangial angles (arrow), deviates from the pericapillary course and covers the mesangium. The interdigitating system of the podocyte (PO) foot processes forms the distal layer of the filtration barrier. Connections between mesangial cell processes and the GBM are prominent at mesangial angles, and are also numerous along the perimesangial GBM. Many of these connections are mediated by microfibrils which are a major constituent of the mesangial matrix (MM). Thus, a mechanical firm linkage of the perimesangial GBM to the contractile apparatus of the mesangial cells is established.
Modified after Kriz et al. (1992).

Mesangial cell-to-GBM contacts are found along the entire perimesangial GBM. The intracellular actin filament bundles are arranged in such a way that segments of the GBM located on opposing sides of the mesangium are interconnected. These connections are most prominent at mesangial angles consisting of tongue-like mesangial processes which establish a bridge between both mesangial angles of the GBM (Figs 43.10, 43.11, and 43.12), (Sakai and Kriz, 1987).

Evidence for the importance of these connections for the structural integrity of the tuft was obtained by selective destruction of the mesangium by experimental application of antibody against the cell surface antigen Thy 1 (Paul et al., 1984; Kriz et al., 2003). In this

situation, the mesangial region as well as the mesangial–endothelial interface is greatly enlarged, leading to a partial 'unfolding' of glomerular capillaries associated with capillary ballooning (Lemley et al., 1992), and profound changes in the glomerular haemodynamics (Blantz et al., 1991).

The large pressure gradients across the GBM represent the crucial challenge to the glomerular tuft. The distending forces acting on the GBM (across the peripheral and across the perimesangial interface) have to be counterbalanced by inwardly directed forces. This comprises two aspects. First, the tuft as a whole, that is, the folding pattern of the GBM providing space for the capillaries, and second, the width of the capillaries have to be maintained (or adapted to varying situations).

The folding pattern of the GBM is supported by the overall centripetal fixation of the GBM to the mesangium (by centripetal contractile forces) (Kriz et al., 1995a). In addition, podocytes are involved; podocyte processes that fill the niches of GBM-infoldings interconnect opposing portions of the GBM from outside, thereby stabilizing the folding pattern of the GBM (Kriz et al., 2003).

The maintenance of the capillary width is supported by the GBM in conjunction with the mesangium. The basic supportive structure of a glomerular capillary wall is represented by (A) the GBM which forms an incomplete cylinder that is open towards the mesangium like a tyre to the rim and (B) by a mesangial cell, which bridges this gap by a contractile cell process fixed to the GBM at both sides. Thus, the GBM (which is an elastic structure (Welling et al., 1995)) together with the mesangial cell bridge form a complete cylinder which is able to develop wall tension and thus to resist distension. Traditionally, the podocyte foot processes have been considered as a kind of pericyte processes contributing to the generation of wall tension; however, this view has recently been challenged (Kriz et al., 2013b).

Thus, the contractile apparatus of the mesangial cells being effective at the GBM appears to be static in nature, operating by isometric or minute isotonic contractions. Whether mesangial cell contractility also plays a role in the regulation of glomerular haemodynamics is still a matter of debate. Because the mesangial–endothelial interface comprises only a small part of the capillary circumference, a contraction of mesangial cells at this site would lead to minor changes in the capillary diameter.

References

Abrahamson, D. (1987). Structure and development of the glomerular capillary wall and basement membrane. *Am J Physiol*, 253, F783–94.

Abrahamson, D. (2012). Role of the podocyte (and glomerular epithelium) in building the GBM. *Semin Nephrol*, 32, 342–9.

Abrahamson, D., Hudson, B. G., Stroganova, L., *et al.* (2009). Cellular origins of type IV collagen networks in developing glomeruli. *J Am Soc Nephrol*, 20, 1471–9.

Alcorn, D., Anderson, W., and Ryan, G. (1986). Morphological changes in the renal macula densa during natriuresis and diuresis. *Renal Physiol*, 9, 335–47.

Appel, D., Kershaw, D. B., Smeets, B., *et al.* (2008). Recruitment of podocytes from glomerular parietal epithelial cells. *J Am Soc Nephrol*, 20, 333–43.

Bachmann, S., Kriz, W., Kuhn, C., *et al.* (1983). Differentiation of cell types of the mammalian kidney by immunofluorescence microscopy using antibodies to intermediate filament proteins and desmoplakins. *Histochemistry*, 77, 365–94.

Bakker, W., Borghuis, T., Harmsen, M. C., *et al.* (2005). Protease activity of plasma hemopexin. *Kidney Int*, 68, 603–10.

Barajas, L. (1997). Cell-specific protein and gene expression in the juxtaglomerular apparatus. *Clin Exp Pharmacol Physiol*, 24, 520–6.

Barajas, L., Salido, E. C., Smolens, P., *et al.* (1989). Pathology of the juxtaglomerular apparatus including Bartter's syndrome. In C. Tisher and B. Brenner (eds.) *Renal Pathology*, pp. 877–912. Philadelphia, PA: Lippincott.

Bek, M., Nüsing, R., Kowark, P., *et al.* (1999). Characterization of prostanoid receptors in podocytes. *J Am Soc Nephrol*, 10, 2093.

Blantz, R., Gabbai, F., and Wilson, C. (1991). Glomerular hemodynamic (GH) response to changes in volume status after lysis of mesangial cells (MC). *J Am Soc Nephrol*, 1, 661.

Border, W., Okuda, S., and Nakamura, T. (1989). Extracellular matrix and glomerular disease. *Semin Nephrol*, 9, 307–17.

Brees, D., Ogle, R., and Williams, J., Jr. (1995). Laminin and fibronectin content of mouse glomerular and tubular basement membrane. *Renal Physiol Biochem*, 18, 1–11.

Breuss, J., Gillett, N., Lu, L., *et al.* (1993). Restricted distribution of integrin b6 mRNA in primate epithelial tissue. *J Histochem Cytochem*, 41, 1521–7.

Bulger, R., Eknoyan, G., Purcell, D., *et al.* (1983). Endothelial characteristics of glomerular capillaries in normal, mercuric chloride-induced, and gentamicin-induced acute renal failure in the rat. *Clin Invest*, 72, 128–41.

Bulger, R. and Hebert, S. (1988). Structural-functional relationships in the kidney. In R. Schrier and A. Gottschalk (eds.) *Diseases of the Kidney*, pp. 3–63. Boston, MA: Little, Brown and Company.

Burridge, K., Fath, K., Kelly, T., *et al.* (1988). Focal adhesion: transmembrane junctions between the extracellular matrix and the cytoskeleton. *Ann Rev Cell Biol*, 4, 487–525.

Castrop, H., Huang, Y., Hashimoto, S., *et al.* (2004). Impairment of tubuloglomerular feedback regulation of GFR in ecto-5'-nucleotidase/CD73-deficient mice. *Clin Invest*, 114, 634–42.

Cosio, F., Sedmak, D., and Nahman, N. (1990). Cellular receptors for matrix proteins in normal human kidney and human mesangial cells. *Kidney Int*, 38, 886–95.

Couchman, J., Beavan, L., and McCarthy, K. (1994). Glomerular matrix: synthesis, turnover and role in mesangial expansion. *Kidney Int*, 45, 328–35.

Daniels, B., Deen, W. M., Mayer, G., *et al.* (1993). Glomerular permeability barrier in the rat. *Clin Invest*, 92, 929–36.

Deen, W., Bohrer, M., and Brenner, B. (1979). Macromolecule transport across glomerular capillaries: application of pore theory. *Kidney Int*, 16, 353–65.

Deen, W., Lazzara, M., and Myers, B. (2001). Structural determinants of glomerular permeability. *Am J Physiol Renal Physiol*, 281, F579–96.

Desjardins, M. and Duling, B. (1990). Heparinase treatment suggests a role for the endothelial cell glycocalyx in regulation of capillary hematocrit. *Am J Physiol*, 258, H647–54.

Drab, M., Verkade, P., Elger, M., *et al.* (2001). Loss of caveolae, vascular dysfunction, and pulmonary defects in caveolin-1 gene-disrupted mice. *Science*, 293, 2449–52.

Drenckhahn, D. and Franke, R. (1988). Ultrastructural organization of contractile and cytoskeletal proteins in glomerular podocytes of chicken, rat, and man. *Lab Invest*, 59, 673–82.

Drumond, M. and Deen, W. (1994a). Structural determinants of glomerular hydraulic permeability. *Am J Physiol*, 266, F1–12.

Drumond, M., Kristal, B., Myers, B., *et al.* (1994b). Structural basis for reduced glomerular filtration capacity in nephrotic humans. *Clin Invest*, 94, 1187–95.

Durbeej, M., Henry, M., and Campbell, K. (1998). Dystroglycan in development and disease. *Curr Opin Cell Biol*, 10, 594–601.

Dworkin, L., Ichikawa, I., and Brenner, B. (1983). Hormonal modulation of glomerular function. *Am J Physiol*, 244, F95–104.

Elger, M., Sakai, T., and Kriz, W. (1998). The vascular pole of the renal glomerulus of rat. *Adv Anat Embryol Cell Biol*, 139, 1–98.

Elger, M., Sakai, T., Winkler, D., *et al.* (1991). Structure of the outflow segment of the efferent arteriole in rat superficial glomeruli. *Contrib Nephrol*, 95, 22–33.

Endlich, K., Kriz, W., and Witzgall, R. (2001a). Update in podocyte biology. *Curr Opin Nephrol Hypertens*, 10, 331–40.

Endlich, N., Nobiling, R., Kriz, W., *et al*. (2001b). Expression and signaling of parathyroid hormone-related protein in cultured podocytes. *Exp Nephrol*, 9, 436–43.

Farquhar, M. (1991). The glomerular basement membrane: a selective macromolecular filter. In E. Hay (ed.) *Cell Biology of Extracellular Matrix*, pp. 365–418. New York: Plenum Press.

Farquhar, M. and Palade, G. (1962). Functional evidence for the existence of a third cell type in the renal glomerulus. Phagocytosis of filtration residues by a distinctive 'third' cell. *J Cell Biol*, 13, 55–87.

Fischer, K., Saueressig, U., Jacobshagen, C., *et al*. (2001). Extracellular nucleotides regulate cellular functions of podocytes in culture. *Am J Physiol Renal Physiol*, 281, F1075–81.

Ford, M., Cauchi, J., Greferath, U., *et al*. (1997). Expression of fibroblast growth factors and their receptors in rat glomeruli. *Kidney Int*, 51, 1729–38.

Fridén, V., Oveland, E., Tenstad, O., *et al*. (2011). The glomerular endothelial cell coat is essential for glomerular filtration. *Kidney Int*, 79, 1322–30.

Fries, J., Sandstrom, D., Meyer, T., *et al*. (1989). Glomerular hypertrophy and epithelial cell injury modulate progressive glomerulosclerosis in the rat. *Lab Invest*, 60, 205–18.

Gelberg, H., Healy, L., Whiteley, H., *et al*. (1996). In vivo enzymatic removal of a26-linked sialic acid from the glomerular filtration barrier results in podocyte charge alteration and glomerular injury. *Lab Invest*, 74, 907–20.

Gibson, M., Kumaratilake, J., and Cleary, E. (1989). The protein components of the 12-nanometer microfibrils of elastic and nonelastic tissues. *J Biol Chem*, 264, 4590–98.

Goormaghtigh, N. (1937). L'appareil neuro-myo-artériel juxta-glomérulaire du rein: ses réactions en pathologie et ses rapports avec le tube urinifère. *C R Seances Soc Biol Fil*, 124, 293–6.

Greka, A. and Mundel, P. (2012). Cell biology and pathology of podocytes. *Ann Rev Physiol*, 74, 299–323.

Grouls, S., Iglesias, D. M., Wentzensen, N., *et al*. (2012). Lineage specification of parietal epithelial cells requires ß-catenin/Wnt signaling. *J Am Soc Nephrol*, 23, 63–72.

Guasch, A. and Myers, B. (1994). Determinants of glomerular hypofiltration in nephrotic patients with minimal change nephropathy. *J Am Soc Nephrol*, 4, 1571–81.

Hansen, K. and Abrass, C. (1999). Role of laminin isoforms in glomerular structure. *Pathology*, 67, 84–91.

Hara, M., Yamamoto, T., Yanagihara, T., *et al*. (1995). Urinary excretion of podocalyxin indicates glomerular epithelial cell injuries in glomerulonephritis. *Nephron*, 69, 397–403.

Hara, M., Yanagihara, T., Takada, T., *et al*. (1998). Urinary excretion of podocytes reflects disease activity in children with glomerulonephritis. *Am J Nephrol*, 18, 35–41.

Haraldsson, B. and Jeansson, M. (2009). Glomerular filtration barrier. *Curr Opin Nephrol Hypertens*, 18, 331–5.

Haraldsson, B. and Nyström, J. (2012). The glomerular endothelium: new insights on function and structure. *Curr Opin Nephrol Hypertens*, 21, 258–63.

Haraldsson, B., Nyström, J., and Deen, W. (2008). Properties of the glomerular barrier and mechanisms of proteinuria. *Physiol Rev*, 88, 451–87.

Hausmann, R., Kuppe, C., Egger, H., *et al*. (2010). Electrical forces determine glomerular permeability. *J Am Soc Nephrol*, 21, 2053–8.

Herman, W., Emancipator, S., and Simonson, M. (1998). Vascular and glomerular expression of endothelin-1 in normal human kidney. *Am J Physiol Renal Physiol*, 275, F8–17.

Hjalmarsson, C., Johansson, B., and Haraldsson, B. (2004). Electron microscopic evaluation of the endothelial surface layer of glomerular capillaries. *Microvasc Res*, 67, 9–17.

Hora, K., Ohno, S., Oguchi, H., *et al*. (1990). Three-dimensional study of glomerular slit diaphragm by quick-freezing and deep-etching replica method. *Eur J Cell Biol*, 53, 402–6.

Horvat, R., Hovorka, A., Dekan, G., *et al*. (1986). Endothelial cell membranes contain podocalyxin- the major sialoprotein of visceral glomerular epithelial cells. *J Cell Biol*, 102, 484–91.

Huang, T. and Langlois, J. (1985). Podoendin. A new cell surface protein of the podocyte and endothelium. *J Exp Med*, 162, 245–67.

Huber, T., Gloy, J., Henger, A., *et al*. (1998). Catecholamines modulate podocyte function. *J Am Soc Nephrol*, 9, 335–45.

Hugo, C., Shankland, S. J., Bowen-Pope, D. F., *et al*. (1997). Extraglomerular origin of the mesangial cell after injury- a new role of the juxtaglomerular apparatus. *Clin Invest*, 100, 786–94.

Ichimura, K., Stan, R., Kurihara, H., *et al*. (2008). Glomerular endothelial cells form diaphragms during development and pathological conditions. *J Am Soc Nephrol*, 19(8), 1463–71.

Jeansson, M. and Haraldsson, B. (2006). Morphological and functional evidence for an important role of the endothelial cell glycocalyx in the glomerular barrier. *Am J Physiol Renal Physiol*, 290, F111–16.

Jorgensen, F. (1966). *The Ultrastructure of the Normal Human Glomerulus*. Copenhagen: Ejnar Munksgaard.

Kaissling, B. and Kriz, W. (1982). Variability of intercellular spaces between macula densa cells: a transmission electron microscopic study in rabbits and rats. *Kidney Int*, 22, 9–17.

Karkavelas, G. and Kefalides, N. (1988). Comparative ultrastructural localization of collagen types III, IV, VI and laminin in rat uterus and kidney. *J Ultrastruct Mol Struct Res*, 100, 137–55.

Kazatchkine, M., Fearon, D. T., Appay, M. D., *et al*. (1982). Immunohistochemical study of the human glomerular C3b receptor in normal kidney and in seventy-five cases of renal diseases. *Clin Invest*, 69, 900–12.

Kerjaschki, D. and Farquhar, M. (1983). Immunocytochemical localization of the Heymann antigen (gp 330) in glomerular epithelial cells of normal Lewis rats. *J Exp Med*, 157, 667–86.

Kerjaschki, D., Sharkey, D., and Farquhar, M. (1984). Identification and characterization of podocalyxin-the major sialoprotein of the renal glomerular epithelial cell. *J Cell Biol*, 98, 1591.

Kiberd, B. (1992). The functional and structural changes of the glomerulus throughout the course of murine lupus nephritis. *J Am Soc Nephrol*, 3, 930–9.

Kirk, K., Bell, P., Barfuss, D., *et al*. (1985). Direct visualization of the isolated and perfused macula densa. *Am J Physiol*, 248, F890–4.

Kobayashi, N., Reiser, J., Kriz, W., *et al*. (1998). Nonuniform microtubular polarity established by CHO1/MKLP1 motor protein is necessary for process formation of podocytes. *J Cell Biol*, 143, 1961–70.

Komlosi, P., Fintha, A., and Bell, P. (2004). Current mechanisms of macula densa cell signaling. *Acta Physiol Scand*, 181, 463–9.

Kreidberg, J. and Symons, J. (2000). Integrins in kidney development, function, and disease. *Am J Physiol Renal Physiol*, 279(2), F233–42.

Kreisberg, J., Venkatachalam, K., and Troyer, D. (1985). Contractile properties of cultured glomerular mesangial cells. *Am J Physiol*, 249, F457–63.

Kriz, W. (2002). Podocyte is the major culprit accounting for the progression of chronic renal disease. *Microsc Res Tech*, 57, 189–95.

Kriz, W. (2007). Ontogenetic development of the filtration barrier. *Nephron Exp Nephrol*, 106, e44–50.

Kriz, W., Elger, M., Lemley, K., *et al*. (1990). Mesangial cell—glomerular basement membrane connections counteract glomerular capillary and mesangium expansion. *Am J Nephrol*, 10, 4–13.

Kriz, W., Elger, M., Mundel, P., *et al*. (1995a). Structure-stabilizing forces in the glomerular tuft. *J Am Soc Nephrol*, 5, 1731–9.

Kriz, W., Hähnel, B., Hosser, H., *et al*. (2003). Pathways to recovery and loss of nephrons in anti-Thy-1 nephritis. *J Am Soc Nephrol*, 14, 1904–26.

Kriz, W., Hähnel, B., Rosener, S., *et al*. (1995b). Long-term treatment of rats with FGF-2 results in focal segmental glomerulosclerosis. *Kidney Int*, 48, 1435–50.

Kriz, W. and Kaissling, B. (1992). Structural organization of the mammalian kidney. In D. Seldin and G. Giebisch (eds.) *The Kidney: Physiology and Pathophysiology*, pp. 707–77. New York: Raven Press.

Kriz, W. and Kaissling, B. (2013a). Structural organization of the mammalian kidney. In R. Alpern, M. Caplan, and O. Moe (eds.) *Seldin and*

Giebisch's The Kidney: Physiology and Pathophysiology, pp. 595–691. Amsterdam: Academic Press Elsevier.

Kriz, W., Shirato, I., Nagata, M., *et al.* (2013b). The podocyte's response to stress: the enigma of foot process effacement. *Am J Physiol Renal Physiol*, 304, F333–47.

Kurtz, A. (2011). Renin release: sites, mechanisms, and control. *Ann Rev Physiol*, 73, 377–99.

Lafayette, R., Druzin, M., Sibley, R., *et al.* (1998). Nature of glomerular dysfunction in pre-eclampsia. *Kidney Int*, 54, 1240–9.

Larsson, L. and Maunsbach, A. (1980). The ultrastructural development of the glomerular filtration barrier in the rat kidney: a morphometric analysis. *J Ultrastruct Res*, 72, 392–406.

Latta, H., Maunsbach, A., and Madden, S. (1960). The centrolobular region of the renal glomerulus studied by electron microscopy. *J Ultrastruct Res*, 4, 455–72.

Lebrun, F., Morel, F., Vassent, G., *et al.* (1992). Cholinergic effects on intracellular free calcium concentration in renal corpuscle: role of parietal sheet. *Am J Physiol*, 262, F248–55.

Lemley, K., Elger, M., Koeppen-Hagemann, I., *et al.* (1992). The glomerular mesangium: capillary support function and its failure under experimental conditions. *Clin Invest*, 70, 843–56.

Lethias, C., Aubert-Foucher, E., Dublet, B., *et al.* (1994). Structure, molecular assembly and tissue distribution of facit collagen molecules. *Contrib Nephrol*, 107, 57–63.

Madri, J., Roll, F., Furthmayr, H., *et al.* (1980). Ultrastructural localization of fibronectin and laminin in the basement membranes of the murine kidney. *J Cell Biol*, 86, G82–7.

Mbassa, G., Elger, M., and Kriz, W. (1988). The ultrastructural organization of the basement membrane of Bowman's capsule in the rat renal corpuscle. *Cell Tissue Res*, 253, 151–63.

Mendrick, D. and Rennke, H. (1988). Induction of proteinuria in the rat by a monoclonal antibody against SGP-115/107. *Kidney Int*, 33, 818–30.

Meuwese, M., Broekhuizen, L. N., Kuikhoven, M., *et al.* (2010). Endothelial surface layer degradation by chronic hyaluronidase infusion induces proteinuria in apolipoprotein E-deficient mice. *PLOS One*, 5, 1–7.

Miner, J. (1999). Renal basement membrane components. *Kidney Int*, 56, 2016–24.

Miner, J. (2011). Glomerular basement membrane composition and the filtration barrier. *Pediatr Nephrol*, 26, 1413–7.

Miner, J., Patton, B. L., Lentz, S. I., *et al.* (1997). The laminin a chanins: expression, development transitions, and chromosomal locations of a1-5, identification of heterotrimeric laminis 8-11, and cloning of a novel a3 isoform. *J Cell Biol*, 137, 685–701.

Miner, J. and Sanes, J. (1994). Collagen IV a3, a4, and a5 chains in rodent basal laminae: Sequence, distribution, association with laminins, and developmental switches. *J Cell Biol*, 127, 879–91.

Mohan, P. and Spiro, R. (1986). Macromolecular organization of basement membranes. *J Biol Chem*, 261, 4328–36.

Morita, M., White, R., and Raafat, F. (1988). Glomerular basement membrane thickness in children. *Pediatr Nephrol*, 2, 190–5.

Müller, E., Burger-Kentischer, A., Neuhofer, W., *et al.* (1999). Possible involvement of heat shock protein 25 in the angiotensin II-induced glomerular mesangial cell contraction via p.38 MAP kinase. *J Cell Physiol*, 181, 462–9.

Mundel, P., Bachmann, S., Bader, M., *et al.* (1992). Expression of nitric oxide synthase in kidney macula densa cells. *Kidney Int*, 42, 1017–9.

Mundel, P., Elger, M., Sakai, T., *et al.* (1988). Microfibrils are a major component of the mesangial matrix in the glomerulus of the rat kidney. *Cell Tissue Res*, 254, 183–7.

Mundel, P. and Kriz, W. (1995). Structure and function of podocytes: an update. *Anat Embryol*, 192, 385–97.

Nagata, M. and Kriz, W. (1992). Glomerular damage after uninephrectomy in young rats. II. Mechanical stress on podocytes as a pathway to sclerosis. *Kidney Int*, 42, 148–60.

Nagata, M., Yamaguchi, Y., and Ito, K. (1993). Loss of mitotic activity and the expression of vimentin in glomerular epithelial cells of developing human kidneys. *Anat Embryol*, 187, 275–9.

Nakamura, T., Ushiyama, C., and Suzuki, S. (2000). Effect of angiotensin-converting enzyme inhibitor, angiotensin II receptor antagonist and calcium antagonist on urinary podocytes in patients with IgA nephropathy. *Am J Nephrol*, 20, 373–9.

Nitschke, R., Henger, A., Ricken, S., *et al.* (2000). Angiotensin II increases the intracellular calcium activity in podocytes of the intact glomerulus. *Kidney Int*, 57, 41–9.

Nitschke, R., Henger, A., Ricken, S., *et al.* (2001). Acetylcholine increases the intracellular calcium activity in prodocytes in intact rat glomeruli via muscarinic M(5) receptors. *J Am Soc Nephrol*, 12, 678–87.

Obeidat, M., Obeidat, M., and Ballermann, B. (2012). Glomerular endothelium: a porous sieve and formidable barrier. *Exp Cell Res*, 318, 964–72.

Osswald, H., Nabakowski, G., and Hermes, H. (1980). Adenosine as a possible mediator of metabolic control of glomerular filtration rate. *Int J Biochem*, 12, 263–7.

Ott, M., Olson, J., and Ballermann, B. (1993). Phenotypic differences between glomerular capillary (GE) and aortic (AE) endothelial cells in vitro. (Abstract). *J Am Soc Nephrol*, 4, 564.

Paul, L., Rennke, H., Milford, E., *et al.* (1984). Thy-1.1 in glomeruli of rat kidneys. *Kidney Int*, 25, 771–7.

Pease, D. (1968). Myoid features of renal corpuscles and tubules. *J Ultrastruct Res*, 23, 304–20.

Persson, A. and Bachmann, S. (2000). Constitutive nitric oxide synthesis in the kidney—functions at the juxtaglomerular apparatus. *Acta Physiol Scand*, 169, 317–24.

Persson, A., Ollerstam, A., Liu, R., *et al.* (2004). Mechanism for macula densa cell release of renin. *Acta Physiol Scand*, 181, 471–4.

Petermann, A., Pippin, J., Krofft, R., *et al.* (2004). Viable podocytes detach in experimental diabetic nephropathy: potential mechanism underlying glomerulosclerosis. *Nephron Exp Nephrol*, 98, 114–23.

Peti-Peterdi, J., Komlosi, P., Fuson, A. L., *et al.* (2003). Luminal NaCl delivery regulates basolateral PGE_2 release from macla densa cells. *Clin Invest*, 112, 76–82.

Pricam, C., Humbert, F., Perrelet, A., and Orci, L. (1974). Gap junctions in mesangial and lacis cells. *J Cell Biol*, 63, 349–54.

Pries, A., Secomb, T., and Gaehtgens, P. (2000). The endothelial surface layer. *Pflugers Archiv*, 440, 653–66.

Raats, C., van den Born, J., and Berden, J. (2000). Glomerular heparan sulfate alterations: mechanisms and relevance for proteinuria. *Kidney Int*, 57(2), 385–400.

Rasch, R. (1979). Prevention of diabetic glomerulopathy in streptozotocin diabetic rats by insulin treatment. Glomerular basement membrane thickness. *Diabetologia*, 16, 319–24.

Rebibou, J., He, C. J., Delarue, F., *et al.* (1992). Functional endothelin-1 receptors on human glomerular podocytes and mesangial cells. *Nephrol Dial Transplant*, 7, 288–92.

Regele, H., Fillipovic, E., Langer, B., *et al.* (2000). Glomerular expression of dystroglycans is reduced in minimal change nephrosis but not in focal segmental glomerulosclerosis. *J Am Soc Nephrol*, 11(3), 403–12.

Rodewald, R. and Karnovsky, M. (1974). Porous substructure of the glomerular slit diophragm in the rat and mouse. *J Cell Biol*, 60, 423–33.

Ronconi, E., Sagrinati, C., Angelotti, M. L., *et al.* (2009). Regeneration of glomerular podocytes by human renal progenitors. *J Am Soc Nephrol*, 20, 322–32.

Rostgaard, J. and Qvortrup, K. (2002). Sieve plugs in fenestrae of glomerular capillaries—site of the filtration barrier? *Cells Tissues Organs*, 170, 132–8.

Ryan, G., Hein, S., and Karnovsky, M. (1976). Glomerular permeability to proteins. Effects of hemodynamic factors on the distribution of endogenous immunoglobulin G and exogenous catalase in the rat glomerulus. *Lab Invest*, 34, 415.

Sagrinati, C., Netti, G. S., Mazzinghi, B., *et al.* (2006). Isolation and characterization of multipotent progenitor cells from the Bowman's capsule of adult human kidneys. *J Am Soc Nephrol*, 17, 2443–56.

Saito, A., Pietromonaco, S., Loo, A., *et al.* (1994). Cloning and sequencing of gp330/megalin, the major Heymann nephritis antigen. *J Am Soc Nephrol*, 5, 767.

Sakai, T. and Kriz, W. (1987). The structural relationship between mesangial cells and basement membrane of the renal glomerulus. *Anat Embryol*, 176, 373–86.

Salmon, A. H., Neal, C. R., and Harper, S. J. (2009). New aspects of glomerular filtration barrier structure and function: five layers (at least) not three. *Curr Opin Nephrol Hypertens*, 18, 197–205.

Salmon, A. and Satchell, S. (2012). Endothelial glycocalyx dysfunction in disease: albuminuria and increased microvascular permeability. *J Pathol*, 226, 562–74.

Satchell, S. (2012). The glomerular endothelium emerges as a key player in diabetic nephropathy. *Kidney Int*, 82, 949–51.

Satchell, S. and Braet, F. (2009). Glomerular endothelial cell fenestration: an integral component of the glomerular filtration barrier. *Am J Physiol Renal Physiol*, 296, F947–56.

Savage, C. (1998). Injury mechanisms in vasculitis. *Kidney Blood Press Res*, 21, 269–70.

Sawada, H., Stukenbrok, H., Kerjaschki, D., *et al.* (1986). Epithelial polyanion (podocalyxin) is found on the sides but not the soles of the foot processes of the glomerular epithelium. *Am J Pathol*, 125, 309–18.

Schlöndorff, D. and Banas, B. (2009). The mesangial cell revisited: No cell is an island. *J Am Soc Nephrol*, 20, 1179–87.

Schnermann, J. (2001). Cyclooxygenase-2 and macula densa control of renin secretion. *Nephrol Dial Transplant*, 16, 1735–8.

Schnermann, J. and Briggs, J. (2013). Tubular control of renin synthesis and secretion. *Pflugers Arch*, 465, 39–51.

Schnermann, J. and Levine, D. (2003). Paracrine factors in tubuloglomerular feedback: adenosine, ATP, and nitric oxide. *Ann Rev Physiol*, 65, 501–29.

Schordan, S., Schordan, E., Endlich, K., *et al.* (2010). Alphav-integrins mediate the mechanoprotective action of osteopontin in podocytes. *Am J Physiol Renal Physiol*, 300, F119–32.

Schwartz, E., Goldfischer, S., Coltoff-Schiller, B., *et al.* (1985). Extracellular matrix microfibrils are composed of core proteins coated with fibronectin. *J Histochem Cytochem*, 33, 268–74.

Schweda, F. and Kurtz, A. (2004). Cellular mechanism of renin release. *Acta Physiol Scand*, 181, 383–90.

Seiler, M., Rennke, H., Venkatachalam, M., *et al.* (1977). Pathogenesis of polycation-induced alteration (fusion) of glomerular epithelium. *Lab Invest*, 36, 48–61.

Sharma, R., Sharma, M., Vamos, S., *et al.* (2001). Both subtype 1 and 2 receptors of angiotensin II participate in regulation of intracellular calcium in glomerular epithelial cells. *J Lab Clin Med*, 138, 40–49.

Sörensson, J., Fierlbeck, W., Heider, T., *et al.* (2002). Glomerular endothelial fenestrae in vivo are not formed from caveolae. *J Am Soc Nephrol*, 13, 2639–47.

Spanidis, A. and Wunsch, H. (1979). *Rekonstruktion einer Goormaghtigh'schen und einer Epitheloiden Zelle der Kaninchenniere*. Dissertation. University of Heidelberg, Heidelberg.

Spath, M., Pavenstädt, H., Müller, C., *et al.* (1995). Regulation of phosphoinositide hydrolysis and cytosolic free calcium induced by endothelin in human glomerular epithelial cells. *Nephrol Dial Transplant*, 10, 1304.

St John, P. and Abrahamson, D. (2001). Glomerular endothelial cells and podocytes jointly synthesize laminin-1 and -11 chains. *Kidney Int*, 60, 1037–46.

Stan, R. (2005). Structure of caveolae. *Biochim Biopyhs Acta*, 1746, 334–48.

Stan, R., Tkachenko, E., and Niesman, I. (2004). PV1 is a key structural component for the formation of the stomatal and fenestral diaphragms. *Mol Biol Cell*, 15, 3615–30.

Steffes, M., Barbosa, J., Basgen, J. M., *et al.* (1983). Quantitative glomerular morphology of the normal human kidney. *Lab Invest*, 49, 82–6.

Sterzel, R., Hartner, A., Schlötzer-Schrehardt, U., *et al.* (2000). Elastic fiber proteins in the glomerular mesangium in vivo and in cell culture. *Kidney Int*, 58, 1588–602.

Stockand, J. and Sansom, S. (1998). Glomerular mesangial cells: Electrophysiology and regulation of contraction. *Physiol Rev*, 78, 723–44.

Taugner, R. and Hackenthal, E. (1989). *The Juxtaglomerular Apparatus*. Heidelberg: Springer-Verlag.

Taugner, R., Schiller, A., Kaissling, B., *et al.* (1978). Gap junctional coupling between the JGA and the glomerular tuft. *Cell Tissue Res*, 186, 279–85.

Thomson, S., Bao, D., Deng, A., *et al.* (2000). Adenosine formed by 5'-nucleotidase mediates tubuloglomerular feedback. *Clin Invest*, 106, 289–98.

Timpl, R. and Dziadek, M. (1986). Structure, development, and molecular pathology of basement membranes. *Int Rev Exp Pathol*, 29, 1–112.

Tisher, C. and Brenner, B. (1989). Structure and function of the glomerulus. In C. Tisher and B. Brenner (eds.) *Renal Pathology*, pp. 92–110. Philadelphia, PA: Lippincott.

Toyoda, M., Najafian, B., Kim, Y., *et al.* (2007). Podocyte detachment and reduced glomerular capillary endothelial fenestration in human type 1 diabetic nephropathy. *Diabetes*, 56, 2155–60.

Van den Berg, J., Aten, J., Chand, M. A., *et al.* (2000). Interleukin-4 and interleukin-13 act on glomerular visceral epithelial cells. *J Am Soc Nephrol*, 11, 413–22.

Vasmant, D., Maurice, M., and Feldmann, G. (1984). Cytoskeleton ultrastructure of podocytes and glomerular endothelial cells in man and in the rat. *Anat Rec*, 210, 17–24.

Vogelmann, S., Nelson, W., Myers, B., *et al.* (2003). Urinary excretion of viable podocytes in health and renal disease. *Am J Physiol Renal Physiol*, 285, F40–8.

Wang, J., Yang, A. H., Chen, S. M., *et al.* (1993). Amelioration of antioxidant enzyme suppression and proteinuria in cyclosporin-treated puromycin nephrosis. *Nephron*, 65, 418–25.

Weil, E., Lemley, K. V., Yee, B., *et al.* (2011). Podocyte detachment in type 2 diabetic nephropathy. *Am J Nephrol*, 33, 21–4.

Welling, L., Zupka, M., and Welling, D. (1995). Mechanical properties of basement membrane. *News Physiol Sci*, 10 (1), 30–5.

Wiggins, R., Fantone, J., and Phan, S. (1989). Mechanisms of vascular injury. In C. Tisher and B. Brenner (eds.) *Renal Pathology*, pp. 965–93. Philadelphia, PA: Lippincott.

Wilcox, C., Welch, W. J., Murad, F., *et al.* (1992). Nitric oxide synthase in macula densa regulates glomerular capillary pressure. *Proc Natl Acad Sci U S A*, 89, 11993–7.

Winkler, D., Elger, M., Sakai, T., *et al.* (1991). Branching and confluence pattern of glomerular arterioles in the rat. *Kidney Int*, 39, S-2–8.

Yamamoto, T., Watanabe, T., Ikegaya, N., *et al.* (1998). Expression of types I, II, and III TGF-beta receptors in human glomerulonephritis. *J Am Soc Nephrol*, 9, 2253–61.

Yang, G. and Morrison, A. (1980). Three large dissectable rat glomerular models reconstructed from wide-field electron micrographs. *Anat Rec*, 196, 431–40.

Yu, D., Petermann, A., Kunter, U., *et al.* (2005). Urinary podocyte loss is a more specific marker of ongoing glomerular damage than proteinuria. *J Am Soc Nephrol*, 16, 1733–41.

Yurchenco, P. and Cheng, Y. (1994). Laminin self-assembly: a three-arm interaction hypothesis for the formation of a network in basement membranes. *Contrib Nephrol*, 107, 47–56.

Zenker, M., Aigner, T., Wendler, O., *et al.* (2004). Human laminin b2 deficiency causes congenital nephrosis with mesangial sclerosis and distinct eye abnormalities. *Hum Mol Genet*, 13, 2625–32.

Zhao, J., Ardaillou, N., Lu, C. Y., *et al.* (1994). Characterization of C-type natriuretic peptide receptors in human mesangial cells. *Kidney Int*, 46(3), 717–25.

Zimmermann, K. (1929). Ueber den Bau des Glomerulus der menschlichen Niere. *Z Mikrosk Anat Forsch*, 18, 520–52.

Zimmermann, K. (1933). Ueber den Bau des Glomerulus der Saeugerniere. *Z Mikrosk Anat Forsch*, 32, 176–278.

CHAPTER 44

Function of the normal glomerulus

Jean-Claude Dussaule, Martin Flamant, and Christos Chatziantoniou

Relationship between glomerular structure and function

The detailed structure of the glomerulus is described in Chapter 43. The glomerular barrier is composed of a layer of endothelial cells, the basal membrane, the foot processes interconnected by the slit diaphragm (SD) (Venkatachalam and Renke, 1978; Kanwar et al., 1991; Jarad and Miner, 2009; Miner, 2011). Despite the lack of open pores, the glomerular barrier can be considered as a semi-permeable membrane due to its structural and biochemical characteristics. Water and soluble molecules of small molecular weight (up to 5 kDa) pass freely depending on the hydrostatic or osmotic pressure. For most authors, the layer of endothelial cells, its cell-coat, the three-dimensional structure of proteoglycans (long and ramified chains of polysaccharides), and the SD are the major physical barriers for the passage of macromolecules (Haraldsson et al., 2008; Satchell and Braet, 2009; Fogo and Kon, 2010; Friden et al., 2011). Recently, a subpodocyte space has been characterized that could contribute to the hydraulic resistance (Salmon et al., 2009). Proteins that are required for the correct function of the SD include podocin, TRPC6, nephrin, and FAT or proteins that interact with the SD complex (CD2AP, Nck, Zona Occludens-1, synaptopodin) (Chuang and He, 2009). The important role of these proteins in ultrafiltration is evidenced by the proteinuria observed when their expression or structure is altered (see the hereditary nephrotic syndromes observed due to mutations of nephrin or podocin genes (Kestila et al., 1998)). The water permeability of this barrier is the highest observed in the organism since it can reach 180 L/24 hours for both kidneys (60 nL/glomerulus/min).

However, the glomerular barrier is not entirely semi-permeable since macromolecules up to 70 kDa can pass through by diffusion across the gel of the basal membrane, depending on their three-dimensional structure and electrical charge (Smithies, 2003). The negative electrostatic properties of the barrier have already been demonstrated. Moreover, a filtration-dependent electrical potential has been measured in micropuncture experiments in *Necturus maculosus* (Hausmanm et al., 2010). This electrical potential that is negative within the Bowman space is likely generated during the filtration process in humans (Hausmanm et al., 2012). Using dextrans (Chang et al., 1975), it has been clearly demonstrated that the percentage of the clearance of a macromolecule relative to a freely filtered substance diminished not only with regards to its size but also to its negative electrical charge (Fig. 44.1). Independently of the charge, proteins or blood microparticles above the limit of 70 kDa are not filtered (Table 44.1). Some of these issues are described further in Chapter 43.

Composition of primitive urine

The primitive urine results from the ultrafiltration of plasma and contains low levels of proteins (100–300 mg/L vs 72 g/L in plasma), which are almost completely reabsorbed in the proximal tubule. In contrast, the concentration in the primitive urine of molecules < 5 kDa is close to their plasma concentration. This concentration can be altered if one of the following applies:

1. Urine does not contain blood proteins and thus a correction factor equal to 7% should be applied for the soluble molecules
2. The retention of proteins (mainly negatively charged) by the glomerular barrier creates a shift in the equilibrium between the diffused anions and cations on both sides of the barrier. According to the Gibbs–Donnan law, anion concentration is higher in the urine than in plasma (and vice versa for cations). The highest the charge of electrolytes, the strongest becomes the shift of equilibrium.
3. Small molecules or ions (like calcium) that are usually bound to proteins undergo incomplete ultrafiltration.

Determinants of glomerular filtration

Single nephron glomerular filtration rate

The pressure leading to the creation of glomerular filtration follows similar principles with the exchanges between vascular and interstitial compartments applied in the rest of the body. In each point of glomerular capillary, the flow towards the Bowman's space is proportional to the difference of hydrostatic and osmotic pressures according to the Starling's equation (Maddox et al., 1975):

$$J = K\,[(Pcap - Pu) - (\pi cap - \pi u)]$$

where Pcap and πcap are the capillary hydrostatic and osmotic pressures, respectively, and Pu and πu are the hydrostatic and osmotic

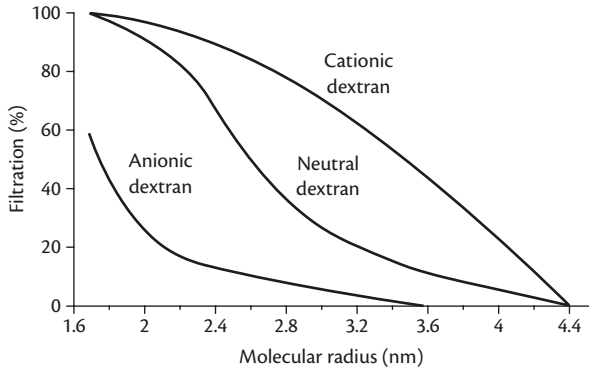

Fig. 44.1 Percent of glomerular filtration of dextran molecules depends on their size and their electric charge.

Table 44.1 Relations between molecular weight, the Stokes's molecular radius, and the percentage of glomerular filtration

Substance	Molecular weight (Da)	Molecular radius (nm)	% of filtration
Water	18	0.10	100
Sodium	23	0.14	100
Urea	60	0.16	100
Glucose	180	0.36	100
Inulin	5500	1.48	100
Myoglobin	17,000	1.95	75
Egg albumin	43,500	2.85	22
Haemoglobin	68,000	3.25	3
Human albumin	69,000	3.55	< 1

pressures of the Bowman's space. Because πu is negligible due to the absence of protein filtration, the equation can be simplified to:

$$J = K\,(\Delta P - \pi\,cap)$$

Glomerular filtration rate (GFR) is proportional to the filtration surface. If k is the permeability coefficient, then the single nephron GFR (SNGFR) is:

$$SNGFR = k \times S\,(\Delta P - \pi cap)$$

k × S is also called the glomerular ultrafiltration coefficient or Kf:

$$SNGFR = Kf\,(\Delta P - \pi cap) = Kf \times Puf$$

where Puf is the mean glomerular ultrafiltration pressure (Brenner et al., 1971).

Considering that each nephron has a similar SNGFR, then:

$$GFR = n \times Kf \times Puf$$

where n is the total number of nephrons and GFR the glomerular filtration rate for both kidneys. In real terms, the above equation is approximate because the filtration surface (and thus Kf) varies among glomeruli (it is 30–50% higher in juxtamedullary compared to cortical glomeruli).

Hydrostatic and osmotic profiles of the glomerulus

Munich Wistar rats provided an excellent experimental model to measure Pcap, πcap and Pu, because they have superficial cortical glomeruli and thus facilitate micropuncture studies (Brenner et al., 1977). An important finding was that Pcap was approximately 50 mmHg under euvolaemia conditions, a value close to that observed in other capillaries. Thus, the high rate of glomerular ultrafiltration compared to vascular-interstitial exchanges of the other organs is essentially due to a higher value of the hydraulic permeability coefficient k (Savin 1983).

In humans, Kf is between 8 and 18 nL/min/mmHg, depending on the method of measurement. Ultrafiltration leads to increasing protein concentration along glomerular capillaries and thus to increase osmotic pressure, πcap, which in turn limits ultrafiltration. In contrast, Pcap and Pu vary little, and in such a way that Puf decreases in the capillary when πcap increases.

Several studies showed that, depending on the experimental conditions, either Puf was cancelled before the capillary extremity (filtration in equilibrium), or in contrast Puf remained positive along the glomerular capillary (filtration out of equilibrium) (Fig. 44.2). In humans under physiological conditions, Puf is positive in most of glomerular capillaries. However, due to the heterogeneity of the length of glomerular capillaries, it is likely that both mechanisms exist. (Remuzzi et al.1992).

Physiological factors modulating glomerular filtration rate

Under physiological conditions, GFR is mainly modulated by renal plasma flow (RPF), Pcap and Kf, and cortical flow distribution. Under pathophysiological conditions, several factors can modify GFR, because all the above parameters can be profoundly altered

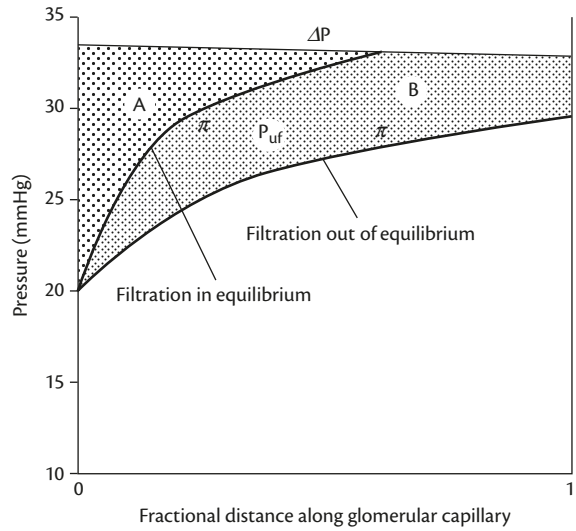

Fig. 44.2 Profile of hydrostatic (ΔP) and osmotic (π) pressures in glomerulus, according to the conditions of filtration with or without equilibrium. Mean pressure of ultrafiltration (P_{UF}) is higher in conditions of disequilibrium (areas A+B) than in conditions of equilibrium (area A).

during the development and progression of renal injuries affecting glomerular structure, protein excretion or tubulointerstitial function.

Renal plasma flow

Simultaneous estimations of GFR and renal plasma flow show that the ratio GFR/RPF called filtration fraction (FF) remains constant between 20% and 25%. When Pcap and Kf are maintained constant, the increase of RPF induces an increase in GFR, especially when the glomerular filtration is in equilibrium (Deen et al., 1972).

Capillary hydrostatic pressure

The hydrostatic pressure inside the Bowman's capsule, Pu, is stable under physiological conditions (10 mmHg), and thus ΔP depends mainly on Pcap variations. Pcap is not sensitive to blood pressure variations because of the renal autoregulation mechanisms (an increase of perfusion pressure induces afferent arteriole vasoconstriction maintaining thus stable the renal blood flows (RBFs) and Pcap). The main factor altering Pcap is the modification of equilibrium between afferent and efferent arteriole resistances, due to the action of local agents or hormones. Afferent arteriole dilation or efferent arteriole constriction increase Pcap thus SNGFR (although in the case of efferent arteriole, its constriction decreases RBF which in turn counteracts Pcap increase). Inversely, afferent arteriole constriction or efferent arteriole dilation decrease Pcap (Fig. 44.3).

Glomerular ultrafiltration coefficient

The coefficient of hydraulic glomerular permeability (k) can slightly decrease when podocytes contract (the foot processes of podocytes contain actin filaments). The decrease of the surface of filtration pores of podocytes leads to the coefficient k decrease. However, most of the variations of Kf are due to the modification of the filtration surface S. Mesangial cells contract under the action of vasoconstrictor agents, and this contraction decreases the filtration surface and thus Kf and SNGFR. The inverse effect is observed when vasodilators act on mesangial cells (Brenner et al., 1971). In conditions of unbalanced glomerular filtration (Puf does not reach 0, along the capillary), Kf variations influence more SNGFR than those of RPF.

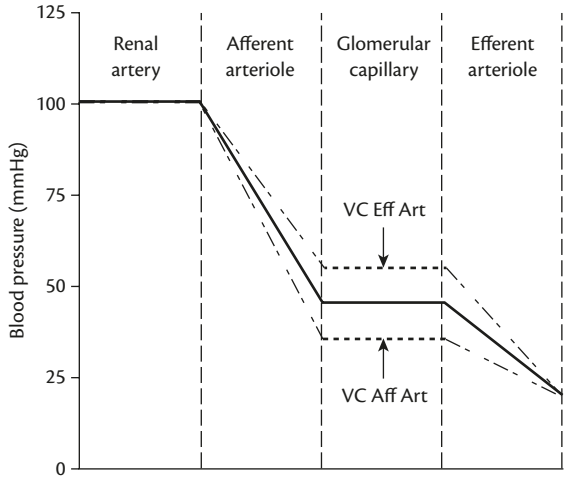

Fig. 44.3 In experimental conditions, vasoconstriction of afferent or efferent arterioles without changes of haemodynamic pressure induces a fall of renal blood flow. In the first case (afferent vasoconstriction), glomerular filtration rate decreases while it does not change when efferent arteriole is vasoconstricted because, in this case, glomerular capillary pressure increases.

Glomerular filtration rate in humans

Techniques for measuring and estimating GFR are described in Chapter 7.

The maximum value of GFR for both kidneys is approximately 120 mL/min/1.73 m². Ageing is associated with a progressive loss of renal function due to a gradual increase of sclerotic glomeruli (5% at 40 years, > 40% at 80 years) (see Chapter 300). There is no actual reference for GFR in the ageing population. The previously proposed notion of a loss of 1 mL/min/1.73 m² per year after the age of 40 years appears excessive. It is however admitted that a value < 60 mL/min/1.73 m² is pathological, independently of age.

Changes in the sodium intake between 20 and 1000 mmol/24 hours induce small variations of GFR. GFR is increased with the protein intake due to increase renal plasma flow by shifting the equilibrium of filtration along the glomerular capillary. The capacity of the kidneys to increase GFR above normal levels is called renal functional reserve (Bosch, 1995). It can be calculated by measuring the difference of GFR before and after protein overload. This process was proposed as predictive marker of renal hypertrophy following nephrectomy, although a direct link was never clearly established.

Regulation of renal blood flow and glomerular filtration rate

The endocrine or paracrine regulation of GFR is a complex process because a variety of local or hormonal factors can interfere and alter the physical parameters of glomerular filtration (Table 44.2).

Table 44.2 Hormones and autacoids modulating GFR and RPF

Vasoconstrictors	Vasodilators
Adenosine	Adenosine
Angiotensin II	Adrenomedullin
Antidiuretic hormone	ATP
ATP	Bradykinin
Endothelin	Dopamine
Growth factors (epidermal growth factor (EGF), platelet-derived growth factor (PDGF)	CGRP (calcitonin gene-related peptide)
Neuropeptide Y	Histamine
Leukotrienes LTC4 and LTD4	Insulin and insulin-like growth factor
Platelet activating factor	Natriuretic peptides (atrial, brain, and C-type (ANP, BNP, CNP))
Norepinephrine	Nitric oxide
Thromboxane (TXA2)	Prostaglandins E_2 and I_2
Vascular endothelial growth factor	PTH (parathyroid hormone)
Vasopressin	PTHrp (PTH-related peptide)
20 hydroxyeicosatetraenoic acid (20 HETE)	Relaxin

Note: adenosine and ATP can be vasodilators in several tissues but they are predominantly vasoconstrictors in renal vessels.

Among these factors, angiotensin II, a vasoconstrictor, nitric oxide, and prostaglandins, vasodilators, and adenosine vasoconstrictor or vasodilator according to the activation of A1 or A2 receptors, play a major role in regulating GFR.

Endocrine and paracrine vasoconstrictors

Angiotensin II

Angiotensin II acts on its cell targets by activating the AT_1 or AT_2 receptors (Ardaillou et al., 1998). AT_1 is the major receptor in the mature kidney and its activation is responsible for most of the vasoconstrictor actions of angiotensin II. Recent studies propose that a part of the effects of angiotensin II is mediated by the transactivation of growth factor receptors (Metha and Griendling, 2007), such as epidermal growth factor and platelet-derived growth factor. AT_2 activation antagonizes the AT_1 signalling and can create a negative retro-control of the AT_1-induced effects. Angiotensin II is catabolized to angiotensin III by cleaving the N-terminal, and then to angiotensin IV, a hexapeptide. Deletion of the C terminal of angiotensin II leads to the formation of angiotensin-(1–7).

Juxtaglomerular cells are the only renal cells capable of producing active renin. Renin synthesis and release by these cells is the limiting step of the activation of the renin–angiotensin system (RAS). In addition, the angiotensin-converting enzyme may act either as an ectoenzyme inserted in the cell membrane of endothelial or epithelial cells or as a plasma circulating enzyme. The renal synthesis of angiotensin II is particularly elevated due to the high local renin secretion and to the presence of the angiotensin-converting enzyme in the arteriolar endothelium, glomeruli, and renal tubular proximal epithelium (Ardaillou and Michel, 1999). Angiotensin II can also act on the renal vasculature as a circulating hormone because it can be also synthesized systemically (Crowley and Coffman, 2012).

When a pressor dose of angiotensin II is injected in a euvolaemic or dehydrated animal, the afferent and efferent arteriolar resistances are increased whereas RBF and Kf decreased. The effect of angiotensin II is predominant in the mesangial and smooth muscle cells of the efferent arterioles and is mediated by the activation of AT_1 receptors leading to an increase of cytosolic calcium. This vasoconstrictor effect has little influence in the glomerular filtration. GFR is decreasing but to a much lesser degree than RBF because of an increased Pcap. This is due to a more pronounced (or to at least equal) vasoconstriction of the efferent compared to the afferent arteriole. Furthermore, when the dose of angiotensin II is sub-pressor (without an increase in systemic blood pressure), GFR is very little altered whereas RBF clearly decreases. Similarly, in conditions of renin release (response to hypovolemia or following induction of renin synthesis), GFR is affected little compared to the major reduction of RBF (Navar 1998) (Fig. 44.4). In experimental conditions, chronic exposition to high levels of angiotensin II leads to a change of phenotype of podocytes that favours the presence of proteinuria (Huby et al., 2009; Palm, 2012)

The dissociation of the effects of angiotensin II on RBF and GFR is better seen in some pathophysiological conditions. For instance, administration of angiotensin-converting enzyme inhibitors to heart failure or renovascular hypertension patients can lead to a decrease of GFR while RBF is normalized. This paradox can be explained by considering a predominant action of angiotensin II on efferent arterioles which in these particular pathological conditions is protective for the glomerular function.

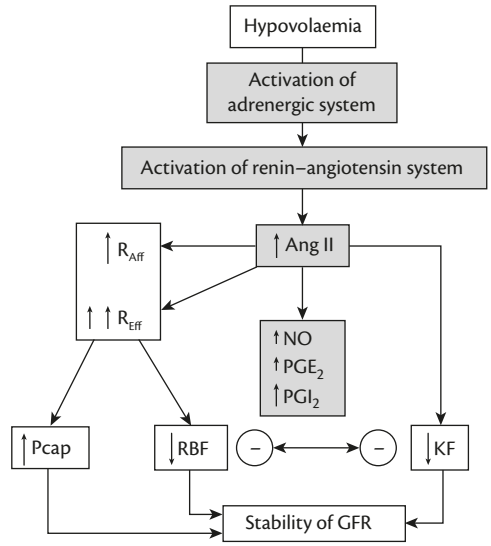

Fig. 44.4 Angiotensin II, in response to severe hypovolaemia, increases renal resistances and decreases renal blood flow (RBF). In these pathophysiological conditions, endocrine regulation overwhelms local autoregulation. Glomerular filtration rate is only slightly affected by the fall of RBF because angiotensin II favours the increase of glomerular Pcap and stimulates NO and prostaglandin production that counteracts its vasoconstrictor action on smooth muscle and mesangial cells.

When the RAS is not activated (euvolaemic conditions) antagonists of AT_1 receptors have little effect on GFR, suggesting that endogenous angiotensin II is not a major regulator of glomerular filtration under normal conditions (Navar et al., 1996).

It is difficult to dissociate the renal vascular effects of angiotensin II from those of nitric oxide (NO) and endogenous prostaglandins (PGE_2 and PGI_2) in clinical conditions in which RAS is activated or during experimental perfusion of angiotensin II. The close and rapid interaction between these vasoactive agents is clearly demonstrated by using cyclooxygenase or NO-synthase inhibitors which exacerbated the angiotensin II-induced decrease of RBF and GFR. Inversely, angiotensin II antagonizes its own action by inducing the synthesis of the above vasodilators (Navar et al., 1996) (Fig. 44.4).

Other vasoconstrictors

Endothelin (ET) is a very potent vasoconstrictor synthesized mainly in endothelial cells. Two of the three existing isoforms, ET-1 and ET-3, are present in the kidney. These peptides act locally in the mesangial and arteriolar smooth muscle cells to activate signalling pathways that are similar to those of angiotensin II. ET-1 synthesis, the major renal isoform, is induced by agents increasing intracellular calcium and activating protein kinase C in the endothelium, by transforming growth factor-β and by shear stress. In contrast, NO inhibits ET-1 synthesis.

Renal perfusion of ET-1, induces a sustained important decrease of RBF and GFR (Guan and Inscho, 2011), after a short initial increase due to the stimulation of NO. This biphasic action is due to the presence of ETB receptors in endothelial cells stimulating NO release and antagonizing the vasoconstrictor effect of ET_A receptors present in smooth muscle cells. During the phase of vasoconstriction, ET-1 increases renal arteriolar resistance and decreases Kf, and its action is more prolonged compared to that of angiotensin II.

The role of ET-1 in renal physiopathology is well described (Hocher et al., 1997), while is more controversial in physiological conditions. Administration of ET_A antagonists does not affect RBF or GFR under normal conditions. Experimental studies suggested that ET-1 interacts with angiotensin II during hypovolemia. Angiotensin II induces ET-1 synthesis which in turn can inhibit renin synthesis (Herizi et al., 1998). Inversely, ET-1 can have an indirect vasodilatory effect through activation of ET_B endothelial receptors and subsequent release of NO. This interaction can explain the mechanisms of action of relaxin, involved in the glomerular hyperfiltration during pregnancy, because its effects are inhibited by the antagonists of ET_B receptors and the blockers of NO synthesis.

ATP and adenosine have paracrine effects on renal circulation (Vallon and Osswald, 2009). ATP is a neuro-modulator present in the renal nerve terminal and a circulating factor released by vascular cells (Jankowski, 2008). ATP when it is bound to P_2y receptors of smooth muscle cells is a vasoconstrictor (Navar, 1998). When it is bound to endothelial P_2y receptors stimulating NO synthesis, ATP is vasodilatory. Adenosine synthesized in macula densa cells acts as vasoconstrictor through A_1 receptor activation leading to decreased cAMP synthesis in the afferent arteriolar smooth muscle cells. Adenosine is mainly involved in the control of tubuloglomerular feedback and can be partly involved in the NaCl–renin interaction. Its local production depends on activity of enzymes under the control of angiotensin II, which leads to a synergistic effect of both vasoconstrictors (Franco et al., 2009). Because at high doses, adenosine can activate A_2 vasodilatory receptors in renal vasculature, it may be hypothesized that these A_2 receptors buffer A_1-induced vasoconstriction of pre- and post-glomerular arterioles (Carlstrom, 2011).

The other vasoconstrictors shown in Table 44.2 play little role in the regulation of GFR under physiological conditions. In contrast, the involvement of several of them, such as thromboxane or leukotrienes sulphido-peptides is well established in pathological conditions.

Endocrine and paracrine vasodilators

Nitric oxide

NO is a major regulator of RBF and GFR under physiological conditions. NO is synthesized in endothelial and epithelial cells from L-arginine and in presence of two NO-synthase isoforms, NOS III and NOS I, respectively (Lamas and Rodriguez-Puyol, 2012). The renal glomerular and arteriolar endothelium is producing NO in response to shear stress and to the action of several vasoactive peptides increasing intracellular calcium (Gabbai and Blantz, 1999). NO secretion raised in macula densa during an increase of NaCl reabsorption. NO acts in the adjacent place to its production site cells, such as smooth muscle cells of afferent and efferent arterioles, mesangial and juxtaglomerular cells (NO is a vasodilator because it induces cGMP production in vascular and glomerular cells). Although its action on juxtaglomerular cells is less understood, most authors agree that NO increase renin synthesis.

The non-selective inhibitors of NO-synthase like L-NAME induce an immediate and sustained reduction of RBF and GFR. These effects are due to a higher increase of resistance of the afferent compared to efferent arteriole and a decrease of Kf (Deng and Baylis, 1993). The effect of NO inhibitors on Pcap is variable and depends on the induction or not of hypertension with the used doses. These results show a vasodilator effect of NO in renal vessels and mesangium, and can explain, at least partly, the moderate increase of GFR during hypervolemia inducing an increased tubular NO synthesis. Some authors have proposed that L-arginine, the natural substrate of NO synthases, is involved in the activation of renal functional reserve during protein overload by increasing NO production in renal vessels. In these circumstances, NO could act in synergy to glucagon induced in pancreas by the amino acids (Bosch, 1995). The interactions of NO with angiotensin II have been described in the above paragraph. The involvement of NO as a major player of GFR regulation is also confirmed by its interaction with other factors inducing NO-synthesis such as kinins, insulin, insulin-like growth factor, calcitonin gene-related peptide (CGRP), and parathyroid hormone-related peptide (PTHrp, sharing structural similarities with the parathyroid hormone).

Other vasodilators

The vasodilator prostaglandins prostacyclin or PGI_2, and PGE_2 are the major products synthesized by the arachidonic acid through activation of an enzymatic cascade involving phospholipase A_2 and cyclooxygenases (constitutive or inducible). PGE_2 acts through activation of its receptors EP2 and EP4 in renal resistance vessels and mesangium. PGI_2 and PGE_2 increase cAMP synthesis and thus induce vasodilatation (Sugimoto et al., 1994). Their main role is to antagonize the vasoconstrictor action of angiotensin II. Use of cyclooxygenase inhibitors has a detrimental effect and decrease RBF and GFR only in conditions in which RAS is activated (Navar et al., 1992).

Perfusion of PGE_2 or of a stable analogue of PGI_2 in the renal artery increases RBF but has little effect on GFR because the decrease of arteriolar resistance is counterbalanced by the decrease of Kf (Schnermann and Briggs 1981). Kf is decreased because both PGs stimulate renin secretion and thus angiotensin II synthesis. A similar dissociation between the alterations of RBF and GFR has been also observed with the other vasodilators, such as kinins, PTH, histamine, CGRP, or dopamine (Brenner et al., 1977).

The natriuretic factors produced in the heart (such as atrial natriuretic peptide (ANP) and brain natriuretic peptide (BNP)) have active receptors in the renal vessels and glomeruli. When infused at pharmacological doses, ANP or BNP increase RBF and GFR. The increase of Kf is more due to the hydraulic permeability modification than to an increase of filtration surface, because the active receptors of ANP and BNP are mainly expressed in podocytes. The cytoskeletal modifications of podocytes result from the ANP- or BNP-induced increase of cytosolic cGMP concentration. Since the pharmacological doses of ANP or BNP perfusion exceed by far their physiological concentrations, it is not clear if natriuretic peptides are involved in the control of GFR under physiological conditions. It cannot be excluded that these peptides participate in the regulation of GFR during hypervolemia conditions which stimulate their cardiac synthesis (Brenner et al., 1990).

Renal nerve regulation

The physiological importance of renal nerve regulation has been reassessed as a result of clinical and experimental data on the effects of renal denervation on blood pressure (Veelken and Schmieder, 2014). Renal innervations consist of efferent sympathetic and afferent sensory nerves (cholinergic innervations are absent). The renal action of afferent sensory nerves is not yet completely understood

while the physiological role of efferent sympathetic renal nerves is well known. Renal sympathetic activity is implied in the response to hypovolemia, including sodium retention, renin secretion, and, to a lesser extent, decrease of RBF and GFR. The sympathetic system innervates cortical vessels including juxtaglomerular apparatus, mesangial cells and macula densa cells among other renal structures (Johns, 1989). The major renal neurotransmitter is norepinephrine. Stimulation of sympathetic nerves induces arterial vasoconstriction and thus decreases RBF and GFR by two mechanisms: a direct vasoconstriction due to α-1-adrenergic receptors stimulation and an indirect effect of β-adrenergic receptors stimulation in the juxtaglomerular cells increasing renin secretion and angiotensin II synthesis. RBF decrease is more pronounced compared to GFR because of the concomitant vasoconstriction of afferent and efferent arterioles (Fig. 44.4) (Kalaitzidis et al., 2013).

Autoregulation of glomerular filtration rate and renal blood flow

Alterations of afferent and efferent arteriole diameters affect RBF. When preglomerular resistance changes in a physiological range, RBF and GFR change to the same direction. When postglomerular resistance increases, RBF decreases but GFR remains almost unchanged due to the increase of glomerular capillary pressure.

RBF does not change significantly for variations of mean arterial pressure between 80 and 180 mm Hg. This process, called 'autoregulation' of RBF, is observed even after denervation or in isolated perfused kidney thus suggesting the existence of internal adaptive mechanism(s) of haemodynamic stability. Autoregulation is considered to be essential for the normal function of the kidneys: due to the existence and efficiency of RBF autoregulation, GFR can remain stable within a large range of changes of perfusion pressure. Micropuncture experiments performed in a rat strain with easily accessible cortical glomeruli suggested that pre-glomerular resistance is a major factor influencing autoregulation (Just, 2007). RBF autoregulation is controlled by two mechanisms, one, very fast, is called myogenic response, and another one, slower, is called tubuloglomerular feedback. Myogenic response is an intrinsic function of vascular wall to contract in response to external stretching force. Tubuloglomerular feedback is a more complex mechanism specific to the kidney that leads to constriction of the afferent arteriole in response to an increase in sodium chloride concentration in the distal tubule and juxtaglomerular apparatus.

Myogenic control of renal blood flow

RBF autoregulation is suppressed by papaverin which inhibits pre-glomerular vessel contractility. Similar inhibition is observed by blockers of voltage-operated type 2 calcium channels indicating the involvement of these channels in the myogenic response. It is noteworthy that the density of calcium channels is high in the pre-glomerular vessels, whereas it is negligible in post-glomerular vessels. The mechanisms leading to the opening of calcium channels during increase of vascular wall stretching are not yet very well known. It appears though that the parietal stretch-induced conformational change of plasma membrane is more important than the action of local paracrine agents. The opening of calcium channels produces calcium influx into the smooth muscle cells leading to cell contraction and to the increase of arteriolar resistance. This increase of vascular resistance maintains stable RBF despite the increase of arterial pressure (Loutzenhiser et al., 2002). Moreover, this autoregulatory capacity plays a protective role against hypertensive renal damage since increased pre-glomerular resistances in response to high blood pressure prevent the parallel increase of glomerular capillary pressure (Bidani et al., 2009).

Tubuloglomerular feedback

The proximity of macula densa with the afferent arteriole suggests a cross-talking between alterations of urinary flow rate or concentration and pre-glomerular resistance. Although several questions still remain unanswered for a complete comprehension of the tubuloglomerular feedback, the general outline of this mechanism is the following. During a sudden increase of perfusion pressure, filtration pressure and GFR are increased instantaneously leading to an increased delivery of sodium concentration to the macula densa (despite the above-described increase of pre-glomerular resistance and the glomerulotubular equilibrium of proximal tubule antagonizing this phenomenon). The increase of NaCl reabsorption by the NaKCC2 co-transporter localized at the apical side of the macula densa cells triggers the release of a vasoconstrictor signal in the afferent arteriole, vasoconstriction leading to the subsequent decrease of RBF, glomerular capillary pressure and GFR (Singh and Thomson, 2010). Recently, experiments on genetically altered mice allowed adenosine to be recognized as the major vasoconstrictor signal by the way of activation of A_1 receptors. Studies on deficient mice in NTPDase1 or ecto-5′-nucleotidase demonstrate that adenosine comes from dephosphorylation of ATP released by tubular cells (Oppermann et al., 2008; Schnermann and Briggs, 2008). Angiotensin II, acting on AT_1 receptors, is a co-factor of the vasoconstriction induced by adenosine in response to increased NaCl uptake by macula densa cells (Franco et al., 2009) while numerous vasodilators such as NO (Carlstrom et al., 2011), carbon monoxide (Ren et al., 2012), prostaglandins (Araujo and Welsh, 2010), and adenosine itself via A_2 receptors (Bell and Welsh, 2009) can negatively modulate this tubular signal.

References

Araujo, M. and Welch, W. J. (2010). Tubuloglomerular feedback is decreased in COX-1 knockout mice after chronic angiotensin II infusion. *Am J Physiol Renal Physiol*, 298, F1059–63.

Ardaillou, R., Chansel, D., Chatziantoniou, C., *et al.* (1998). Biology and functions of renal receptors for angiotensin II and its active fragments. *Adv Nephrol Necker Hosp*, 28, 225–57.

Ardaillou, R. and Michel, J. B. (1999). The relative roles of circulating and tissue renin-angiotensin systems. *Nephrol Dial Transplant*, 14, 283–6.

Bell, T. D. and Welch, W. J. (2009). Regulation of renal arteriolar tone by adenosine: novel role for type 2 receptors. *Kidney Int*, 75, 769–71.

Bidani, A. K., Griffin, K. A., Williamson, G., *et al.* (2009). Protective importance of the myogenic response in the renal circulation. *Hypertension*, 54, 393–8.

Bosch, J. P. (1995). Renal reserve: a functional view of glomerular filtration rate. *Semin Nephrol*, 15, 381–5.

Brenner, B. M., Ballermann, B. J., Gunning, M. E., *et al.* (1990). Diverse biological actions of atrial natriuretic peptide. *Physiol Rev*, 70, 665–99.

Brenner, B. M., Bohrer, M. P., Baylis, C., *et al.* (1977). Determinants of glomerular permselectivity: insights derived from observations in vivo. *Kidney Int*, 12, 229–37.

Brenner, B. M., Troy, J. L., and Daugharty, T. M. (1971). The dynamics of glomerular ultrafiltration in the rat. *J Clin Invest*, 50, 1776–80.

Bröchner-Mortensen, J. (1972). A simple method for the determination of glomerular filtration rate. *Scand J Clin Lab Invest*, 30, 271–4.
Carlstrom, M., Wilcox, C. S., and Welch, W. J. (2011). Adenosine A2A receptor activation attenuates tubuloglomerular feedback responses by stimulation of endothelial nitric oxide synthase. *Am J Physiol Renal Physiol*, 300, F457–64.
Chang, R. L., Ueki, I. F., Troy, J. L., *et al.* (1975). Permselectivity of the glomerular capillary wall to macromolecules. II. Experimental studies in rats using neutral dextran. *Biophys J*, 15, 887–906.
Chuang, P. Y. and He, J. C. (2009). Signaling in regulation of podocyte phenotypes. *Nephron Physiol*, 111, 9–15.
Cockcroft, D. W. and Gault, M. H. (1976). Prediction of creatinine clearance from serum creatinine. *Nephron*, 16, 31–41.
Coresh, J., Astor, B. C., McQuillan, *et al.* (2002). Calibration and random variation of the serum creatinine assay as critical elements of using equations to estimate glomerular filtration rate. *Am J Kidney Dis*, 39, 920–9.
Crowley, S. D. and Coffman, T. M. (2012). Recent advances involving the renin-angiotensin system. *Exp Cell Res*, 318, 1049–56.
Deen, W. M., Robertson, C. R., and Brenner, B. M. (1972). A model of glomerular ultrafiltration in the rat. *Am J Physiol*, 223, 1178–83.
Deng, A. and Baylis, C. (1993). Locally produced EDRF controls preglomerular resistance and ultrafiltration coefficient. *Am J Physiol*, 264, F212–15.
Fawaz, A. and Badr, K. F. (2006). Measuring filtration function in clinical practice. *Curr Opin Nephrol Hypertens*, 15, 643–7.
Flamant, M., Haymann, J. P., Vidal-Petiot, E., *et al.* (2012). GFR estimations using the Cokcroft-Gault, MDRD study and CKD-EPI equations in the elderly. *Am J Kidney Dis*, 60, 847–9.
Flamant, M., Vidal-Petiot, E., Metzger, M., *et al.* (2013). Performance of GFR estimating equations in African Europeans: basis for a lower race-ethnicity factor than in African Americans. *Am J Kidney Dis*, 62, 182–4.
Fogo, A. B. and Kon, V. (2010). The glomerulus—a view from the inside—the endothelial cell. *Int J Biochem Cell Biol*, 42, 1388–97.
Franco, M., Perez-Mendez, O., and Martinez, F. (2009). Interaction of intrarenal adenosine and angiotensin II in kidney vascular resistance. *Curr Opin Nephrol Hypertens*, 18, 63–7.
Friden, V., Oveland, E., Tenstad, O., *et al.* (2011). The glomerular endothelial cell coat is essential for glomerular filtration. *Kidney Int*, 79, 1322–30.
Froissart, M., Rossert, J., Jacquot, C., *et al.* (2005). Predictive performance of the modification of diet in renal disease and Cockcroft-Gault equations for estimating renal function. *J Am Soc Nephrol*, 16, 763–73.
Gabbai, F. B. and Blantz, R. C. (1999). Role of nitric oxide in renal hemodynamics. *Semin Nephrol*, 19, 242–50.
Guan, Z. and Inscho, E. W. (2011). Endothelin and the renal vasculature. *Contrib Nephrol*, 172, 35–49.
Haraldsson, B., Nystrom, J., and Deen, W. M. (2008). Properties of the glomerular barrier and mechanisms of proteinuria. *Physiol Rev*, 88, 451–87.
Hausmann, R., Grepl, M., Knecht, V., *et al.* (2012). The glomerular filtration barrier function: new concepts. *Curr Opin Nephrol Hypertens*, 21, 441–9.
Hausmann, R., Kuppe, C., Egger, H., *et al.* (2010). Electrical forces determine glomerular permeability. *J Am Soc Nephrol*, 21, 2053–8.
Herizi, A., Jover, B., Bouriquet, N., *et al.* (1998). Prevention of the cardiovascular and renal effects of angiotensin II by endothelin blockade. *Hypertension*, 31, 10–14.
Hocher, B., Thone-Reineke, C., Rohmeiss, P., *et al.* (1997). Endothelin-1 transgenic mice develop glomerulosclerosis, interstitial fibrosis, and renal cysts but not hypertension. *J Clin Invest*, 99, 1380–9.
Huby, A. C., Rastaldi, M. P., Caron, K., *et al.* (2009). Restoration of podocyte structure and improvement of chronic renal disease in transgenic mice overexpressing renin. *PLoS One*, 4, e6721.
Inker, L. A., Schmid, C. H., Tighiouart, H., *et al.* (2012). Estimating glomerular filtration rate from serum creatinine and cystatin C. *N Engl J Med*, 367, 20–9.
Jankowski, M. (2008). Purinergic regulation of glomerular microvasculature and tubular function. *J Physiol Pharmacol*, 59 Suppl 9, 121–35.
Jarad, G. and Miner, J. H. (2009). Update on the glomerular filtration barrier. *Curr Opin Nephrol Hypertens*, 18, 226–32.
Johns, E. J. (1989). Role of angiotensin II and the sympathetic nervous system in the control of renal function. *J Hypertens*, 7, 695–701.
Just, A. (2007). Mechanisms of renal blood flow autoregulation: dynamics and contributions. *Am J Physiol Regul Integr Comp Physiol*, 292, R1–17.
Kalaitzidis, R. G., Karasavvidou, D. and Siamopoulos, K. C. (2013). Renal sympathetic denervation and renal physiology. *Curr Clin Pharmacol*, 8(3), 189–96.
Kanwar, Y. S., Liu, Z. Z., Kashihara, N., *et al.* (1991). Current status of the structural and functional basis of glomerular filtration and proteinuria. *Semin Nephrol*, 11, 390–413.
Kestila, M., Lenkkeri, U., Mannikko, M., *et al.* (1998). Positionally cloned gene for a novel glomerular protein—nephrin—is mutated in congenital nephrotic syndrome. *Mol Cell*, 1, 575–82.
Lamas, S. and Rodriguez-Puyol, D. (2012). Endothelial control of vasomotor tone: the kidney perspective. *Semin Nephrol*, 32, 156–66.
Levey, A. S. (1990). Measurement of renal function in chronic renal disease. *Kidney Int*, 38, 167–84.
Levey, A. S., Bosch, J. P., Lewis, J. B., *et al.* (1999). A more accurate method to estimate glomerular filtration rate from serum creatinine: a new prediction equation. Modification of Diet in Renal Disease Study Group. *Ann Intern Med*, 130, 461–70.
Levey, A. S., Coresh, J., Greene, T., *et al.* (2007). Expressing the Modification of Diet in Renal Disease Study equation for estimating glomerular filtration rate with standardized serum creatinine values. *Clin Chem*, 53, 766–72.
Lewis, J., Agodoa, L., Cheek, D., *et al.* (2001). Comparison of cross-sectional renal function measurements in African Americans with hypertensive nephrosclerosis and of primary formulas to estimate glomerular filtration rate. *Am J Kidney Dis*, 38, 744–53.
Loutzenhiser, R., Bidani, A. and Chilton, L. (2002). Renal myogenic response: kinetic attributes and physiological role. *Circ Res*, 90, 1316–24.
Maddox, D. A., Bennett, C. M., Deen, W. M., *et al.* (1975). Determinants of glomerular filtration in experimental glomerulonephritis in the rat. *J Clin Invest*, 55, 305–18.
Mariat, C., Alamartine, E., Barthelemy, J. C., *et al.* (2004). Assessing renal graft function in clinical trials: can tests predicting glomerular filtration rate substitute for a reference method? *Kidney Int*, 65, 289–97.
Mehta, P. K. and Griendling, K. K. (2007). Angiotensin II cell signaling: physiological and pathological effects in the cardiovascular system. *Am J Physiol Cell Physiol*, 292, C82–97.
Miner, J. H. (2011). Glomerular basement membrane composition and the filtration barrier. *Pediatr Nephrol*, 26, 1413–17.
Nankivell, B. J., Gruenewald, S. M., Allen, R. D., *et al.* (1995). Predicting glomerular filtration rate after kidney transplantation. *Transplantation*, 59, 1683–9.
Navar, L. G. (1998). Integrating multiple paracrine regulators of renal microvascular dynamics. *Am J Physiol*, 274, F433–44.
Navar, L. G., Inscho, E. W., Majid, S. A., *et al.* (1996). Paracrine regulation of the renal microcirculation. *Physiol Rev*, 76, 425–536.
Olbrich, O., Ferguson, M. H., Robson, J. S., *et al.* (1950). Simplified procedure for determining the renal clearance of inulin and diodone. *Lancet*, 2, 565–7.
Oppermann, M., Friedman, D. J., Faulhaber-Walter, R., *et al.* (2008). Tubuloglomerular feedback and renin secretion in NTPDase1/CD39-deficient mice. *Am J Physiol Renal Physiol*, 294, F965–70.
Palm, F. (2012). The dark side of angiotensin II: direct dynamic regulation of the glomerular filtration barrier permeability to macromolecules. *Am J Physiol Renal Physiol*, 303, F789.
Remuzzi, A., Brenner, B. M., Pata, V., *et al.* (1992). Three-dimensional reconstructed glomerular capillary network: blood flow distribution and local filtration. *Am J Physiol*, 263, F562–72.

Ren, Y., D'ambrosio, M. A., Wang, H., *et al.* (2012). Mechanisms of carbon monoxide attenuation of tubuloglomerular feedback. *Hypertension*, 59, 1139–44.

Salmon, A. H., Neal, C. R., and Harper, S. J. (2009). New aspects of glomerular filtration barrier structure and function: five layers (at least) not three. *Curr Opin Nephrol Hypertens*, 18, 197–205.

Satchell, S. C. and Braet, F. (2009). Glomerular endothelial cell fenestrations: an integral component of the glomerular filtration barrier. *Am J Physiol Renal Physiol*, 296, F947–56.

Savin, V. J. (1983). Ultrafiltration in single isolated human glomeruli. *Kidney Int*, 24, 748–53.

Schnermann, J. and Briggs, J. P. (1981). Participation of renal cortical prostaglandins in the regulation of glomerular filtration rate. *Kidney Int*, 19, 802–15.

Schnermann, J. and Briggs, J. P. (2008). Tubuloglomerular feedback: mechanistic insights from gene-manipulated mice. *Kidney Int*, 74, 418–26.

Singh, P. and Thomson, S. C. (2010). Renal homeostasis and tubuloglomerular feedback. *Curr Opin Nephrol Hypertens*, 19, 59–64.

Smithies, O. (2003). Why the kidney glomerulus does not clog: a gel permeation/diffusion hypothesis of renal function. *Proc Natl Acad Sci U S A*, 100, 4108–13.

Stevens, L. A., Claybon, M. A., Schmid, C. H., *et al.* (2011). Evaluation of the Chronic Kidney Disease Epidemiology Collaboration equation for estimating the glomerular filtration rate in multiple ethnicities. *Kidney Int*, 79, 555–62.

Stevens, L. A., Coresh, J., Greene, T., *et al.* (2006). Assessing kidney function—measured and estimated glomerular filtration rate. *N Engl J Med*, 354, 2473–83.

Sugimoto, Y., Namba, T., Shigemoto, R., *et al.* (1994). Distinct cellular localization of mRNAs for three subtypes of prostaglandin E receptor in kidney. *Am J Physiol*, 266, F823–8.

Vallon, V. and Osswald, H. (2009). Adenosine receptors and the kidney. *Handb Exp Pharmacol*, 443–70.

Veelken, R. and Schmieder, R. E. (2014). Renal denervation – implications for chronic kidney disease. *Nat Rev Nephrol*, 10, 305–13.

Venkatachalam, M. A. and Rennke, H. G. (1978). The structural and molecular basis of glomerular filtration. *Circ Res*, 43, 337–47.

CHAPTER 45

Mechanisms of glomerular injury: overview

Neil Turner

Introduction

Seen as a cartoon, the glomerulus is composed of three main cell types plus its glomerular basement membrane (GBM) (Fig. 45.1). The different pathological processes that damage glomeruli usually target one of these, although other cells and structures may be injured in bystander damage, or altered by the response to injury.

Processes that may lead to glomerular injury include genetic, toxic, immune, and metabolic. Genetic conditions affecting the podocyte and GBM are summarized in Chapter 327 and Chapter 320 respectively, and toxic diseases in Chapter 82.

The intention here is to give an overview of the type of processes leading to glomerular damage. The paradigm is particularly useful for understanding the primary glomerulopathies which are mostly autoimmune mediated, but metabolic, toxic, and genetic diseases can be understood in the same framework (Fig. 45.1).

Mechanisms involved in individual diseases (e.g. autoantibodies in membranous nephropathy and anti-GBM disease, tissue injury in vasculitis and lupus) are considered in detail in chapters on individual diseases.

Two extremes define a spectrum

The glomerulus has a limited repertoire of responses to injury. It can leak protein; it can leak blood; it can lose filtration function; it can cause hypertension. These are the key features of glomerular disease.

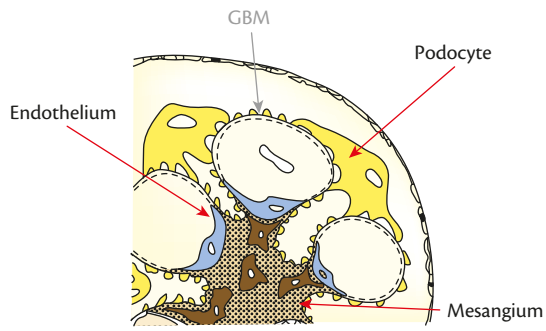

Fig. 45.1 Schematic diagram of a segment of a glomerulus. The podocyte (yellow) lies in the urinary space and is disordered in proteinuric diseases. In order to create glomerular haematuria, the red blood cell shown in the central capillary loop has to break through the GBM via defects created by pathological process (usually inflammatory).

It is useful to distinguish the pathological processes that cause proteinuria from those that cause haematuria. This distinction is clear at the initiation of most glomerular diseases, but some blurring occurs as it becomes chronic and the architecture of the glomerulus becomes increasingly abnormal. In particular, diseases that cause haematuria cause proteinuria if they leave scarring or progress. Nevertheless it remains a helpful distinction.

Most glomerular disease can be positioned at an approximate point on a spectrum with these two quite different modes of glomerular injury at the extremes (Fig. 45.2). At one end, proteinuria, and at the other end, haematuria; 'nephrotic' versus 'nephritic' (see Table 42.1, Chapter 42). Some diseases are so protean that they cannot be put at a single point, they may present in a number of different ways.

Diseases that cause proteinuria

It has become clear from studies of genetics, *in vitro* cell biology, and *in vivo* models that the podocyte is central to proteinuria. The three primary causes of pure nephrotic syndrome are all associated with some type of podocyte pathology:

- Minimal change disease (see Chapter 55)—podocyte dysfunction
- Membranous nephropathy (see Chapter 61)—podocyte attack by autoantibodies
- Focal segmental glomerulosclerosis (see Chapter 57)—podocyte injury or death.

So are most of the genetic causes of nephrotic syndrome, which almost all involve podocyte genes (see Chapter 327).

The major metabolic and systemic diseases causing nephrotic syndrome are associated with alterations to the matrix in the environment of podocytes through deposition of abnormal components and/or architectural changes. It is likely that this will disturb the function of these highly specialized, highly differentiated cells. In amyloidosis (see Chapter 152), deposition of fibrils affects the GBM directly beneath podocytes. In diabetes (see Chapter 149), a variety of abnormal matrix proteins are deposited. It is also possible that podocytes are directly injured by high glucose levels or by abnormally glycosylated proteins. An early change in diabetic nephropathy is increased deposition of normal GBM proteins (including collagen IV 345 network; see Chapter 320), similar to the changes seen in early membranous nephropathy, where the injury is mediated by autoantibodies. The podocyte may be responding to injury in diabetic nephropathy too.

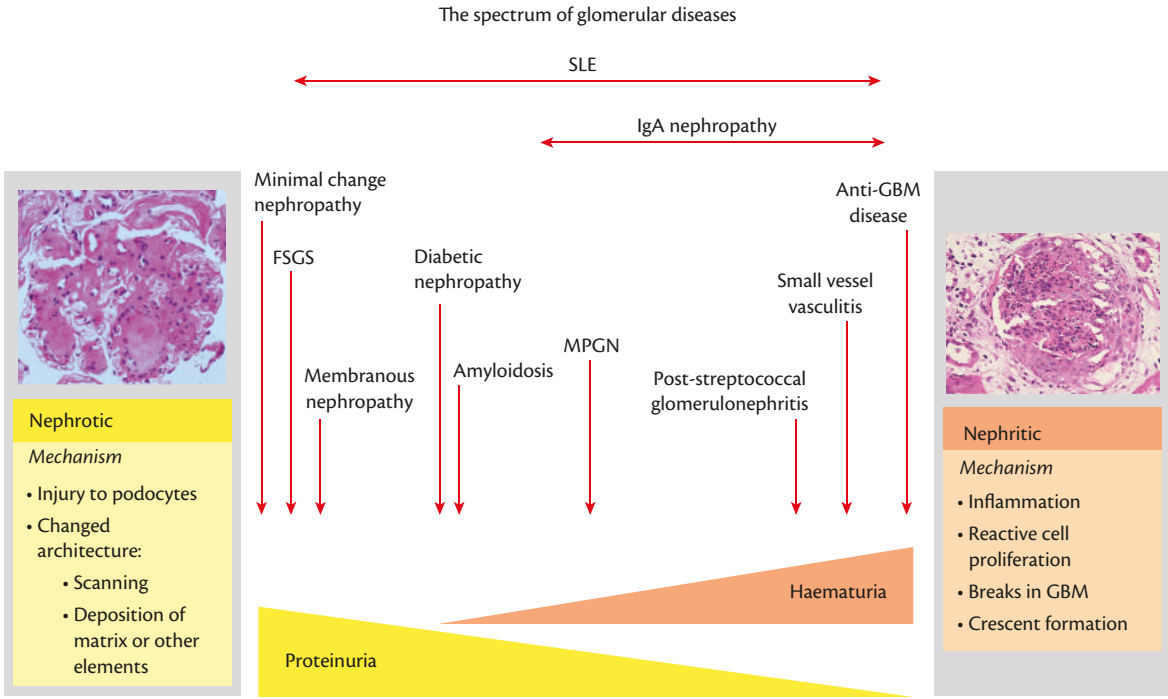

Fig. 45.2 The spectrum of glomerular diseases. It is useful to contrast the pathological processes causing proteinuria ('nephrosis') on the left, from those causing glomerular haematuria ('nephritis') on the right. Common causes of acquired nephropathy are shown at the point on the spectrum at which they generally present, but genetic and other diseases can also be fitted into this concept. Further details in text.

Similar alteration of glomerular matrix may occur in post-inflammatory scarring caused by diseases that are burnt out, or active at low level, causing their position on the spectrum to shift from mostly haematuric to more of a mixture (see below).

Low-level haematuria may occur in diabetes mellitus and some other diseases usually associated with nephrotic syndrome. This usually appears to be because of GBM fragility caused by deposition of abnormal material.

Most rarer causes of nephrotic syndrome can also be explained by these mechanisms.

Modulating proteinuria via the podocyte

Proteinuria is caused by podocyte dysfunction, and closely associated with progressive loss of renal function. There is evidence that some of the therapies we use now may be acting directly on podocytes, and understanding this may lead to new therapies.

Contrary to the impression that can be created by electron micrographs, it emerges that podocytes are dynamic, active cells that are studded with scores of receptor types enabling them to respond to external mediators. For example Winn et al. (2006) listed 25 heptahelical receptors of 11 types, and there are many other classes. The slit diaphragm is not a static barrier to protein, it is susceptible to a wide range of external influences both chemical and mechanical (Table 45.1).

Ransom et al. (2005) produced the first hard evidence that corticosteroids, one of our key immunosuppressive agents and the mainstay of therapy for minimal change nephrotic syndrome, might have direct structure-protective effects on podocytes. Faul et al. (2008) showed that the immunosuppressive calcineurin inhibitor tacrolimus also had direct effects on podocytes. This helps to explain why calcineurin inhibitors seem able to reduce proteinuria regardless of the cause (see Chapter 50; and described further in Chapter 52). Both of these drugs had previously been assumed to be acting through the immune system when used to treat proteinuric diseases (Mathieson, 2008). The observations raise the questions not only of whether minimal change disease is an immune disorder at all, but more broadly whether shared pathways in immune cells and podocytes might underlie some of the antiproteinuric effects of other immunomodulating agents.

Schießl and Castrop (2013) showed in rats that angiotensin II directly modulates glomerular permeability to albumin largely independently of the perfusion pressure. This action was mediated by angiotensin II type 1 (AT_1) receptors and partially attenuated by stimulation of AT_2 receptors. Other *in vitro* and *in vivo* evidence points the same way and suggests that a direct effect on podocytes may be an important mode of action of agents that block the renin–angiotensin system.

The literature includes a large amount of evidence around other mediators too (Table 45.1). Early evidence that targeting B7-1 may alter outcome of some nephrotic diseases (Yu et al., 2013) is exciting. We must hope that further additional therapeutic strategies are possible.

Diseases that cause haematuria

Haematuric diseases are characterized by breaks in the GBM. This can be caused by having a fragile GBM for genetic or other reasons, but usually it is caused by inflammatory disruption of the GBM. The majority of haematuric conditions are slowly or rapidly

Table 45.1 Agents that alter podocyte phenotype or behaviour *in vitro* or *in vivo*. Derangement and differentiation are shorthand terms. 'Deranged' phenotype seems likely to be physiologically important, possibly for motility, filter de-clogging, or other purposes, but if sustained may be associated with proteinuria

Promote derangement	Promote differentiation	Comment and key citations
Unknown factor in minimal change disease		
Circulating factor in idiopathic FSGS		
Angiotensin	ACE inhibitors, ARBs	Schießl and Castrop (2012)
Endothelin		
Insulin		
Anti-VEGF therapy	VEGF	
Stretch/tension		
TRPC6 signalling		
TLR4 signalling		Stimulated by LPS
B7-1 signalling	Abatacept	In patients with post-transplant FSGS, Yu et al. (2014)
TGF-β		
	Corticosteroids	Ransome et al. (2005)
	Calcineurin inhibitors (tacrolimus, ciclosporin)	Faul et al. (2008)
	Aldosterone antagonists	
	All-trans retinoic acid	
	PPAR-γ signalling	
	PPAR-α signalling?	Fenofibrate, Ting and Keech (2013)
	CDK2 inhibition	
	Vitamin D	
Any cellular injury		
Abnormal matrix		

ACE = angiotensin-converting enzyme; ARB = angiotensin receptor blocker; CKD2 = cyclin-dependent kinase 2; FSGS = focal segmental glomerulosclerosis; LPS = lipopolysaccharide; PPAR = peroxisome proliferator-activated receptor; TGF-β = transforming growth factor beta; VEGF = vascular endothelial growth factor.

destructive diseases associated with infiltration of inflammatory cells and proliferation of endogenous cells of the glomerulus, probably in attempts at repair. When the injury is very recent or very minor (e.g. early glomerulonephritis or Alport syndrome), haematuria may occur alone, without substantial proteinuria.

Crescentic nephritis and rapidly progressive nephritis

Crescentic nephritis can occur on a background of any of the 'nephritic' conditions. It is nephritic disease at its most severe and usually associated with the clinical syndrome of rapidly progressive nephritis (see Chapter 70). It reflects a response to severe injury, not a specific immune phenomenon. Evidence for this comes from its occasional occurrence in non-inflammatory causes of haematuria such as Alport syndrome and amyloidosis.

Nephrotic/nephritic diseases

Conditions such as membranoproliferative glomerulonephritis typically present with both haematuria and proteinuria but show some variation. These are inflammatory diseases, so sometimes the proteinuria may be due to scarring and alteration of the milieu for podocytes. However, in some there may be direct damage to the podocyte.

Diseases that cannot be pinned to one region of the spectrum

Immunoglobulin A nephropathy may present with haematuria alone, but commonly also presents at a late stage by which time urinary abnormalities have progressed as a consequence of glomerular damage and scarring (see Chapter 66). However, it is always associated with some haematuria.

Lupus nephritis may present with very different pathologies, so it may present with 'pure' nephrotic syndrome (no haematuria; e.g. lupus membranous), or with the most severe, crescentic disease, or with subacute disease when there is both haematuria and proteinuria (see Chapter 161).

Progressive renal disease after glomerular injury

Mechanisms to explain the progression of renal disease after glomerular injury, and its close relationship to proteinuria, are summarized in Chapter 136. Three broad mechanisms are proposed to explain this and while not mutually exclusive, they have different implications for therapy and new drug targets:

- Haemodynamic—increased glomerular perfusion pressure or stretch is the primary problem, angiotensin-converting enzyme inhibitors work by reducing this.
- Podocyte—podocyte stress and death is the engine of progression (see Chapter 139) (Zhou and Turner, 2010). If so, potential therapies are listed in Table 45.1.
- Toxicity of proteinuria—proteinuria creates progression by its effects on the tubulointerstitium (see Chapter 137).

These hypotheses are not mutually exclusive, and concepts such as disordered repair and replacement of cells must be important too (see Chapter 140). Proven ways to delay or arrest progression in clinical practice are discussed in Chapter 99.

References

Faul, C., Donnelly, M., Merscher-Gomez, S., *et al.* (2008). The actin cytoskeleton of kidney podocytes is a direct target of the antiproteinuric effect of cyclosporine A. *Nat Med*, 14, 931–8.

Mathieson, P. W. (2008). Proteinuria and immunity—an overstated relationship? *N Engl J Med*, 359, 2492–94.

Ransom, R. F., Lam, N. G., Hallett, M. A., *et al.* (2005). Glucocorticoids protect and enhance recovery of cultured murine podocytes via actin filament stabilization. *Kidney Int*, 68, 2473–83.

Schießl, I. M. and Castrop, H. (2013). Angiotensin II AT2 receptor activation attenuates AT1 receptor-induced increases in the glomerular filtration of albumin: a multiphoton microscopy study. *Am J Physiol Renal Physiol*, 305, F1189–200.

Ting, R. D. and Keech, A. (2013). Fenofibrate and renal disease. *Clin Lipidol*, 8, 669–80

Winn, M. P., Daskalakis, N., Spurney, R. F., *et al*. (2006). Unexpected role of TRPC6 channel in familial nephrotic syndrome: does it have clinical implications? *J Am Soc Nephrol*, 17, 378–87.

Yu, C.-C., Fornoni, A., Weins, A., *et al*. (2013). Abatacept in B7-1–positive proteinuric kidney disease. *N Engl J Med*, 369, 2416–23.

Zhou, Y. S. and Turner, A. N. (2010). New ways of thinking about proteinuria and progression of renal damage. *Nephron Exp Nephrol*, 16, e1–2.

CHAPTER 46

The patient with haematuria

John Neary and Neil Turner

The origin of haematuria

Haematuria is a common primary care problem and in the majority of cases is not caused by renal disease (Fig. 46.1). It is also a common finding in screening programmes or routine medical examinations. Many algorithms have therefore been developed to aid management at primary care level.

Age and whether the haematuria is visible (macroscopic) or invisible (microscopic) are commonly used as a primary sorting method. This is because the great majority of causes of visible, red blood in the urine fall into the province of the urologist, and similarly, with increasing age broadly urological causes are more likely than renal disease. This is an age gradient, not accurately defined by a fixed cut-off age, but US and UK guidelines suggest that for patients over the age of 40 the 'urological pathway' should usually be the first choice for investigation of even microscopic (non-visible) haematuria. Although the risks of urological malignancy are much lower with microscopic haematuria, in older patients this is usually the most important diagnosis to exclude initially.

Recommended pathways differ according to resource availability, regional differences in incidence of causes, and simply variation in policies. Comparing the United Kingdom with the United States, recommendations are largely similar but the US guidelines specify computed tomography urography (CTU) as the imaging method of choice (Grossfeld et al., 2001a, 2001b).

Epidemiology of haematuria

Studies looking at the prevalence of haematuria in the general population are complicated by the fact that results are method dependent, that is, whether dipsticks or microscopy have been used, or whether a single or multiple tests are used, and suggest widely different but relatively high prevalences of between 0.8% and 16%. The figure increases markedly with age (Table 42.1).

The most informative study to date comes from screening data on over 1.2 million 16–25-year-olds screened for fitness for military service in Israel (Vivante et al., 2011), and followed for an average of 21 years by linkage to the end-stage renal disease (ESRD) database. 0.3% (3690; 0.4% of males, 0.2% of females) had persistent non-visible haematuria (>5 red blood cells per high power field (HPF), measured if dipstick positive, see below; identified on three separate occasions). Those with proteinuria > 200 mg per 24 hours were excluded. End-stage renal failure (ESRF) occurred in 34 per 100,000 person years in those with haematuria and two per 100,000 patient years without. The main causes of ESRD in this group were Alport syndrome (n = 4), immunoglobulin A (IgA) nephropathy (n = 4), and other glomerular disease (n = 7); 11 were attributed to other conditions. Only one had cystic renal disease. The lower overall rate of haematuria in this study, and the increased risk associated with haematuria, is likely to be due to the high threshold used.

The data from most of the studies in Table 46.1 reflect a belief that screening is valuable in identifying future risk. Its place is better established for proteinuria than haematuria (see Chapter 50), but even that is not an accepted screening test in most countries. The US taskforce (Grossfeld et al., 2001a, 2001b) found no case for haematuria screening. Some have questioned that following the Vivante (2011) study, but to put it in perspective, only 26 of the 565 (4.6%) cases of ESRF in that study came from those with persistent microscopic haematuria, and in 21 years only 26 of the 3690 haematuric individuals, 0.7%, developed ESRF. The test was therefore neither sensitive nor specific. Rate of ESRF did seem to be climbing in a linear manner however, suggesting that the lifetime rate of ESRF in those individuals will be at least three to four times higher.

In Japan, screening urinalysis has been mandatory for workers and school-age children since the 1970s, extended in 1983 to include all adults aged 40 years or older (Iseki, 2012). Participants are classified based on the results of a single dipstick test. Results from 100,000 individuals in Okinawa in 1983 (Iseki et al., 1996) show that the incidence of haematuria measured this way rises quite steeply with age, and is significantly higher at all ages in women than men (7% versus 1% in 20s; 14% versus 6% in 70s). In 10 years of follow-up, 193 patients started dialysis. Proteinuria was a 10-fold stronger risk factor for ESRF than haematuria alone, but the combination of both conveyed approximately twice the risk of proteinuria alone.

Testing for haematuria

Urinalysis techniques are described in Chapter 6. Dipsticks are the most common method for testing.

Detecting non-visible haematuria

Dipsticks depend on the peroxidase activity of haem proteins and employ buffered tetramethylbenzidine and an organic peroxide to create a colour change. The detection limit is 5–20 intact red cells per microlitre or 15–600 micrograms per litre of free haemoglobin. Myoglobin will produce the same changes (Lamb and Price, 2011).

Definitions of haematuria have traditionally emanated from microscopy, definitions varying between 1 and 10 red cells per HPF, but typically five. Dipstick testing can pick up blood in urine at significantly lower levels, one to two red blood cells/HPF, thus picking up cases which would not normally meet the criteria. False-positive results can occur with dipsticks if myoglobinuria or haemoglobinuria is present, and can also occur with the presence of semen in the

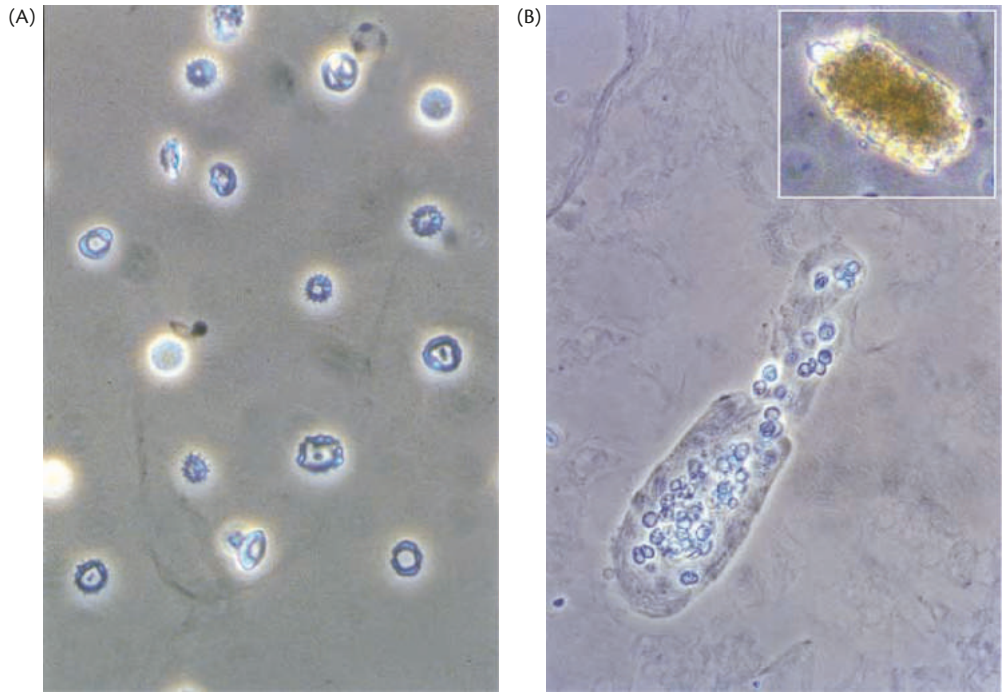

Fig. 46.1 Glomerular red cells in urine—different types of acanthocytes (left) and a red cell cast (right; inset shows a haemoglobin cast).

Table 46.1 Prevalence of haematuria in population studies

Study and reference	Prevalence (%)
Children	
Dodge et al. (1976)	0.6 (girls), 0.3 (boys)
Vehaskari et al. (1979)	1.0–4.0
Armed forces recruits 18–33 years	
Froom et al. (1984)	5.2
Vivante et al. (2011); see text	0.4 (males), 0.2 (females)
Older adults (> 50 years)	
Mariani et al. (1989)	13 (males and females)
Mohr et al. (1986)	13 (males),14 (females)
Ritchie et al. (1986)	2.5 (males, age not stated)
Thompson (1987)	4 (males, aged 40–90 years)
Messing et al. (1982)	13 (males)
Britton et al. (1992)	18 (males)
Iseki et al. (1996)	6 (males), 14 (females)
Elderly (> 75 years)	
Mohr et al. (1986)	13 (males), 9 (females)

Different criteria were used to define the upper limit of 'normal' and hence the lower limit of 'microhaematuria' in all of these studies, as discussed in the text. Original papers should be consulted for details.

urine or a strongly alkaline urine. If free myoglobin or haemoglobin is present in urine, microscopy on fresh urine will be negative for red cells. False negative findings can be found in patients on high doses of vitamin C (Brigden et al., 1992).

Blood-coloured urine

In the setting of macroscopic haematuria, it is useful to spin down the urine and examine the sediment and supernatant. In haematuria, the supernatant should be clear. If the supernatant is still coloured, it would suggest ingestion of foodstuffs such as beetroot/food colourings, drug use with rifampicin or phenolphthalein, or the presences of porphyrin or urates (for a list of substances that may colour urine see Chapter 6, Table 6.1).

Evaluation of haematuria

Exclude simple causes

Red urine may be a consequence of some foods, drugs (see Chapter 6). Positivity of stick tests during menstruation is normal. Urinary tract infection commonly causes invisible haematuria (dipstick test for nitrite, leucocytes; microscopy and culture).

Visible haematuria

Even a single episode of visible haematuria is significant unless there is an immediately apparent cause (Fig. 46.2); investigations as indicated in Fig. 46.3 are justified, unless the patient is suspected of having an aggressive glomerulonephritis. In those circumstances the quickest investigation is fresh urine microscopy for casts or fragmented and deformed red cells (see Chapter 6), along with scrutiny of the progression of tests of renal function and other signs of intrinsic renal disease. Presence of clots indicates non-glomerular bleeding.

Non-visible haematuria

For non-visible haematuria it is important to repeat the test, consider other explanations, then test for infection by culture and microscopy. Non-visible haematuria is often transient and can be

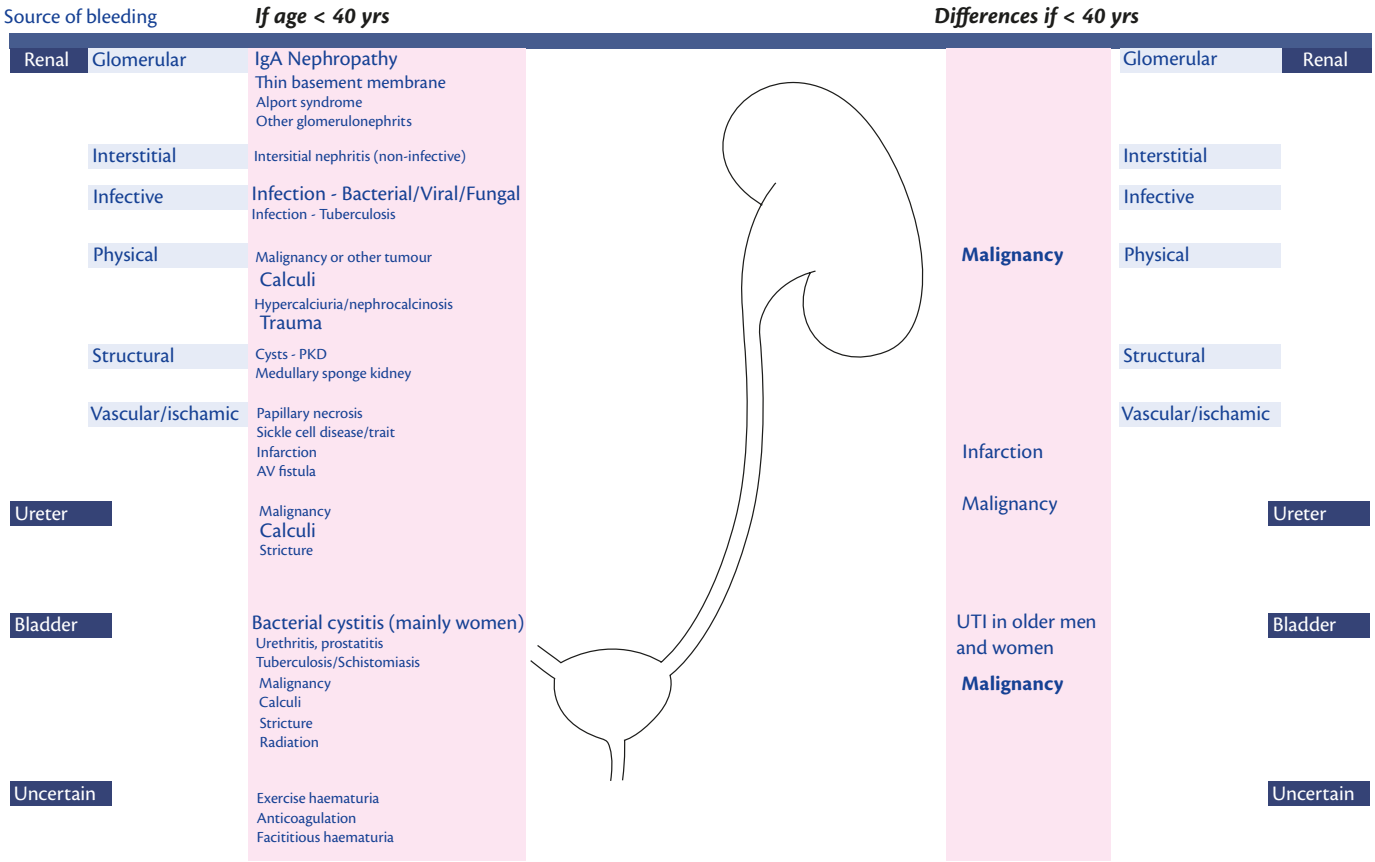

Fig. 46.2 Causes of haematuria. More common causes are shown in larger font. On the right hand column are shown causes that differ in frequency in older patients. The division at 40 years is arbitrary but is chosen by some guidelines.

related to exercise, trauma, sexual activity, or menstruation. In large population studies it has been shown that up to 40% of people might expect to have dipstick haematuria on at least one occasion, but the number positive is approximately halved if the test is repeated (Froom et al., 1984; Loo et al., 2013).

Few bacteriological laboratory microscopists use phase contrast microscopy or are alert to features of renal disease such as red cell morphology and nature of urinary casts, so this is rarely a means of picking out renal haematuria. As above, microscopy may be negative for red cells—this does not make the diagnosis of non-visible haematuria incorrect. Autoanalysers and automated image analysis may improve the diagnostic utility of this step in the future.

If non-visible haematuria is persistent, the algorithm in Fig. 46.3 is relevant. Regardless of age, key features suggesting a renal explanation include:

- proteinuria—elevated urinary albumin:creatinine ratio is a useful pointer to renal disease in young patients who do not have comorbid conditions, but less useful in older patients
- renal impairment
- hypertension—though this becomes less informative with age as the incidence of 'essential' hypertension is very high.

History should not only seek to exclude reversible causes such as urine infection but also explore possible family history of kidney disease (particularly Alport syndrome/ thin glomerular basement membrane nephropathy; see Chapters 321 and 323), and seek risk factors for urothelial cancers (age, smoking history, occupational risks—in particular working with dyes, rubber, or chemicals, drug exposure including cyclophosphamide, aristocholic acid in herbal remedies, and analgesics).

Travel history should ask about travel to areas where schistosomiasis is endemic (Chapter 181).

Physical examination should include blood pressure and look for any physical manifestations of renal or other disease.

Testing for urine protein and serum creatinine should be undertaken.

Urine cytology no longer appears in the diagnostic pathway in the United Kingdom. While it has reasonably good sensitivity for bladder cancers (80%), it is less good for upper urothelial tract cancer. These sensitivities are not high enough to rule out malignancy so cystoscopy is required; cystoscopy is also required if it is positive. It is no longer a first-line test.

Patients on anticoagulants

Anticoagulation has historically been said to be a cause of haematuria, but with careful monitoring of anticoagulation, this should not be the case. Patients on anticoagulation treatment should be investigated with the same concerns as any other patients with haematuria (unless international normalized ratio (INR) is significantly > 3 or there is bleeding from other sites. Patients with glomerulonephritis on anticoagulants may also be at increased risk of acute kidney injury (AKI) related to glomerular bleeding (see below).

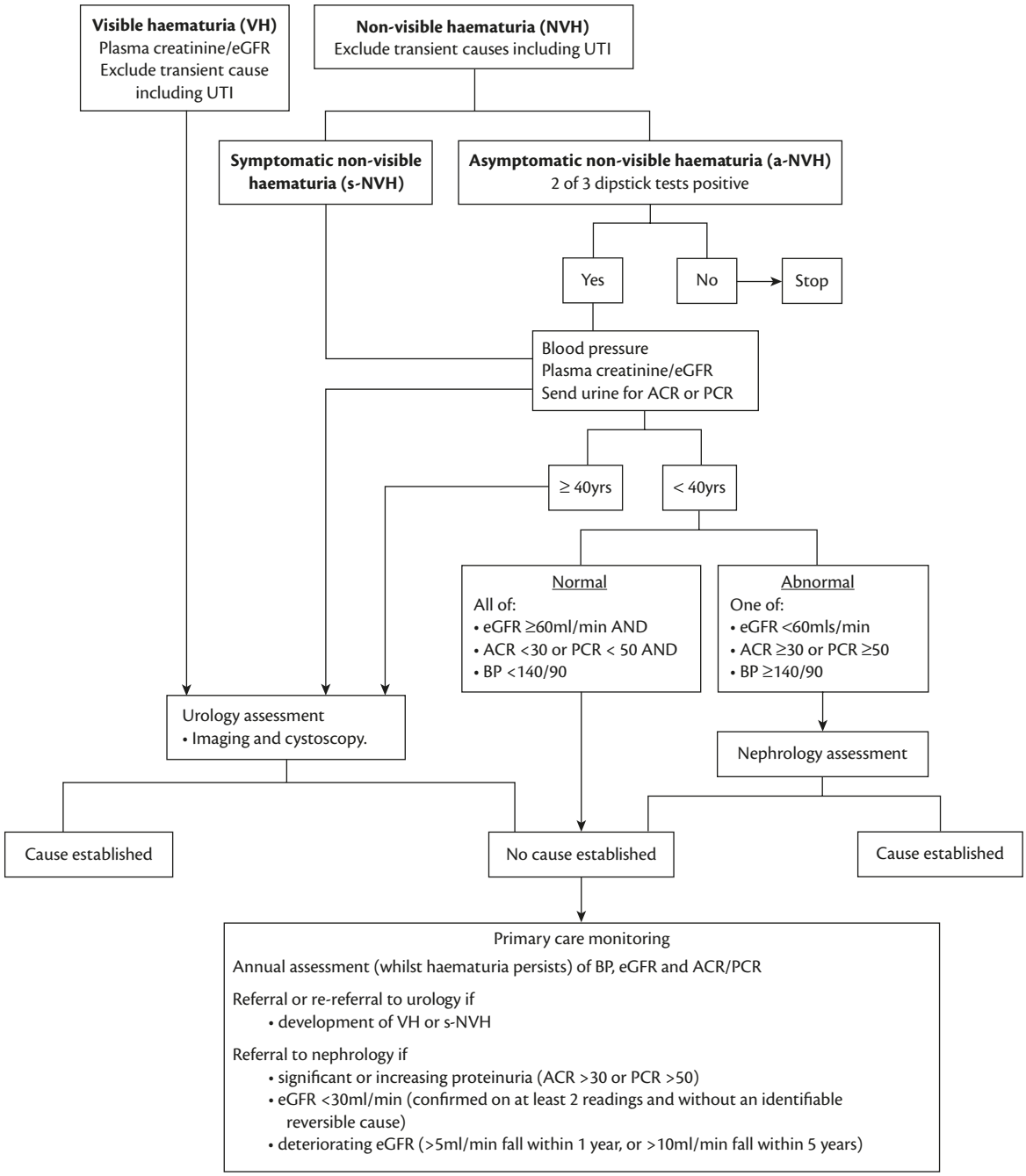

Fig. 46.3 UK recommendations for the management of haematuria. Joint guideline from the Renal Association and the British Association of Urological Surgeons. Further commentary and detail is provided in Anderson et al. (2008).

Patients who live in or visit the tropics

In some parts of the world, schistosomiasis (see Chapter 181) is the dominant cause of haematuria. Pathways for assessment will recognize this with urine microscopy for ova, or empirical treatment, as initial steps. Recognizing travellers who have picked up schistosomiasis and then returned to non-endemic areas is important but difficult as they may not recall their travel, or its significance. Serum antibody for schistosomal exposure may be useful to exclude the diagnosis in this group.

Management when investigations are negative

Visible haematuria with negative investigations

Investigations for visible haematuria should usually include cystoscopy and imaging of kidneys, ureters, and bladder. CTU is now the preferred imaging technique if available as it has superior sensitivity and specificity compared to intravenous urography (IVP)

(Maher et al., 2004; O'Connor et al., 2008) and one study comparing CTU to IVP demonstrated a sensitivity of 100% versus 61% and a specificity of 97% versus 91% (Gray Sears et al., 2002).

Renal ultrasound may be useful in determining size of kidney and any large masses, but is less sensitive than CTU , especially with lesions < 2 cm—sensitivity 26–60% (Warshauer et al., 1998). There are circumstances when CTU cannot be carried out—renal impairment, pregnancy, and hypersensitivity to contrast medium. In these settings, ultrasound or consideration of magnetic resonance urography would be reasonable alternatives.

If these investigations have been carried out and are negative after a single episode of visible haematuria, reassessment after a period may be indicated. If they are negative and significant visible haematuria is still occurring, testing for rarer causes of bleeding is indicated. This may include cannulation of ureters to localize bleeding, angiography to identify renal arteriovenous malformations, and possibly consideration of nutcracker phenomenon (see Chapter 48), for example.

Persistent non-visible haematuria with negative investigations

Between 19% and 68% of patients with non-visible haematuria remain undiagnosed (Howard and Golin, 1991; Khadra et al., 2000; Cohen and Brown, 2003). There is much debate as to how these patients should be managed long term, but a reasonable approach is that they should be followed in 6 months then annually. At each visit, checks of blood pressure, serum creatinine, urine protein:creatinine ratio, and dipstick should be made. If the haematuria settles on two consecutive urinalyses and no other features have developed, then they can be discharged. If the patient develops proteinuria, renal impairment, hypertension, or visible haematuria they should be re-evaluated.

In patients with microscopic haematuria in the absence of proteinuria or renal impairment, or a family history, the most common abnormalities on renal biopsy in developed world series are normal findings, IgA nephropathy, or thin basement membrane disease. The management of none of these conditions is altered by knowing this; monitoring remains the usual management (Richards et al., 1994; Sparwasser et al., 1994; Topham et al., 1994; McGregor et al., 1998; Hall et al., 2004; Choo et al., 2013). Most nephrologists therefore do not recommend renal biopsy unless there are particular reasons for seeking greater diagnostic certainty.

Acute kidney injury caused by glomerular haematuria

The phenomenon of AKI during profuse glomerular bleeding causing macroscopic haematuria was first reported in 1983 (Kincaid-Smith et al., 1983). Since then there have been numerous reports, mostly relating to IgA nephropathy in relatively young patients (reviewed in Moreno et al., 2012); indeed, IgA nephropathy seems over-represented, it is rarely recognized in other conditions. AKI can be severe requiring temporary dialysis, and while most cases resolve, recovery of glomerular filtration rate (GFR) may be incomplete. Biopsies tend to show many red cell casts and it is presumed to have a mixed obstructive-toxic aetiology (see Chapter 221). Free haem is well known to cause AKI in haemoglobinuria of various aetiologies, and also in rhabdomyolysis, and this may be part of the aetiology. Possible mechanisms of tubular damage are reviewed by Moreno et al. (2012).

Patients on anticoagulants may be at increased risk of developing this complication of glomerular bleeding. A number of reports have identified AKI more severe than glomerular changes would predict in patients on anticoagulants, usually warfarin, attracting the label warfarin-related nephropathy (Brodsky et al., 2009). Patients affected have usually had INR well over the therapeutic range. A later study (Brodsky et al., 2011) looked at creatinine changes retrospectively in 4006 patients developing an INR > 3, raising the possibility that the phenomenon might occur in patients without glomerular disease, but this is speculative. Outcomes in case reports have tended to be poor, with incomplete recovery of GFR.

References

Anderson, J., Fawcett, D., Feehally, J., *et al.* (2008). *Joint Consensus Statement on the Initial Assessment of Haematuria*. [Online] <http://www.baus.org.uk/AboutBAUS/publications/haematuria-guidelines>

Brigden, M. L., Edgell, D., McPherson, M., *et al.* (1992). High incidence of significant urinay ascorbic acid concentrations in a West Coast population—implication for routine analysis. *Clin Chem*, 38, 426–31.

Britton, P. J., Dowell, A. C., Whelan, P., *et al.* (1992). A community study of bladder cancer screening by the detection of occult urinary bleeding. *J Urol*, 148, 788–90.

Brodsky, S. V., Satoskar, A., Chen, J., *et al.* (2009). Acute kidney injury during warfarin therapy associated with obstructive tubular red blood cell casts: a report of 9 cases. *Am J Kidney Dis*, 54, 1121.

Brodsky, S. V., Nadasdy, T., Rovin, B. H., *et al.* (2011). Warfarin-related nephropathy occurs in patients with and without chronic kidney disease and is associated with an increased mortality rate. *Kidney Int*, 80, 181.

Choo, B. S., Hahn, W. H., Cheong, H. I., *et al.* (2013). A nationwide study of mass urine screening tests on Korean school children and implications for chronic kidney disease management. *Clin Exp Nephrol*, 17, 205–10.

Cohen, R. A. and Brown, R. S. (2003). Clinical practice. Microscopic hematuria. *N Engl J Med*, 348(23), 2330–8.

Froom, P., Ribak, J., and Benbassat, J. (1984). Significance of microhaematuria in young adults. *Br Med J (Clin Res Ed)*, 288, 20–2.

Gray Sears, C. L., Ward, J. F., Sears, S. T., *et al.* (2002). Prospective comparison of computerized tomography and excretory urography in the initial evaluation of asymptomatic microhaematuria. *J Urol*, 168, 2457.

Grossfeld, G. D., Litwin, M. S., Wolf, J. S., Jr., *et al.* (2001a). Evaluation of asymptomatic microscopic hematuria in adults: the American Urological Association best practice policy—Part I: Definition, detection, prevalence, and etiology. *Urology*, 57, 599–603.

Grossfeld, G. D., Litwin, M. S., Wolf, J. S., Jr., *et al.* (2001b). Evaluation of asymptomatic microscopic hematuria in adults: the American Urological Association best practice policy. II. Patient evaluation, cytology, voided markers, imaging, cytoscopy, nephrology evaluation, and follow-up. *Urology*, 57, 604–10.

Hall, C. L., Bradley, R., Kerr, A., *et al.* (2004). Clinical value of renal biopsy in patients with asymptomatic microscopic hematuria with and without low-grade proteinuria. *Clin Nephrol*, 62(4), 267–72.

Howard, R. S. and Golin, A.L. (1991). Long-term follow up of asymptomatic microhematuria. *J Urol*, 145, 335–6.

Iseki, K. (2012). Evidence for asymptomatic microhematuria as a risk factor for the development of ESRD. *Am J Kid Dis*, 60, 12–14.

Iseki, K., Iseki, C., Ikemiya, Y., *et al.* (1996). Risk of developing end-stage renal disease in a cohort of mass screening. *Kidney Int*, 49, 800–5.

Khadra, M. H., Pickard, R. S., Charlton, M., *et al.* (2000). A prospective analysis of 1,930 patients with hematuria to evaluate current diagnostic practice. *J Urol*, 163, 524–7.

Kincaid-Smith, P., Bennet, W. M., Dowling, J. P., *et al.* (1983). Acute renal failure and tubular necrosis associated with hematuria due to glomerulonephritis. *Clin Nephrol*, 19, 206–10.

Lamb, E. J. and Price, C. P. (2011). Kidney function tests. In C. A. Burtis, E. R. Ashwood, and D. E. Bruns (eds.) *Tietz Textbook of Clinical Chemistry and Molecular Diagnostics* (5th ed.), pp. 669–707. St. Louis, MO: Elsevier,

Loo, R. K., Lieberman, S. F., Slezak, J. M., *et al.* (2013). Stratifying risk of urinary tract malignant tumors in patients with asymptomatic microscopic hematuria. *Mayo Clin Proc*, 88(2), 129–38.

Maher, M. M., Kalra, M. K., Rizzo, S., *et al.* (2004). Multidetector CT urography in imaging of the urinary tract in patients with hematuria. *Korean J Radiol*, 5(1), 1–10.

Mariani, A. J., Mariani, M. C., Macchioni, C., *et al.* (1989). The significance of adult hematuria: 1,000 hematuria evaluations including a risk benefit and cost-effectiveness analysis. *J Urol*, 141, 350–5.

McGregor, D. O., Lynn, K. L., Bailey, R. R., *et al.* (1998). Clinical audit of the use of renal biopsy in the management of isolated microscopic hematuria. *Clin Nephrol*, 49, 345–8.

Messing, E. M., Young, T. B., Hunt, V. B., *et al.* (1992). Home screening for hematuria: results of a multiclinic study. *J Urol*, 148, 289–92.

Mohr, D. N., Offord, K. P., Owen, R. A., *et al.* (1986). Asymptomatic microhematuria and urologic disease: a population-based study. *JAMA*, 256, 224–9.

Moreno, J. A., Martín-Cleary, C., Gutiérrez, E., *et al.* (2012). AKI associated with macroscopic glomerular hematuria: clinical and pathophysiologic consequences. *Clin J Am Soc Nephrol*, 7, 175–84.

O'Connor, O. J., McSweeney, S. E., and Maher, M. M. (2008). Imaging of hematuria. *Radiol Clin North Am*, 46, 113–32.

Richards, N. T., Darby, S., Howie, A. J., *et al.* (1994). Knowledge of renal histology alters patient management in over 40% of cases. *Nephrol Dial Transplant*, 9, 1255–9.

Sparwasser, C., Cimniak, H. U., Treiber, U., *et al.* (1994). Significance of the evaluation of asymptomatic microscopic haematuria in young men. *Br J Urol*, 74, 723–9.

Sutton, J. M. (1990). Evaluation of hematuria in adults. *JAMA*, 263, 2475–80.

Topham, P. S., Harper, S. J., Furness, P. N., *et al.* (1994). Glomerular disease as a cause of isolated microscopic haematuria. *QJM*, 87, 329–35.

Vivante, A., Afek, A., Frenkel-Nir, Y., *et al.* (2011). Persistent asymptomatic isolated microscopic hematuria in Israeli adolescents and young adults and risk for end-stage renal disease. *JAMA*, 306, 729–36.

Warshauer, D. M., McCarthy, S. M., Street, L., *et al.* (1998). Detection of renal masses: sensitivities and specificities of excretory urography/linear tomography, US, and CT. *Radiology*, 169, 363–5.

CHAPTER 47

Loin pain haematuria syndrome

John Neary

Introduction

Loin pain haematuria syndrome (LPHS) was first described by Little and colleagues in 1967 (Little et al., 1967) who described three young females with recurrent bouts of flank pain and haematuria which occurred in the absence of identifiable or relevant urinary tract disease. Since then, a number of case series have been published amounting to approximately 300 cases worldwide, meaning it remains a rare condition. However, it is likely that significant under-reporting occurs.

Clinical features

The most obvious clinical feature is that of loin pain which is most often unilateral although can progress to bilateral pain. This may or may not be associated with haematuria which is generally microscopic. Renal function is normal, no significant proteinuria is present, and hypertension is usually absent.

This is a diagnosis of exclusion, so no other obvious cause of haematuria or pain should be found on urological or nephrological investigation—in particular, no evidence of infection, obstruction, arteriovenous malformations, or malignancy.

Patients described in the literature tend to be young (mostly < 30 years), Caucasian (94%), and female (74%). They often have a background of renal calculi (~ 50%) or less commonly immunoglobulin A nephropathy (~ 20%). Patients with a history of calculi should have no evidence of obstruction on imaging at the time of presentation with LPHS. Patients with an underlying glomerular disorder are given the diagnosis of secondary LPHS (Spetie et al., 2006).

By the time they are seen by a nephrologist, patients have often seen a number of physicians, had multiple investigations, have frequently developed serious disability due to chronic pain, and may be opiate dependent (Coffman, 2009). Some authors feel there is a large element of somatization involved in the syndrome and point to peaks of pain associated with episodes of parental illness or increased pain with psychological triggers (Lucas et al., 1995; Lall et al., 1997). Many of these patients also meet the criteria for somatoform pain disorder but the same authors acknowledge the fact that the psychological symptoms may be secondary to long-standing pain and frustration at lack of an effective cure (Bass et al., 2007). Another small series reported use of a number of pain scoring systems which suggest that LPHS patients have a predominant or significant neuropathic element to their pain (Smith and Bajwa, 2012).

Investigations

Patients with LPHS are often found to have either microscopic or less commonly macroscopic haematuria, with dysmorphic red cells (acanthocytes) on urine microscopy suggesting a glomerular aetiology. In general, renal biopsy is not often performed due to lack of evidence of kidney disease with normal creatinine and lack of proteinuria. However, if renal biopsy is undertaken, blood is often seen in the renal tubular cross-sections (~7% LPHS vs 2% healthy controls) (Hebert et al., 1996; Spetie et al., 2006).

A series of other findings have been reported, but importantly, none of these changes are specific or sensitive and none of them can confirm or rule out the diagnosis. For example, the renal vasculature may show C3 deposition in the arteriolar walls (Naish et al., 1975; Spitz et al., 1997). Some early groups had suggested abnormalities in the renal vasculature (Little et al., 1967; Burden et al., 1979; Fletcher et al., 1979), but this does not feature in recent reports. It has been suggested that these earlier angiographic abnormalities were due to contrast-induced spasm (Bergroth et al., 1987). The largest series of biopsies in LPHS reported a wide variation in glomerular basement membrane (GBM) width between thin, thick, and normal basement membrane (~ 1/3 in each group) (Spetie et al., 2006). This partially confirmed an earlier finding of increased prevalence of thin basement membrane in these patients (Hebert et al., 1996).

Other investigators report a high prevalence of hypercalciuria and hyperuricosuria in these patients and have suggested that, in combination with glomerular haematuria causing tubular obstruction, this may lead to intratubular microcrystal formation (Praga et al., 1998).

It is difficult to find a clearly defined diagnosis of LPHS in the earliest studies, and there remains a large variation in clinical features in reported patients to date. Some patients have a history of renal calculi, some of somatoform disorder, and presence of haematuria is variable. This heterogenous group of patients may account for the variation in investigative findings in the patients studied. However, one group has recently suggested diagnostic criteria for primary LPHS (Spetie et al., 2006), including renal pain which is constant or recurrent over a 6-month period, haematuria present on almost all urinalyses, and absence of obstruction on imaging if past history of calculi.

Aetiology/pathology

An important observation is that many of the associated abnormalities can explain the finding of blood in the urine, but cannot easily explain the pain. The great majority of patients with glomerular haematuria do not experience similar pain. It has been suggested that LPHS is part of a general somatoform pain disorder with some renal involvement (Lucas et al., 1995; Lall et al., 1997; Bass et al., 2007). Some of the common longitudinal features of the condition, such as recurrence despite autotransplantation, may seem to support that.

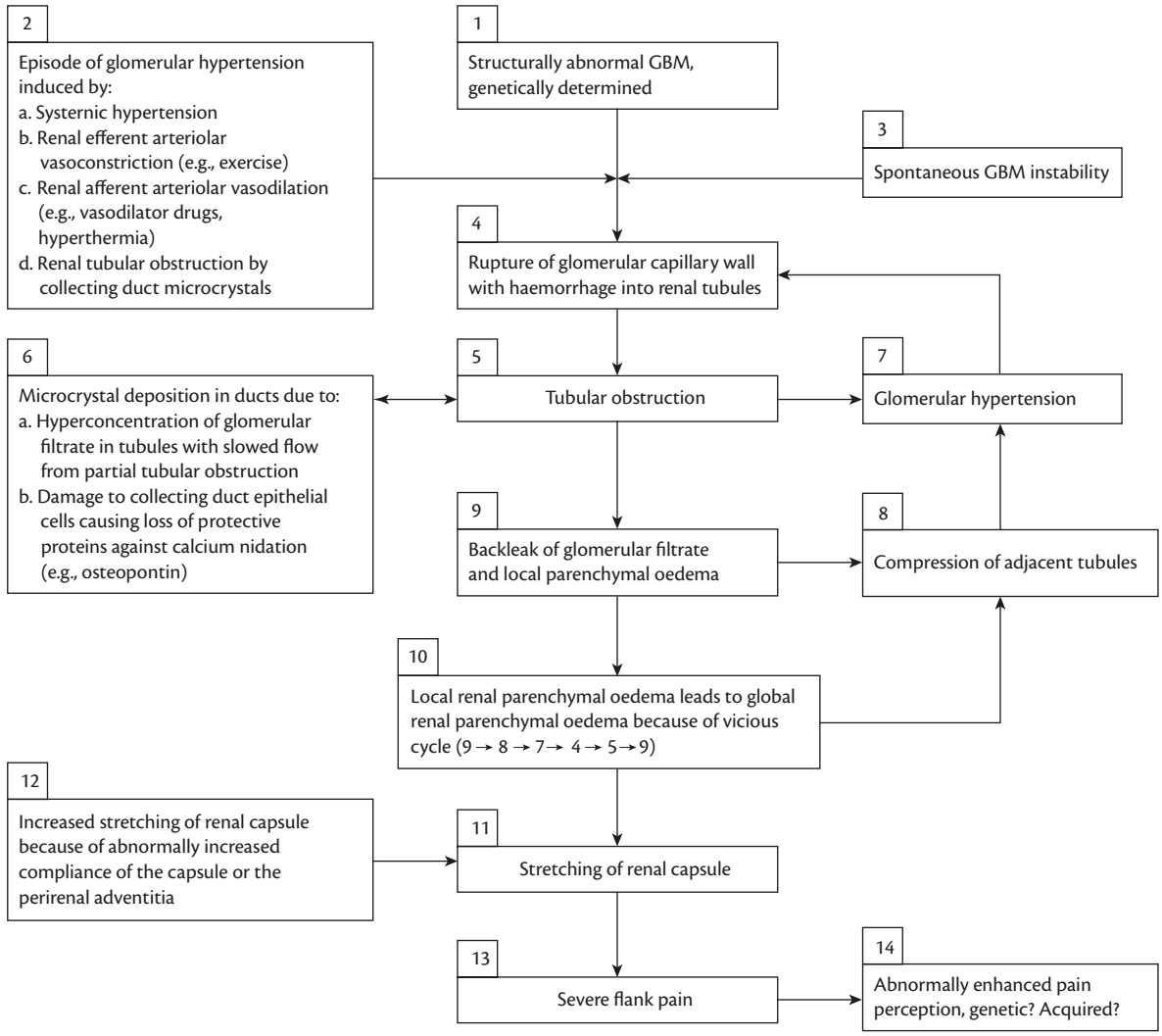

Fig. 47.1 Proposed pathogenesis of pain in idiopathic loin pain haematuria syndrome. Note that the process is initiated by glomerular bleeding.
From Spetie et al. (2006).

A number of potential aetiologies for the associated haematuria have been proposed including abnormal platelet activation and prostacyclin production, complement activation, clotting abnormalities, renal tubular calcification, oestrogen-containing oral contraceptive pill, or somatization (Weisberg et al., 1993; Smith and Bajwa, 2012). These are reported in individual case studies or small case series only.

The presence of dysmorphic red cells is common and would seem to point to a glomerular source of bleeding. As in others with 'benign' haematuria, there is a suggestion that variation in GBM thickness may have a role. This has resulted in a hypothesis that abnormal GBM leads to glomerular bleeding which results in tubular obstruction by red cells. This in turn leads to back-leak and parenchymal oedema, with subsequent glomerular hypertension, which predisposes to further glomerular bleeding and so on (Spetie et al., 2006). However, this process occurs in the great majority of patients with thin GBM nephropathy (see Chapter 325) without causing any symptoms. Fig. 47.1 illustrates a proposed mechanism (Spetie et al., 2006).

It is interesting that of the many case reports of nutcracker syndrome (see Chapter 48), a condition that is generally associated with macroscopic haematuria, pain is rarely a mentioned feature.

It has been hypothesized that this cycle may be exacerbated by the presence of hypercalciuria and hyperuricosuria leading to microcrystal formation (Praga et al., 1998). Tubular obstruction and interstitial oedema then results in renal parenchymal oedema and renal capsular stretching, causing renal pain. Enhanced pain perception in these patients may also play a role.

Treatment

A number of pharmacologic treatments, which have been directed towards correction of platelet activation or clotting abnormalities including aspirin and warfarin, have been unsuccessful (Weisberg et al., 1993). Use of renin–angiotensin system inhibition to reduce glomerular hypertension has been proposed and this led to some improvement in symptoms in four of seven patients treated with enalapril (Hebert et al., 1996). In patients with a history of

nephrolithiasis, adherence to stone prevention fluid and diet guidelines should be followed, if not doing so already.

It should also be noted that a number of cases of LPHS resolve spontaneously as patients get older, with approximately 30% of cases resolving at a mean of 3.5 years (Weisberg et al., 1993).

The cornerstone of treatment of LPHS remains analgesia management and for many patients the best results may be achieved through management by a multidisciplinary team including psychiatric or psychological and expert pain management input. A conservative approach, with long-term support and continuity of care with as few practitioners as possible, can have beneficial results, with up to a 38% improvement rate, even in long-standing cases (Bass et al., 2007).

A number of surgical treatments have also been attempted although the poor overall results of these are attested to in many case reports:

- Intrarenal capsaicin injections were initially reported to have an approximately 60% improvement in symptoms, but this was only short term (mean duration 2–17 weeks) and the side effects were significant including bladder pain, worsening renal pain, and deterioration in renal function. Most worryingly, a nephrectomy rate of between 20% and 67% was reported and thus use of capsaicin is no longer encouraged (Uzoh et al., 2009).
- A trial of ureteric bupivacaine infusion (Ahmed et al., 2010) reported 95% improvement rate at 1 year in a cohort of 17 patients, with 23% reporting complete resolution after one treatment. Several patients required repeat treatments (mean number of treatments = 2.9) and a well-validated pain-scoring method was not used. A larger trial is required.
- Denervation of the kidney, with surgical stripping of the renal pedicle and renal capsulotomy, has been reported with an approximately 30% cure rate, but some groups have reported recurrence of symptoms secondary to re-innervation in approximately 70% cases at a mean of 11 months, development of pain in the contralateral kidney, and, of concern, a large percentage of patients, 38%, subsequently proceeding to nephrectomy (Andrews et al., 1997; Greenwell et al., 2004).
- Autotransplantation of the kidney (with coincident renal denervation) has also been reported with good success in some groups reporting up to 76% of patients pain free at 1 year with follow-up of up to 8.4 years (Chin et al., 1998; Sheil et al., 1998), although other groups have had less success, with up to 75% recurrence and significant morbidity including occasional graft loss or recurrence of pain in contralateral kidney (Gibson et al., 1994; Harney et al., 1994; Parnham et al., 1996). Even in the programmes reporting success with autotransplantation, there is significant morbidity with a 7% rate of graft loss due to surgical complications and an 11% rate of nephrectomy following autotransplant (Chin et al., 1998).

There is some debate as to why the denervation seen in autotransplantation seems to have better success than standard denervation, as regrowth of nerves is seen in both groups. It has been proposed that use of interposed polytetrafluroethylene (PTFE) arterial grafts should reduce re-innervation but experience with this technique is limited (Blacklock et al., 1999).

The majority of authors would propose that autotransplantation is only an option of last resort (Dube et al., 2006), while others feel that due to the high risk of morbidity and/or recurrence of pain, this procedure should not be considered. As negative results are less likely to get published, it is also likely that many unsuccessful cases of surgical treatment have not been reported.

Potential other treatments include intrathecal opiates (Prager et al., 1995), paraspinal nerve root stimulation (Goroszeniuk et al., 2009), and finally catheter-based renal denervation (Gambaro et al., 2013), but many of these are isolated case reports or small case series.

Summary

In summary, the relatively small numbers of patients with LPHS and the wide heterogeneity in clinical findings may account for the varying degree of success of treatment options. Anecdotal successful reports may be due in part to placebo effect or other confounding variables. In future studies, clearly defined patient categories may need to be identified before trialling treatments in order to identify which treatments may be successful.

References

Ahmed, M., Acher, P., and Deane, A. M. (2010). Ureteric bupivicaine infusion for loin pain haematuria syndrome. *Ann R Coll Surg Engl*, 92(2), 139–41.

Andrews, B. T., Jones, N. F., and Browse, N. L. (1997). The use of surgical sympathectomy in the treatment of chronic renal pain. *Br J Urol*, 80, 6–10.

Bass, C. M., Parrott, H., Jack, T., *et al.* (2007). Severe unexplained loin pain (loin pain haematuria syndrome): management and long-term outcome. *QJM*, 100, 369–81.

Bergroth, V., Konttinen, Y. T., Nordstrom, D., *et al.* (1987). Loin pain and haematuria syndrome: possible association with intrarenal arterial spasms. *Brit Med J*, 294(6558), 1657.

Blacklock, A. R., Raabe, A. L., and Lam, F. T. (1999). Renal auto-transplantation with interposed PTFE arterial graft: not necessarily a cure for loin pain/haematuria syndrome. *J R Coll Surg Edinb*, 44(2), 134.

Burden, R. P., Dathan, J. R., Etherington, M. D., *et al.* (1979). The loin pain/haematuria syndrome. *Lancet*, 1(8122), 897–900.

Chin, J. L., Kloth, D., Pautler, S. E., *et al.* (1998). Renal autotransplantation for the loin pain hematuria syndrome: long-term follow-up of 26 cases. *J Urol*, 160, 1232–5.

Coffman, K. L (2009). Loin pain hematuria syndrome: a psychiatric and surgical conundrum. *Curr Opin Org Transplant*, 14, 186–90.

Dube, G. K., Hamilton, S. E., Ratner, L. E., *et al.* (2006). Loin pain hematuria syndrome. *Kidney Int*, 70, 2152–5.

Fletcher, P., Al-Khader, A. A., Parsons, V., *et al.* (1979). The pathology of intrarenal vascular lesions associated with the loin-pain-haematuria syndrome. *Nephron*, 24(3), 150–4.

Gambaro, G., Fulignati, P., Spinelli, A., *et al.* (2013). Percutaneous renal sympathetic nerve ablation for loin pain haematuria syndrome. *Nephrol Dial Transplant*, 28, 2393–95.

Gibson, P., Winney, R. J., Masterton, G., *et al.* (1994). Bilateral nephrectomy and haemodialysis for the treatment of severe loin pain haematuria syndrome. *Nephrol Dial Transplant*, 9, 1640–41.

Goroszeniuk, T., Khan, R., and Kothari, S. (2009). Lumbar sympathetic chain neuromodulation with implanted electrodes for long-term pain relief in loin pain haematuria syndrome. *Neuromodulation*, 12, 284–291.

Greenwell, T. J., Peters, J. L., Neild, G. H., *et al.* (2004). The outcome of renal denervation for managing loin pain haematuria syndrome. *BJU Int*, 93(6), 818–21.

Harney, J., Rodgers, E., Campbell, E., *et al.* (1994). Loin pain-hematuria syndrome: how effective is renal autotransplantation in its treatment? *Urology*, 44, 493–6.

Hebert, L. A., Betts, J. A., Sedmak, D. D., *et al*. (1996). Loin pain-hematuria syndrome associated with thin glomerular basement membrane disease and hemorrhage into renal tubules. *Kidney Int*, 49(1), 168–73.

Lall, R., Mailis, A., and Rapoport, A. (1997). Hematuria-loin pain syndrome: its existence as a discrete clinicopathological entity cannot be supported. *Clin J Pain*, 13, 171–7.

Little, P. J., Sloper, J. S., and de Wardener, H. E. (1967). A syndrome of loin pain and haematuria associated with disease of peripheral renal arteries. *QJM*, 36, 253–9.

Lucas, P. A., Leaker, B. R., Murphy, M., *et al.* (1995). Loin pain and haematuria syndrome: a somatoform disorder. *QJM*, 88, 703–9.

Naish, P. F., Aber, G. M., and Boyd, W. N. (1975). C3 deposition in renal arterioles in the loin pain and haematuria syndrome. *Br Med J*, 3, 746.

Parnham, A. P., Low, A., Finch, P., *et al.* (1996). Recurrent graft pain following renal autotransplantation for loin pain hematuria syndrome. *Br J Urol*, 78, 25–8.

Praga, M., Martinez, M. A., Andres, A., *et al.* (1998). Association of thin basement membrane nephropathy with hypercalciuria, hyperuricosuria and nephrolithiasis. *Kidney Int*, 54, 915–20.

Prager, J. P., DeSalles, A., Wilkinson, A., *et al*. (1995). Pain hematuria syndrome: pain relief with intrathecal morphine. *Am J Kidney Dis*, 25, 629–31.

Sheil, A. G., Chui, A. K., Verran, D. J., *et al*. (1998). Evaluation of the loin pain/hematuria syndrome treated by renal autotransplantation or radical renal neurectomy. *Am J Kidney Dis*, 32, 215–20.

Smith, H.S. and Bajwa, Z. H. (2012). Loin pain hematuria syndrome-visceral or neuropathic pain syndrome? *Clin J Pain*, 28, 646–51.

Spetie, D. N., Nadasdy, T., Nadasdy, G., *et al.* (2006). Proposed pathogenesis of idiopathic loin pain-hematuria syndrome. *Am J Kidney Dis*, 47, 419–27.

Spitz, A., Huffman, J. L., and Mendez, R. (1997). Autotransplantation as an effective therapy for the loin pain-hematuria syndrome: case reports and a review of the literature. *J Urol*, 157(5), 1554–9.

Uzoh, C. C., Kumar, V., and Timoney, A. G. (2009). The use of capsaicin in loin pain-haematuria syndrome. *BJU Int*, 103, 236–9.

Weisberg, L. S., Bloom, P. B., Simmons, R. L. *et al*. (1993). Loin pain hematuria syndrome. *Am J Nephrol*, 13, 229–37.

CHAPTER 48

Nutcracker syndrome and phenomenon

John Neary and Neil Turner

Introduction

Obstruction of venous return by pressure of the superior mesenteric artery on the left renal vein was noted as a possible cause of varicocele in 1950 (El-Sadr and Mina, 1950). The term nutcracker was introduced over 20 years later. The phenomenon and syndrome are well reviewed by Kurklinsky and Rooke (2010). A distinction between the anatomical nutcracker phenomenon (NCP) and nutcracker syndrome, in which specific symptoms are attributed to NCP, is useful (Kurklinsky and Rooke, 2010). See also Chapters 46, 47, and 51.

Anatomy and incidence of nutcracker phenomenon

Figs 48.1 and 48.2 illustrate the NCP. Gonadal and lumbar veins may be distended in association with the narrowing and varicocele and vulval varicosities have been attributed to NCP.

Cross-sectional imaging techniques show narrowing and dilatation of the left renal vein. The phenomenon can also be demonstrated by Doppler ultrasound, which can show haemodynamic effects of narrowing. However, the findings on Doppler ultrasound are prone to variation depending on posture, patient anatomy, and operator. Detailed studies are more likely to be performed in patients with symptoms, which complicates assessment of unblinded studies performed using this technique.

It is difficult to find neutral studies that look at the incidence of NCP in the absence of symptoms. However, in a useful study of 99 consecutive adult potential renal transplant donors by computed tomography angiography (Grimm et al., 2013), there was an average 35% reduction in diameter of the left renal vein as it crossed the aorta; 27% of patients had substantial (> 50%) compression of the left renal vein including 8% with > 70% narrowing. Dilated gonadal or lumbar veins were also common (16% or 24% respectively) and this finding was unrelated to the degree of renal vein compression. These were healthy, symptom-free individuals but five (5%) had dipstick haematuria; this was not associated with renal vein compression. There was no significant age or sex influence on the degree of NCP in this selected group.

The phenomenon has mostly been described in young adults and older children, but there are reports at all ages. It has been suggested that low body mass index (BMI) increases the likelihood of NCP as reduced abdominal fat reduces the angle between superior mesenteric artery and aorta. However, there is not a good correlation between BMI and abdominal fat, and there is not enough evidence to prove or disprove this hypothesis (discussed by Park and Shin, 2013).

Haematuria

Haematuria is the most commonly associated renal feature of NCP. Episodic macroscopic haematuria is the symptom that most commonly leads to the imaging studies in which NCP is identified. Occasional patients have been described in whom bleeding has necessitated blood transfusion, but this is rare. Cystoscopy may reveal left-sided bleeding (illustrated by Matsubara et al., 2013).

There are numerous case reports in which surgical intervention or stenting of the left renal vein has been followed by cessation of bleeding episodes (e.g. Vince et al., 2011; Chen et al., 2012). However, spontaneous improvement may also occur (e.g. Matsubara et al., 2013).

The precise location of bleeding, other than that it seems to come from the kidney itself, is not described. The red cells do not have characteristics of glomerular haematuria. It is interesting that in reported cases autotransplantation or stenting do not seem to halt bleeding immediately but over a period of days to weeks.

It is not clear that persistent microscopic haematuria can be attributed to NCP.

Pain

Few case reports of NCP with episodic haematuria report pain as a prominent feature, although some do (e.g. Vince et al., 2011).

A variety of pain syndromes have been attributed to NCP, with symptoms in the abdomen, flank (presumably left flank), and sometimes buttock. Overt haemorrhage may lead to left renal colic (clot colic). Pelvic pain syndromes have sometimes been attributed to NCP but the case for this is usually weak. It is an unlikely cause of classic loin pain haematuria syndrome as this is more typically associated with glomerular bleeding (see Chapter 47).

Postural proteinuria

While the relationship with episodic visible haematuria seems firmly based, a possible relationship between NCP and proteinuria remains uncertain. It was suggested as a cause of postural proteinuria (see Chapter 51) in 1958, and a number of unblinded studies using Doppler ultrasound have found an apparently persuasive

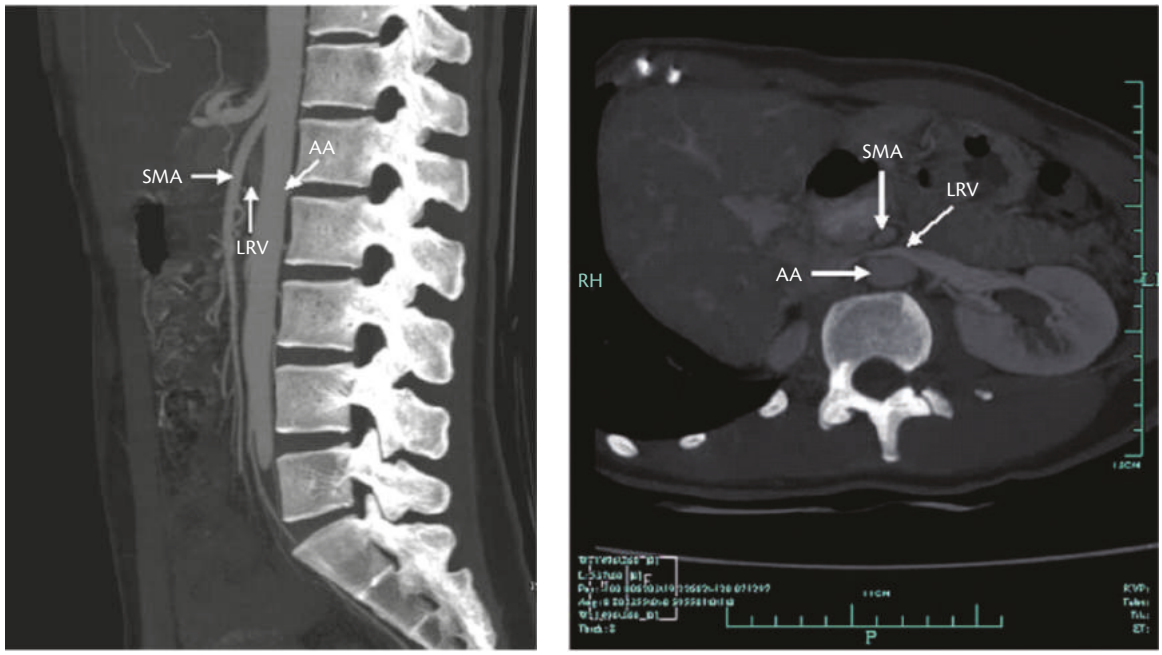

Fig. 48.1 Computed tomography angiography of a 29-year-old woman with a 4-year history of intermittent visible haematuria. Cystoscopy showed bleeding from the left ureter. There is compression of the left renal vein between the abdominal aorta and the superior mesenteric artery. An endovascular stent was implanted and the haematuria ceased two weeks later.
From Song et al. (2013).

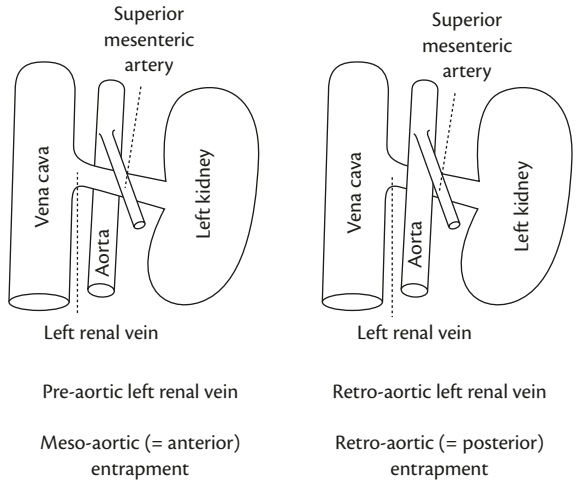

Fig. 48.2 Anatomy of the left renal vein, showing normal anatomy (left) and the mechanism by which the renal vein can be entrapped: and (right) a variation seen in approximately 3% of the population that can be associated with a posterior entrapment.
From Mazzoni et al. (2011).

relationship (Mazzoni et al., 2011). Problems with the interpretation of these studies are discussed in Chapter 51.

As outcomes of orthostatic proteinuria appear generally good, and there are usually no symptoms, intervention to alter anatomy cannot be easily justified for this reason. One study found that angiotensin-converting enzyme inhibitors lowered protein excretion in postural proteinuria (Ha and Lee, 2006).

Management

Reported treatments have included nephrectomy, autotransplantation, vascular bypass procedures, and more recently intravascular or extravascular stenting (Kurlinsky and Rooke, 2010; Chen et al., 2012). Almost always these treatments are potentially more harmful than the condition itself, and as described above, the relationship of NCP to symptoms is often uncertain. Conservative management is therefore appropriate for the great majority of patients, as is remaining open to an alternative diagnosis. Intervention might be indicated for severe recurrent haematuria, but haematuria may also remit spontaneously (e.g. Matsubara et al., 2013).

Interestingly, several reports describe episodes of haemorrhage as reducing not immediately but over days to weeks after stenting. Spontaneous recovery may also occur.

Intervention can rarely be justified where it is suspected that nutcracker syndrome might explain proteinuria.

References

El-Sadr, A. R. and Mina, E. (1950). Anatomical and surgical aspects in the operative management of varicocele. *Urol Cutaneous Rev*, 54, 257–62.

Ha, T. S. and Lee, E. J. (2006). ACE inhibition can improve orthostatic proteinuria associated with nutcracker syndrome. *Pediatr Nephrol*, 21, 1765–8.

Chen, S., Zhang, H., Tian, L., *et al.* (2012). Endovascular management of nutracker syndrome after migration of a laparoscopically placed extravascular stent. *Am J Kidney Dis*, 50, 322–6.

Grimm, L. J., Engstrom, B. I., Nelson, R. C., *et al.* (2013). Incidental detection of nutcracker phenomenon on multidetector CT in an asymptomatic population: prevalence and associated findings. *J Comput Assist Tomogr*, 37, 415–18.

Ha, T. S. and Lee, E. J. (2006). ACE inhibition can improve orthostatic proteinuria associated with nutcracker syndrome. *Pediatr Nephrol*, 21, 1765–8.

Kurklinsky, A. K. and Rooke, T. W (2010). Nutcracker phenomenon and nutcracker syndrome. *Mayo Clin Proc*, 85, 552–9.

Matsubara, T., Ogawa, O., and Yanagita, M. (2013). Physical finding of nutcracker phenomenon. *Kidney Int*, 83, 335.

Mazzoni, M. B., Kottanatu, L. K., Simonetti, G. D., *et al.* (2011). Renal vein obstruction and orthostatic proteinuria: a review. *Nephrol Dial Transplant*, 26, 562–5.

Park, S. J. and Shin, J. I. (2013). Low body mass index in nutcracker phenomenon: an underrecognized condition. *Kidney Int*, 84, 1287.

Song, Y., Wu, J. Y., and Chen, J. H. (2013). Haematuria and the nutcracker syndrome. *QJM*, 106, 879–80.

Vince, H. B., Tomson, C. R., Loveday, E. J., *et al.* (2011). Nutcracker syndrome presenting as loin pain haematuria syndrome. *NDT Plus*, 4, 418–20.

CHAPTER 49

Exercise-related pseudonephritis

Neil Turner

Introduction

The effects of exercise on urine sediment were recognized well over a century ago but the phenomena still resist complete explanation. The findings can appear extreme and the term pseudonephritis coined by Gardner (1956) is accurate.

Exercise-related haematuria and haemoglobinuria

March haematuria, first documented in 1881 (reviewed by Gilligan and Blumgart, 1941), has from the first reports probably been a mix of exercise-induced haemoglobinuria, exercise-related myoglobinuria, and exercise-related haematuria. In the earliest literature, many cases are described in which no blood cells could be seen despite positive tests for globin; some cases are more suggestive of recurrent rhabdomyolysis. Others are more suggestive of immunoglobulin A nephropathy in young adults, while yet others hint at a urological origin in bladder or urethra.

Tobal et al. (2008) described an informative series of cases from Uruguay related to prolonged, intense, hand drumming (candombe drumming). Apparently rust-coloured urine following carnival is widely recognized. Over 2000 drummers participate; over 6 years, five patients were admitted with acute kidney injury (AKI) following these events, with two of these requiring dialysis. In a series of 45 individuals studied after drumming, rust-coloured urine was noted in 20% and urinary abnormalities on analysis in 64%. All of those with rust-coloured urine had evidence of intravascular haemolysis. Only one patient in the series had detectable myoglobinuria. Of the 20 with rust-coloured urine, six had urine samples collected 48–72 hours after drumming and all had returned to normal. No cases of even mild AKI were seen in their study group, so this must be a rare event.

The most common scenario in which these changes are seen now seems to be long-distance running, after which some see visible haematuria and the prevalence of abnormal dipstick tests may be 25% (Siegel et al., 1979; Kallmeyer and Miller, 1993). It is usually asymptomatic and resolves within 72 hours. There is again evidence that this can be related to red cell trauma and lysis (Telford et al., 2003). However, phase contrast microscopy is reported to show increased excretion of red blood cells which not only have the characteristics of glomerular red cells, but are also commonly

Fig. 49.1 'The Start at Iffley' from Tom Brown at Oxford, 1861.

accompanied by red cell casts (Barach, 1910; Gardner, 1956; Fassett et al., 1982). It is not at all clear what the explanation for glomerular bleeding is.

Others find evidence for a non-glomerular origin in the bladder or urethra (Kallmeyer and Miller, 1993; and others discussed in Kincaid-Smith, 1982), and it is not satisfactorily resolved which origin is more common.

Exercise-related proteinuria

Proteinuria is less visually obvious after strenuous exercise but has an equally distinguished history, first described by Leube in 1878 and lucidly described by Collier in 1907. Collier described proteinuria appearing in the urine shortly after exercise and usually gone again the following day, only to recur again following further exercise. He found albumin in the post-exercise urine of 57% of 156 Oxford student rowers in intensive training for college races, with higher proportions in the more successful boats (Fig. 49.1). Relating these findings to prior observations on the longevity of boat race participants he concluded that it was a benign phenomenon, going against then prevalent views on the implications of proteinuria. Others (reviewed by Poortmans, 1985) have since described in detail an early post-exercise peak in protein excretion which drops over hours, the magnitude depending on the intensity of the exercise. The protein is mostly albumin.

The mechanism of exercise-induced proteinuria is unknown. Most of the individuals in these studies did not have haematuria or smoky urine; the proteinuria effect seems to be encountered after lesser durations of exercise than haematuria, and in types of exertion less likely to involve trauma to red cells.

References

Barach, J. H. (1910). Physiological and pathological effects of severe exertion (the Marathon Race) on the circulatory and renal systems. *Arch Intern Med*, 5, 382–405.

Collier, W. (1907). Functional albuminuria in athletes. *Br Med J*, i, 4–6.

Fassett, R. G., Owen, J. E., Fairley, J., *et al*. (1982). Urinary red-cell morphology during exercise. *Br Med J*, 285, 1455–7.

Gardner, K. D. (1956). 'Athletic pseudonephritis'—alteration of the urine sediment by athletic competition. *JAMA*, 161, 1613–17.

Gilligan, D. R. and Blumgart, H. L (1941). March hemoglobinuria: studies of the clinical characteristics, blood metabolism and mechanism: with observations on three new cases, and review of literature. *Medicine*, 20, 341–95.

Kallmeyer, J. C. and Miller, N. M. (1993). Urinary changes in ultra long-distance marathon runners. *Nephron*, 64, 119–21.

Kincaid-Smith, P. (1982). Haematuria and exercise-related haematuria. *Br Med J*, 285, 1595–7.

Poortmans, J. R. (1985). Postexercise proteinuria in humans: facts and mechanism. *JAMA*, 253, 236–40.

Siegel, A. J., Hennekens, C. H., Solomon, H. S., and Van Boeckel, B. (1979). Exercise-related hematuria: findings in a group of marathon runners. *JAMA*, 241, 391–2.

Telford, R. D., Sly, G. J., Hahn, A. G., *et al*. (2003). Footstrike is the major cause of hemolysis during running. *J Appl Physiol*, 94, 38–42.

Tobal, D., Olascoaga, A., Moreira, G., *et al*. (2008). Rust urine after intense hand drumming is caused by extracorpuscular hemolysis. *Clin J Am Soc Nephrol*, 3, 1022–7.

Von Leube, W. (1878). Über ausscheidung von Eiweiss in harn des gesunden menschen. *Virchows Arch Pathol Anat Physiol*, 72, 145–7.

CHAPTER 50

Proteinuria

Neil Turner and Stewart Cameron

Introduction

Richard Bright's outstanding clinical and autopsy observations in 1827 demonstrated the association between proteinuria and fatal kidney disease, laying the ground for the specialty of nephrology (see Chapter 42). He built his observations on many going before him, but his insights pushed proteinuria into the forefront of medical thinking in the 1800s. Measuring proteinuria became a regular part of clinical assessment. By the end of the nineteenth century the implications of proteinuria as a risk factor were being considered by insurance companies as well as by doctors (Collier, 1907; Barringer, 1912; Turner, 2013).

The origin of proteinuria

Unlike haematuria, which can come from any level in the urinary tract, proteinuria almost always originates in the kidney. Pathological mechanisms of proteinuria mainly affect the glomerulus, and specifically the podocyte, as outlined in Chapter 45.

The glomerulus filters only very small amounts of serum albumin, but proteins smaller than 30 kDa filter almost freely. These lower-molecular-weight proteins and the small amounts of albumin that are filtered are mostly reabsorbed in the proximal tubule (Fig. 50.1).

Although there has been some debate about the permeability of the glomerular barrier to albumin (discussed further in Chapter 137), the weight of evidence confirms that little albumin is filtered in health (Haraldsson and Tanner, 2014).

Internalization of filtered proteins in the proximal tubule is a process that involves the cell surface receptors megalin and cubulin (Amsellem et al., 2010; Haraldsson, 2010). It is then degraded in lysosomes, a process that can be beautifully visualized *in vivo* (Slattery et al., 2008). Ovunc et al. (2011) described an interesting family with autosomal inherited cubulin mutations causing intermittent proteinuria up to 2 g/day. Mutations in cubulin have also been associated with proteinuria and megaloblastic anaemia because of failure to internalize the intrinsic factor–vitamin B_{12} complex. Polymorphisms in tubulin may account for variance in the albumin:creatinine ratio (ACR) in the general population (Böger et al., 2011). Cubulin interacts with megalin to function. Mice with deletion of megalin survive poorly, but one abnormality is severe rickets from failure of tubular cells to absorb filtered vitamin D in complex with its binding protein.

In Dent disease (see Chapter 41), a failure of lysosomal function appears to be responsible for a widespread failure of tubular reabsorption.

What are the proteins in normal urine?

Albumin is the most abundant protein in normal urine, as in serum. It makes up a little less than half of average daily urinary protein, about 30 mg.

Proteins in normal urine fall into three groups: filtered proteins that have not been reabsorbed such as albumin; proteins actively secreted into urine; and proteins lost from cells in the nephron and urinary tract, particularly via extruded exosomes, small cell-derived vesicles that can be identified in all biological fluids. Many of these proteins are present at very low level and analysis is a challenging biochemical task. The most abundant components of normal urine are serum proteins (albumin, α_1-antitrypsin, zinc α_2-glycoprotein, α_1-microglobulin), but there is also a large group of kidney secreted and structural proteins, of which uromodulin (Tamm–Horsfall protein) is a leading representative. Uromodulin is secreted into the thick ascending limb of the loop of Henle and early distal tubule, and is a major component of urinary casts (Rustecki et al., 1971; Hoyer and Sieler, 1979; Scherberich, 1990). Its gene is implicated in familial juvenile hyperuricaemic nephropathy and in medullary cystic kidney disease (see Chapter 318).

By two-dimensional gel electrophoresis the complexity of urinary proteins can be seen, but a typical review was able to confirm identities of only 275 of 1118 spots representing 82 proteins. Many of the uncharacterized species were low-abundance, low-molecular-weight proteins (Candiano et al., 2010). The proportion of serum proteins increases dramatically in nephrotic syndrome.

Modern mass spectrometry pushes the boundaries further, but probably with different selection bias; 868 identified proteins are listed in one study (Liu et al., 2012).

Biomarkers: albumin remains the one to beat

Many groups are working to find specific molecules that might be informative about particular disease processes, but few candidates have survived tests of clinical utility. Albumin remains as good a predictor of most outcomes as many putative biomarkers—a fascinating and important observation.

Pathological proteinuria

Proteinuria in excess of the usual modest limit can come about by:

1. Glomerular proteinuria: the glomerular filter becomes more permeable to proteins of large molecular size. This is common.
2. Tubular proteinuria: the proximal tubule is damaged so that proteins that are normally reabsorbed (principally of low molecular

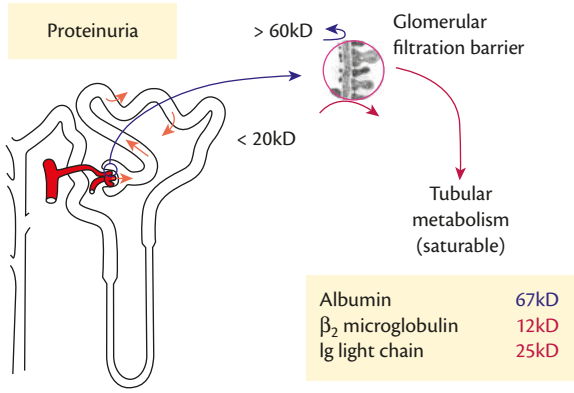

Fig. 50.1 Handling of protein in the nephron. Proteins the size of albumin (67 kD) are largely excluded at the glomerular filtration barrier. Proteins smaller than approximately 20 kD pass freely through it into the filtrate. Proteins between these sizes are filtered progressively less well as size increases. Most filtered proteins are internalized into proximal tubular cells by a process involving the cell surface receptors cubulin and megalin, and degraded in lysosomes. Albuminuria is the defining characteristic of increased glomerular permeability (*glomerular proteinuria*). β_2-microglobulin is an example of a freely filtered protein that is found in the urine in increased quantities if the internalization process into proximal tubular cells is disrupted (*tubular proteinuria*). If immunoglobulin light chains are overproduced they filter fairly freely (*overflow proteinuria*) and some light chains may be nephrotoxic through aggregation in the tubular lumen or in proximal tubular cells after internalization.

weight) pass into the urine. This usually occurs as part of the Fanconi syndrome of multiple proximal tubular dysfunction (see Chapter 41). This is much less common.

3. Overflow proteinuria: an increase in the plasma concentration of a filterable protein, so that the amount filtered exceeds the reabsorptive capacity of the proximal tubule. Immunoglobulin (Ig) light chains or fragments, and lysozyme in monomyelocytic leukaemia, are the only clinical examples.

Most significant proteinuria is glomerular.

Discerning the origin of proteinuria

Glomerular origin of proteinuria can be inferred by the quantity, ratio of albumin to other components, or by specific assays for tubular proteins.

Quantity

Tubular proteinuria rarely exceeds 1.5–2 g per 24 hours (protein:creatinine ratio (PCR) 150–200 mg/mol) and is usually less than this. Therefore (almost) all patients with higher excretion rates, and all patients with nephrotic syndrome (see Chapter 52), have a glomerular leak, primarily.

Ratio

The simplest test is to compare the results of a specific albumin assay test with a total protein result; ACR versus PCR (see 'Tests'). A ratio of > 0.4 suggests glomerular proteinuria. A ratio of < 0.4 suggests non-albumin proteinuria (Methven et al., 2012; Smith et al., 2012). A historic way to show tubular or 'overflow' protein in urine was to compare the sulphosalicylic acid precipitation test (see 'Tests'), which precipitates many proteins, with the results of dipstick tests, which are more sensitive to albumin.

Specific assays for tubular proteins

Specific assays enable the identification of low-molecular-weight proteins that would normally be internalized and destroyed, but pass through the nephron if tubules are damaged. β_2-microglobulin (molecular weight (MW) 12 kDa) is a sensitive indicator of tubular damage, but the protein is unstable in urine of normal pH (5–6.5) and even alkalinization of urine to pH 7 or above immediately on voiding may not stop degradation. Normally, < 0.4 micrograms/L of β_2-microglobulin is present in urine. Also useful, but rarely used, are assays for retinol-binding protein (21 kDa) (Tomlinson et al., 1997), α_1-microglobulin (30 kDa), and lysozyme (15 kDa, normal excretion < 1 mg/mmol creatinine) (Barratt, 1983). For further details see Chapter 7.

Tests for overflow or exogenous proteins

Immunofixation for Ig light chains (Bence Jones protein) remains an important test. Analysis of serum and/or urine for free light chains is more sensitive and also useful in monitoring light chain dyscrasias (see Chapter 150) and their response to treatment.

Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) gel electrophoresis is not a routine technique but it is very useful for investigating unusual proteinuria. It can clearly show the low-molecular-weight proteins of tubular proteinuria overflow proteins, as well as shining light on rare examples where exogenous proteins have been used to mimic proteinuria. Very occasionally, proteinuria may be factitious (Tojo et al., 1990), egg albumin or other protein being added to the urine. Even more rarely parents may add protein to their child's urine, as a variety of the Munchausen syndrome by proxy (Meadow, 1977).

Epidemiology of proteinuria

Incidence

The method used to screen for proteinuria affects both the proportion found to have it, and the strength of the association with outcomes. It is the same for haematuria (see Chapter 46), but proteinuria is a much stronger predictor of poor long-term outcomes, and risk is graded according to severity of proteinuria. This reduces the problem of false classification by a positive/negative test.

Table 50.1 summarizes results from a number of surveys internationally. They show a high prevalence of simple positivity with varying cut-offs. Some report detailed results of quantitation in very large numbers, for example, the Okinawa studies (Iseki et al., 1996, 2003).

Prognostic significance of proteinuria in population studies

At the first meeting of the Assurance Medical Society in 1894, the importance of measuring albuminuria in assessing insurance risk was discussed. In 1912, Barringer described increased mortality in 396 New York men who had been found to have proteinuria, but otherwise normal health, 10 years previously (Barringer, 1912). He also cited insurance company data from larger numbers of patients that suggested that mortality was more than doubled in individuals with a small amount of albuminuria and urinary casts.

Chapter 97 summarizes the risks measured in population studies. It is interesting that the implications of proteinuria in population studies (cardiovascular outcomes more likely than renal) are quantitatively different from the outcomes in patients known

Table 50.1 Prevalence of dipstick or sulphosalicylic acid-positive proteinuria in population studies

Study and reference	Prevalence (%)
Schoolchildren	
10 studies from 1950 (Vehaskari and Rapola, 1982)	0.6–6.4 (weighted mean 2.2)
1000–50,000 children	
Lin et al. (2000) dipstick confirmed by SSA	5
Young adults—armed forces recruits	
McLean (1919)	5.6
Murphy (1944)	3.0
Sinniah (1977)	0.9
College students, both sexes	
Diehl and McKinney (1944)	5.3
Burden (1933)	26.0
Lee (1920)	5.0
Adults, both sexes	
Blatherwick (1942) (> 20 mg/dL)	1.7
Alwall (1973)	1.7
Yudkin (1988) (microalbuminuria)	9.4
Bigazzi (1992) (microalbuminuria > 20–50 mg/L)	10.0
Ritz et al. (1994) (> 20 mg/L)[a]	3.0
Iseki et al. (1996, 2003) dipstick tests:	
+ or higher	5.1
++ or higher	1.3
+++ or higher	0.3
Adults > 60 years	
Sawyer (1988)	6.6
Casiglia (1993)	10.0
Adults > 80 years	
Casiglia (1993)	16.1

For details of pre-1994 references see Ritz et al. (1994) and Vehaskari and Rapola (1982)

SSA = sulphosalicylic acid turbidimetry test, see Table 50.3.

to have renal disease, where renal outcomes are more likely. See, for instance, long-term outcomes of the Modification of Diet in Renal Disease (MDRD) study in which the great majority of participants developed end-stage renal failure (ESRF) (see Chapter 99). The nature of the association of proteinuria with renal outcomes is discussed further below. In the Okinawa study of adult patients screened in 1983, and reviewed by inspecting the end-stage renal disease register at the end of 2000, the risk of ESRF was 0.2% overall, 0.4% if the screening result was +/1, 1.4% if +, 7% if ++, and 15% if it was +++ (Iseki et al., 2003). The risk for patients with haematuria as well as proteinuria was approximately double that for patients with proteinuria (all levels) alone (Iseki et al., 1996).

Testing for proteinuria

The 24-hour excretion of protein is 80 ± 24 mg (mean ± standard deviation (SD)) in normal individuals, so that 128 mg/24 hours (Peterson et al., 1969; Berggård, 1970) represents the mean ± 2SD, a rate of excretion of 103 micrograms/minute total protein. This represents < 11 mg/mmol creatinine (PCR) in spot urine samples (0.1 mg/mg). The normal range is usually taken to be up to 150 mg/day, for PCR up to 15 mg/mmol, significantly above this level. This avoids misclassification, but means that some levels within the normal range may in fact be elevated.

Normal levels and different ways of measuring and expressing proteinuria are summarized in Table 50.2. Further laboratory details of tests are shown in Chapter 7.

Dipsticks

Total protein

Early tests for protein based on heating and/or acidifying urine were superseded for most clinical purposes by dry stick tests (dipsticks) in the 1950s (Turner, 2013). In these, protein detection is based on colour reactivity with tetrabromphenol blue, which is yellow in the absence of protein at pH 3 and green in its presence. Very alkaline urine will interfere with this reaction, and other substances may sometimes interfere with different measurement methods. The lower limit of detection is about 150–300 mg/L. Depending on urine concentration therefore, normal levels of urinary protein may give weakly positive dipstick tests. This reagent is more sensitive to albumin than to some other proteins, so that most light chains (Bence Jones protein) and mucoproteins are less sensitively detected (Lamb and Price, 2011).

Albuminuria

Dipsticks specific for albumin, based on more specific chemical or on immunodetection methods, are available, but are more

Table 50.2 Approximately equivalent levels of proteinuria estimated by PCR/ACR. For accurate conversions between units: to convert mg/mmol to mg/g, multiply by 8.8. To convert mg/g to mg/mmol, multiply by 0.113

24-hour excretion[a]	PCR mg/mmol[b] (mg/g)	ACR mg/mmol[c] (mg/g)	Paediatric equivalent[d]
150 mg	> 15 (150)	> 2.5/3.5 (M/F)	> 4 mg/m^2/h;
0.5 g	50 (500)	30 (300)	100 mg/m^2/day
1 g	100 (1000)	70 (700)	PCR < 20 mg/mmol
3 g	300 (3000)	250 (2500)	> 40 mg/m^2/h; 1 g/m^2/day

[a] Normal 24-hour protein excretion for adults varies in different studies and 150 mg is unequivocally elevated, for example, the mean + 2SD was 128 mg according to Peterson et al. (1969) and Berggård (1970).

[b] Protein:creatinine ratio.

[c] Albumin:creatinine ratio.

[d] First-morning samples are particularly favoured in children as this helps to distinguish postural proteinuria. The upper limit of normal 24-hour excretion for children defines a large proportion as having postural proteinuria, which appears usually to be associated with normal health (see Chapter 51) (Hladunewich and Schaefer, 2011).

expensive. For detection of microalbuminuria an ACR or timed excretion must be tested.

PCR and ACR

Measuring protein excretion rates by 24-hour collection is inconvenient and error prone. The correlation between 24-hour protein or albumin excretion and the protein or albumin to creatinine ratio (PCR or ACR) is reported to be high (although the 95% confidence limits are wide), and this has led to 'spot' urines being recommended for routine practice in preference to 24-hour collections (Chitalia et al., 2001; Johnson et al., 2012; National Institute for Health and Care Excellence, 2014). Individual test results may vary more between measurements than 24-hour collection results. However, most feel that because ACR or PCR can be checked more easily and frequently, and because 24-hour collections are notoriously difficult to complete, some variability in results is an acceptable price. Clearly major treatment decisions should not be made on moderate changes seen in only one test.

Most nephrologists use spot tests most of the time but there continues to be some debate on the question (Ginsberg et al., 1983; Guy et al., 2009; Hebert et al., 2009; Naresh et al., 2013).

Point-of-care devices are available that measure both creatinine and either protein or albumin on a single stick. These can give rapid results but are costly for routine use if compared to bulk analysis in a laboratory.

Lab and other methods including selectivity of proteinuria

Laboratory and other methods and their features are shown in Table 50.3.

Historically, tests for selectivity of proteinuria were sometimes used to provide evidence that patients with nephrotic syndrome were likely to have minimal change disease and that therefore a trial of steroid therapy would be reasonable without a biopsy. The test has fallen out of use, but evidence for it, and that selectivity may predict remission in membranous nephropathy, is discussed by D'Amico and Bazzi (2003).

The test compared the clearance of a larger molecule with that of a smaller: IgG, IgM, or α_2-macroglobulin, against that of albumin or transferrin. A clearance of IgG > 20% (0.20) of transferrin or albumin represents 'non-selective' proteinuria; < 0.10 is 'highly selective' and suggests that a minimal change lesion may be present. The range between 0.10 and 0.20 is of little discriminatory value.

Evaluation of proteinuria

Many entirely healthy individuals have protein at 'trace' or '+' levels, as described above (and see Table 50.1). Two to five per cent of children, 5% of young adults, and up to 16% of the elderly will show proteinuria on testing of a single sample.

Confirm and quantitate

Note whether haematuria is also present. Exclude infection by dipstick for nitrite, leucocytes, and by microscopy and culture.

As for non-visible haematuria (see Chapter 46), simply repeating the test makes these figures fall, but quantitation by PCR can distinguish normal from abnormal levels.

Chyluria is a rare cause of non-renal proteinuria caused by lymphatic disease. It is usually obvious because urine is cloudy or milky. Leg swelling may be caused by lymphatic disease rather than proteinuria. Urine contains large numbers of lymphocytes (Cheng et al., 2006).

Table 50.3 Methods for measuring urine protein. The sulphosalicylic acid test (0.5 mL of 3% solution to 0.5 mL urine, can be graded manually or read in a densitometer) has been largely superseded but was used in some population studies as the primary method, or to verify dipstick results

Method	Description	Detection limit	Comments
Kjeldahl	Remove non-protein nitrogen, digest protein, measure protein nitrogen	10–20 mg/L	Reference and research method
Biuret	Copper reagent, measures peptide bonds	50 mg/L	Requires precipitation of proteins, used for 24-hour measurement in some laboratories
Turbidimetric (trichloracetic acid, sulphosalicylic acid)	Addition of trichloracetic or sulphosalicylic acids alters colloid properties and produces turbidity to be read in densitometer. Benzethomecin also used	50–100 mg/L	Imprecise, different readings for albumin and globulin
Dye-binding	Indicator changes colour in presence of protein (e.g. Coomassie brilliant blue)	50–100 mg/L	Different proteins bind differently; several different dyes in use; used in many laboratories for 24-hour excretion
Nephelometric	Specific antialbumin antibody used		Measures albumin excretion not total protein. Does not detect globulins or any other proteins
Stick tests	Impregnated with indicator dye which changes colour in the presence of protein	100 mg/L	Reacts poorly with globulins. Usual clinical screening test

Exclude transient proteinuria

There are several causes of transient proteinuria that usually do not convey the same connotations of renal disease and risk as sustained proteinuria:

- Postural proteinuria (see Chapter 51), the most common diagnosis in children and young people. Test a first-in-morning sample.

- Post-exercise proteinuria (see Chapter 49). Occurs within hours of exercise, magnitude depends on exertion.
- Transient proteinuria during fever (Marks et al., 1970).
- Heart failure (Albright et al., 1983).

Factitious and pseudoproteinuria

Factitious proteinuria was mentioned above. Electrophoresis of the urine can demonstrate foreign proteins such as ovalbumin. Pseudoproteinuria may be noted in patients receiving infusions of gelatin (MW 30 kDa)-based volume expanders such as 'gelofusine', which are readily filtered at the glomerulus (Jones et al., 1999), if the molybdate pyrogallol or possibly other dye-binding methods are used. The presence of blood interferes with protein assays variably, but usually only at quite high levels of blood content.

Assess the degree of proteinuria

Nephrotic range proteinuria

Proteinuria at nephrotic levels (Table 50.2), > 3 g/day, 40 mg/m^2/day in children, PCR > 300 mg/mmol or 3000 mg/g, almost always comes from glomerular disease. Unless the cause is readily apparent, investigation including renal biopsy is usually indicated. Diagnosis and management of nephrotic syndrome are considered in Chapter 52. For patients with nephrotic range proteinuria who fall short of nephrotic syndrome, many will require the same investigation and management. There may be exceptions when comorbidity makes it unlikely that disease-specific therapies could be tolerated.

Proteinuria at levels of 1–3 g/day

A PCR of 100 mg/mmol (1000 mg/g), approximately equivalent to 1 g proteinuria per day, is suggested in the United Kingdom as a suitable threshold for referral to a nephrology service, as above this level diagnosable renal disease becomes more likely. This was also the level selected in the MDRD study to define patients with significant proteinuria, who benefited most from blood pressure control, and for whom renal outcomes were substantially worse. Of course the risk of proteinuria is graded, there is no threshold at 1 g. Because of the duration at risk, younger people with lower levels of proteinuria may be at substantial lifetime risk of ESRF, justifying review at lower levels. Conversely, elderly patients with comorbid conditions may be unlikely to have their management altered significantly by nephrological review at this level of proteinuria.

If significant haematuria coexists with proteinuria, renal risk is substantially higher. Nephrological assessment is justified at lower levels of proteinuria. Renal impairment, or even more important, worsening GFR, are indications for further assessment.

The diseases causing proteinuria are increasingly likely to be recognizable glomerular conditions as the levels of proteinuria rise. Towards the lower end of this range diagnoses are less likely to alter management, except possibly in relatively young patients.

At levels of proteinuria below 2–2.5g consider tubular proteinuria. If a glomerular cause is not apparent measure both ACR and PCR; a ratio of albumin:protein of < 0.4 suggests non-glomerular proteinuria.

In all patients consider the possibility of plasma cell dyscrasias. Estimation of urinary light chains by immunofixation is the most widely used test, and though not as sensitive as assays for free Ig light chains it will certainly be positive if the explanation for proteinuria at this level.

Protein at levels of 0.3–1.0 g/day

The chances of identifying a specific glomerular disease become substantially lower unless there are pointers or known risk factors (e.g. genetic, infective, and systemic disease), but considerations are the same as above for 1–3 g/day. The coexistence of haematuria greatly increases the probability of identifiable glomerular disease. Lower thresholds should be considered relevant in young patients because they face longer at risk.

Microalbuminuria

Microalbuminuria is presumed to be a consequence of derangement of function of the glomerular filtration barrier, although this has not been formally proven in all circumstances. This would explain the early appearance of microalbuminuria in glomerulopathies, as first demonstrated in diabetes (Mogensen and Christensen, 1984; Viberti, 1988) (see Chapter 149). However the origin of microalbuminuria in many patients with cardiovascular disease (see Chapters 97 and 98) has not been satisfactorily explained, and in theory it could alternatively be caused by reduced capacity of proximal tubules to reabsorb tubular proteins from filtrate.

In young patients without comorbid conditions, microalbuminuria is likely to reflect early glomerular disease. It can be a useful test in patients with isolated dipstick haematuria. In older patients it is less specific as a test for primary renal disease.

Assess other renal risk factors

These include family history, drug history, hypertension, and low or (even more important) reducing estimated glomerular filtration rate.

Assess and manage cardiovascular risk

Patients with proteinuria are at significantly increased risk of cardiovascular disease and death (see Chapter 98). Like the renal risk, this is graded with increasing severity of proteinuria. However, in the general population without an established renal diagnosis, cardiovascular endpoints are more likely than renal (discussed in Chapter 99).

Proteinuria and progression of renal diseases

The association between proteinuria and population outcomes was known before 1900. In the late 1970s, it was first appreciated how strongly the outcome of diverse renal diseases was dependent on proteinuria, and that this seemed even more important than the nature of the renal diagnosis (Fig. 50.2) (Cameron, 1979). This experience was extended across other diseases including those in which proteinuria was not an initial feature such as reflux nephropathy (Kincaid-Smith and Becker, 1979), and during the 1980s it was found that the association of poor outcome with proteinuria seemed universal (Williams et al., 1988; Cameron, 1990; D'Amico, 1991). Poorer outcomes in the group of patients with > 1 g of proteinuria were illustrated again in the long-term results of the MDRD study (Peterson et al., 1995; Menon et al., 2009). These observations hold true. Moriyama et al. (2014) show the renal survival curves of patients with IgA nephropathy and different degrees of proteinuria (Fig. 50.3). Gutiérrez et al. (2012) confirm excellent long-term outcomes of proven IgA disease in which there is haematuria with no or minimal proteinuria.

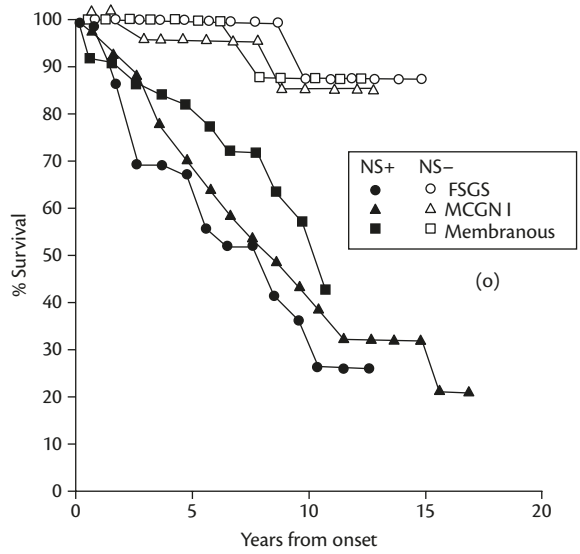

Fig. 50.2 Proteinuria predicts outcome more strongly than histological type of glomerulonephritis. 196 patients with three different histological diagnoses (90 FSGS, 40 Mesangiocapillary glomerulonephritis type 1, 66 Membranous nephropathy) were divided into NS+ (proteinuria > 2 g/24 hours) and NS− (proteinuria < 2 g/24 hours).
From Cameron (1979).

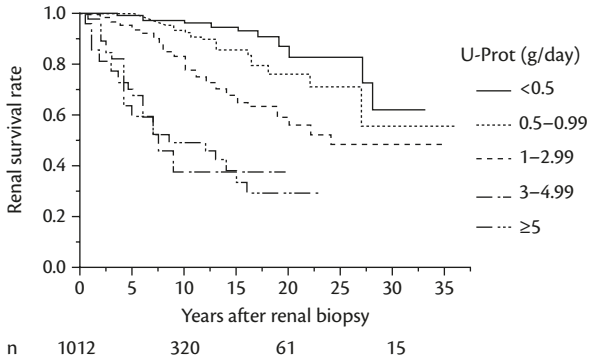

Fig. 50.3 Long-term outcomes of 1012 patients with IgA nephropathy stratified by degree of proteinuria.
From Moriyama et al. (2014).

Angiotensin-converting enzyme inhibitors, which happen to lower proteinuria, show additional renoprotection beyond that conveyed by blood pressure control. This was first shown in diabetes but later also in other diseases associated with proteinuria (see Chapter 98). It has not been proven whether this association with proteinuria is causative, or simply a useful epiphenomenon. Early research focused on possible direct toxicity of filtered proteins on cells of the nephron and the interstitium, but an equally viable hypothesis is that proteinuria is an indication of podocyte damage, and it is podocyte death that determines the outcome of scarred or stressed glomeruli. These concepts are reviewed in Chapter 136.

Attempts to lower proteinuria further by adding additional agents with proteinuria-lowering effects have had disappointing results so far, but there are other candidates to test (see Chapters 45 and 99).

References

Albright, P., Brensilver, J., and Cortell, S. (1983). Proteinuria in congestive heart failure. *Am J Med*, 3, 272–5.

Amsellem, S., Gburek, J., Hamard, G., *et al.* (2010). Cubulin is essential for albumin reabsorption in the renal proximal tubule. *J Am Soc Nephrol*, 21, 1859–67

Barratt, T. M. (1983). Proteinuria. *Br Med J*, 287, 1489–90.

Barringer, T. B. (1912). The prognosis of albuminuria with or without casts. *Arch Int Med*, 9, 657–64.

Berggård, I. (1970). Plasma proteins in normal urine. In H. Manuel, H. Bétuel, and J. -P. Revillard (eds.) *Proteins in Normal and Pathological Urine*, p. 719. Basel: Karger.

Böger, C. A., Chen, M. H., Tin, A., *et al.* (2011). CUBN is a gene locus for albuminuria. *J Am Soc Nephrol*, 22, 555–70.

Cameron, J. S. (1979). Clinicopathological correlations in glomerular disease. In J. Churg, B. H. Spargo, F. K. Mostofi *et al.* (eds.) *Kidney Disease: Present Status by 16 Authors*, pp. 76–97. Baltimore, MD: Williams and Wilkins.

Cameron, J. S. (1990). Proteinuria and progression in human glomerular diseases. *Am J Nephrol*, 10, Suppl 1, 81–7.

Candiano, G., Santucci, L., Petretto, A., *et al.* (2010). 2D-electrophoresis and the urine proteome map: where do we stand? *J Proteomics*, 73, 829–44.

Cheng, J. T., Mohan, S., Nasr, S. H., *et al.* (2006). Chyluria presenting as milky urine and nephrotic-range proteinuria. *Kidney Int*, 70, 1518–22.

Chitalia, V. C., Kothari, J., Wells, E. J., *et al.* (2001). Cost–benefit analysis and prediction of 24-hour proteinuria from the spot urine protein–creatinine ratio. *Clin Nephrol*, 55, 436–47.

Collier, W. (1907). Functional albuminuria in athletes. *Br Med J*, i, 4–6.

D'Amico, G. (1991). The clinical role of proteinuria. *Am J Kidney Dis*, 17, 48–52.

D'Amico, G. and Bazzi, C. (2003). Pathophysiology of proteinuria. *Kidney Int*, 64, 809–25.

Ginsberg, J. M., Chang, B. S., Matarese, R. A., *et al.* (1983). Use of single voided urine samples to estimate quantitative proteinuria. *N Engl J Med*, 309, 1543–6.

Gutiérrez, E., Zamora, I., Ballarin, J. A., *et al.* (2012). Long-term outcomes of IgA nephropathy presenting with minimal or no proteinuria. *J Am Soc Nephrol*, 23, 1753–60.

Guy, M., Borzomato, J. K., Newall, R. G., *et al.* (2009). Protein and albumin-to-creatinine ratios in random urines accurately predict 24 h protein and albumin loss in patients with kidney disease. *Ann Clin Biochem*, 46, 468–76.

Haraldsson, B. (2010). Tubular reabsorption of albumin: it's all about cubulin. *J Am Soc Nephrol*, 21, 1810–12.

Haraldsson, B. and Tanner, G. A. (2014). Zeroing in on the albumin glomerular sieving coefficient. *Am J Physiol Renal Physiol*, 306(6), F577–8.

Hebert, L. A., Birmingham, C. J., Shidham, G., *et al.* (2009). Random spot urine protein/creatinine ratio is unreliable for estimating 24-hr proteinuria in individual SLE nephritis patients. *Nephron Clin Pract*, 113, 177–82.

Hladunewich, M. A. and Schaefer, F. (2011) Proteinuria in special populations: pregnant women and children. *Adv Chronic Kidney Dis*, 18, 267–72.

Hoyer, J. and Seiler, M. W. (1979). Pathophysiology of Tamm–Horsfall protein. *Kidney Int*, 16, 279–89.

Johnson, D. W., Jones, G. R., Mathew, T. H., *et al.* (2012). Australasian Proteinuria Consensus Working Group. Chronic kidney disease and measurement of albuminuria or proteinuria: a position statement. *Med J Aust*, 197, 224–5.

Jones, C. R., Sumeray, M., Heys, A., *et al.* (1999). Pseudo-proteinuria following gelofusine infusion. *Nephrol Dial Transplant*, 14, 944–5.

Kincaid-Smith, P. and Becker, G. (1979). Reflux nephropathy and chronic atrophic pyelonephritis. *J Infect Dis*, 138, 774–80.

Iseki, K., Ikemiya, Y., Iseki, C., *et al.* (2003). Proteinuria and the risk of developing end-stage renal disease. *Kidney Int*, 63, 1468–74.

Iseki, K., Iseki, C., Ikemiya, Y., *et al.* (1996). Risk of developing end-stage renal disease in a cohort of mass screening. *Kidney Int*, 49, 800–5.

Lamb, E. J. and Price, C. P. (2011). Kidney function tests. In C. A. Burtis, E. R. Ashwood, and D. E. Bruns (eds.) *Tietz Textbook of Clinical Chemistry and Molecular Diagnostics* (5th ed.), pp. 669–707. St. Louis, MO: Elsevier.

Lin, C. -H., Sheng, C. -C, Chen, C. -H., *et al.* (2000). The prevalence of heavy proteinuria and progression risk factors in children undergoing urinary screening. *Pediatr Nephrol*, 14, 953–9.

Liu, X., Shao, C., Wei, L., *et al.* (2012). An individual urinary proteome analysis in normal human beings to define the minimal sample number to represent the normal urinary proteome. *Proteome Sci*, 10, 70.

Marks, M. L., McLaine, P. N., and Drummond, K. N. (1970). Proteinuria in children with febrile illnesses. *Arch Dis Child*, 45, 250–3.

Meadow, R. (1977). Munchausen syndrome by proxy. The hinterland of child abuse. *Lancet*, ii, 343–5.

Menon, V., Kopple, J. D., Wang, X., *et al.* (2009) Effect of a very low protein diet on outcomes: long-term follow-up of the MDRD study. *Am J Kidney Dis*, 53, 208–17.

Methven, S., Traynor, J. P., O'Reilly, D. S., *et al.* (2012) Urine albumin:protein ratio as a predictors of patient outcomes in CKD. *Nephrol Dial Transplant*, 27, 3372–3.

Mogensen, C. E. and Christensen, C. K. (1984). Predicting diabetic nephropathy in insulin-dependent patients. *N Engl J Med*, 311, 89–93.

Moriyama, T., Tanaka, K., Iwasaki, C., *et al.* (2014). Prognosis in IgA nephropathy: 30-year analysis of 1,012 patients at a single center in Japan. *PLOS One*, 9(3), e91756.

Naresh, C. N., Hayen, A., Weening, A., *et al.* (2013). Day-to-day variability in spot urine albumin-creatinine ratio. *Am J Kidney Dis*, 62, 1095–101.

National Institute for Health and Care Excellence (2014). *Chronic Kidney Disease: Early Identification and Management of Chronic Kidney Disease in Adults in Primary and Secondary Care.* [Online] <http://www.nice.org.uk/guidance/cg182/resources/guidance-chronic-kidney-disease-pdf>

Ovunc, B., Otto, E. A., Vega-Warner, V., *et al.* (2011). Exome sequencing reveals cubulin mutation as a single-gene cause of proteinuria. *J Am Soc Nephrol*, 22, 1815–20.

Peterson, P. A., Evrin, P. E., and Berggård, I. (1969). Differentiation of glomerular, tubular, and normal proteinuria: determinations of urinary excretion of beta-2-macroglobulin, albumin, and total protein. *J Clin Invest*, 48, 1189–98.

Peterson, J. C., Adler, S., Burkart, J. M., *et al.* (1995). Blood pressure control, proteinuria, and the progression of renal disease. The Modification of Diet in Renal Disease Study. *Ann Intern Med*, 123, 754–62.

Ritz, E., Nowicki, M., Fliser, D., *et al.* (1994). Proteinuria and hypertension. *Kidney Int*, 47, Suppl 47, s76–80.

Rustecki, G. J., Goldsmith, C., and Schreiner, G. E. (1971). Characterization of proteins in urinary casts. Fluorescent antibody identification of Tamm–Horsfall protein in matrix and serum proteins in granules. *N Engl J Med*, 284, 1049–52.

Slattery, C., Lee, A., Zhang, Y., *et al.* (2008). In vivo visualization of albumin degradation in the proximal tubule. *Kidney Int*, 74, 1480–6.

Smith, E. R., Cai, M. M., McMahon, L. P., *et al.* (2012). The value of simultaneous measurements of urinary albumin and total protein in proteinuric patients. *Nephrol Dial Transplant*, 27, 1534.

Turner, A. N. (2013). *Proteinuria: A Bad Thing Since 400 BC.* [Online] <http://historyofnephrology.blogspot.co.uk/2013/01/proteinuria-bad-thing-since-400-bc.html>

Tojo, A., Nanba, S., Kimura, K., *et al.* (1990). Factitious proteinuria in a young girl. *Clin Nephrol*, 33, 299–302.

Tomlinson, P. A., Dalton, R. N., Hartley, B., *et al.* (1997). Low molecular weight protein excretion in glomerular disease: a comparative analysis. *Pediatric Nephrol*, 11, 285–90.

Vehaskari, V. M. and Rapola, J. (1982). Isolated proteinuria: analysis of a school-age population. *J Pediatr*, 101, 661–8.

Viberti, G. F. (1988). Etiology and prognostic importance of proteinuria in diabetes. *Diabetes Care*, 11, 840–5.

Williams, P. S., Fass, G., and Bone, J. M. (1988). Renal pathology and proteinuria determine progression in untreated mild/moderate renal failure. *QJM*, 67, 343–54.

CHAPTER 51

Postural proteinuria (benign orthostatic proteinuria)

Neil Turner

Introduction

Postural proteinuria was defined as one of the relatively benign variants of 'cyclical' or recurrent proteinuria in studies in the nineteenth century, following widespread testing of Bright's ominous association of proteinuria with serious renal disease. Pavy (1886) gives a clear account of the disorder in three patients. He tested the effect of delayed rising from bed, and ascertained that it was posture that kept the proteinuria away rather than cold exposure causing it, by comparing the effect of a cold bath.

Epidemiology

At least 5% of school-age children show positivity for protein on screening (Dodge et al., 1976; Brandt et al., 2010). Most of these children do not go on to develop evidence of progressive renal disease, and postural proteinuria is the diagnosis in the majority (Dodge et al., 1976).

The definition of postural proteinuria is increased daily protein excretion, but with normal levels of protein in first-morning urine. The currently accepted upper limit of normal for protein excretion in children is > 100 mg/m^2/24 hours, higher in neonates (Hogg et al., 2000). Using this definition, postural proteinuria is common. Brandt's small study of 91 healthy children and young people aged 6–19 years found 20% of them qualified for a diagnosis of postural proteinuria using these criteria, others (e.g. Dodge et al., 1976) are closer to 5%. This does raise the question of whether this is simply physiology at this age, rather than pathology. Perhaps the accepted norm is too low.

Some studies have suggested that the incidence may be higher in children with obesity and hypertension, or it may simply be that they have slightly higher urine excretion so meet the criteria more easily. Other authors have suggested that gain in body mass may reduce obstruction to the left renal vein (nutcracker phenomenon, see 'Relationship to nutcracker phenomenon') when/if this explains proteinuria.

Mechanism

Diurnal variation of protein excretion has been identified in those with pathological explanations for proteinuria (Wan et al., 1995), and also in those with proteinuria in the normal range (Brandt et al., 2010). This may be related to postural changes in renal haemodynamics and could also be related to changes in renal vein compression (see 'Relationship to nutcracker phenomenon').

Relationship to nutcracker phenomenon

There is a contentious relationship in the literature between nutcracker phenomenon/syndrome (see Chapter 48) and postural proteinuria, a suggestion first raised in 1958 (reviewed by Mazzoni et al., 2011). Nutcracker syndrome is more commonly associated with macroscopic haematuria, but modern imaging methods are no doubt picking up this anatomical feature more frequently.

A positive review of published reports of this association (Mazzoni et al., 2011) found that five studies included 229 patients aged 5–17 years. Nutcracker syndrome was identified by Doppler ultrasound in 68%, versus reports of the same venous anatomical features (in different studies) in up to 5% of controls. These studies were mostly undertaken in Asia, and the analyses were not blinded. A reassessment of 13 of their own patients 6 years later was described as showing that both nutcracker syndrome and proteinuria had disappeared in nine patients, in three patients both had persisted, and in one patient the proteinuria had persisted but nutcracker syndrome resolved (Milani et al., 2010).

However, in a study of 99 consecutive adult potential renal transplant donors by computed tomography angiography (Grimm et al., 2013), a high incidence of asymptomatic nutcracker phenomenon was found. Twenty-seven per cent of patients had substantial (> 50%) compression of the left renal vein, and the finding of dilated gonadal or lumbar veins was common in these healthy individuals who had neither haematuria nor proteinuria.

Lee et al. (1997) studied two girls aged 11 and 12 with marked postural proteinuria (24-hour excretion > 40 mg/m^2/hour, 10 times the upper limit of normal) extensively. Both had marked left renal vein obstruction with collateral vein formation. Bilateral ureteral catheterisation was undertaken and showed that only urine from the left kidney contained increased protein.

The relationship remains uncertain. Issues around diagnosis of nutcracker syndrome are discussed in Chapter 48. However, as outcomes of orthostatic proteinuria appear generally good, intervention to alter the anatomy cannot be easily justified for this reason.

Management

Outcomes are reported to be uniformly good, with little suggestion that there is extra long-term risk associated. However, large and very long studies would probably be required to identify such outcomes, given the size and duration of study required to show, for example, adverse long-term outcomes from microscopic haematuria (see Chapter 46). There are a few long-term reports (Levitt,

1967; Antoine et al., 1968; McLaine and Drummond, 1970), including a remarkable follow-up of patients seen by over 40 years previously Thomas Addis (Rytand and Spreiter, 1981). If there is extra long-term risk it is likely to be very low.

If morning samples have normal levels of protein and there are no other pointers to renal disease (including normal blood pressure and no haematuria), no further investigations are warranted. The simplest monitoring technique is occasional measurement of protein:creatinine or albumin:creatinine ratios in first-in-morning urine samples.

Investigation seeking to demonstrate nutcracker phenomenon (see Chapter 48) is not recommended. The results may be misleading and results are highly unlikely to alter management.

Where total protein excretion is very high, or there are other pointers to disease, further investigation may be considered. It has been observed that angiotensin-converting enzyme inhibitors can reduce proteinuria in this condition as in proteinuria of other causes (Ha and Lee, 2006).

References

Antoine, B., Symvoulidis, A., and Dardenne, M. (1968). La stabilité évolutive des états de proteinurie permanente isolée. *Nephron*, 6, 526–36.

Brandt, J. R., Jacobs, A., Raissy, H. H., *et al.* (2010). Orthostatic proteinuria and the spectrum of diurnal variability of urinary protein excretion in healthy children. *Pediatr Nephrol*, 25, 1131–17.

Dodge, W. F., West, E. F., Smith, E. H., *et al.* (1976). Proteinuria and hematuria in schoolchildren: epidemiology and early natural history. *J Pediatrics*, 88, 327–47.

Grimm, L. J., Engstrom, B. I., Nelson, R. C., *et al.* (2013). Incidental detection of nutcracker phenomenon in an asymptomatic population: prevalence and associated findings. *J Comput Assist Tomogr*, 37, 415–18.

Hogg, R. J., Portman, R. J., Milliner, D., *et al.* (2000). Evaluation and management of proteinuria and nephrotic syndrome in children: recommendations from a pediatric nephrology panel established at the National Kidney Foundation conference on proteinuria, albuminuria, risk, assessment, detection, and elimination (PARADE). *Pediatrics*, 105, 1242.

Ha, T. S. and Lee, E. J. (2006). ACE inhibition can improve orthostatic proteinuria associated with nutcracker syndrome. *Pediatr Nephrol*, 21, 1765–8.

Lee, S. J., You, E. S., Lee, J. E., *et al.* (1997). Left renal vein entrapment syndrome in two girls with orthostatic proteinuria. *Pediatr Nephrol*, 11, 218–20.

Levitt, J. I. (1967). The prognostic significance of proteinuria in young college students. *Ann Internal Med*, 66, 685–96.

McLaine, P. N. and Drummond, K. N. (1970). Benign persistent asymptomatic proteinuria in childhood. *Pediatrics*, 46, 548–52.

Mazzoni, M. B., Kottanatu, L. K., Simonetti, G. D., *et al.* (2011). Renal vein obstruction and orthostatic proteinuria: a review. *Nephrol Dial Transplant*, 26, 562–5.

Milani, G. P., Mazzoni, M. B., Burdick, L., *et al.* (2010). Postural proteinuria associated with left renal vein entrapment: a follow-up evaluation. *Am J Kidney Dis*, 55, e29–31.

Pavy, F. W. (1886). A further contribution on cyclical albuminuria; with observations on the effect of various conditions upon the diurnal appearance of albumen. *Lancet*, 127, 437–8.

Rytand, D. A. and Spreiter, S. (1981). Prognosis in postural (orthostatic) proteinuria. Forty to fifty-year follow-up of six patients after diagnosis by Thomas Addis. *N Engl J Med*, 305, 618–21.

Wan, L. L., Yano, S., Hiromura, K., *et al.* (1995). Effects of posture on creatinine clearance and protein excretion in patients with various renal diseases. *Clin Nephrol*, 43, 312–17.

CHAPTER 52

Nephrotic syndrome

Premil Rajakrishna, Stewart Cameron, and Neil Turner

Introduction

Richard Bright's cases of advanced renal disease causing dropsy were not examples of simple nephrotic syndrome, but by drawing attention to the significance of urinary protein he led other physicians to study the problem. Robert Christison in Edinburgh quickly identified patients with episodes of dropsy associated with proteinuria that recovered—later described by physicians as nephrosis, and subsequently as nephrotic syndrome (Cameron and Hicks, 2002; Turner, 2010; see Chapter 42).

Definition

Criteria for diagnosis of nephrotic syndrome are inconsistent, and this is understandable (Glassock et al., 2015). The essential elements are that there should be high-level proteinuria with lowered serum albumin. Most definitions also include oedema. However, oedema appears at different levels of proteinuria in different individuals, and correlates poorly with serum albumin. Age and salt intake may explain some of this variation. Setting strict limits on proteinuria or serum albumin for diagnosis is, however, problematic.

We commonly use the term nephrotic range proteinuria, usually meaning > 3.5 g/24 hours, (see Chapter 50), but many patients only develop low serum albumin and oedema at levels of proteinuria appreciably higher than this. Others have such severe nephrotic syndrome that serum albumin is very low, and protein excretion may fall as a consequence of this. It may also fall because of hypovolaemia and acute kidney injury (AKI). We also have to make our decisions based on measurements of known variability or unreliability such as spot urinary protein:creatinine ratio (PCR) and 24-hour urine collections. The clinical implications of nephrotic syndrome are broadly proportional to the severity of the protein leak and the oedema, and not defined by whether a particular threshold is reached.

The concept of nephrotic syndrome includes the immediate symptomatic consequences, notably oedema, but also the increased risk of infection, thrombosis, hyperlipidaemia, and disturbances of circulating volume.

Clinical features

Proteinuria itself is symptomless in most individuals. Frothy urine may be caused by heavy proteinuria, but is not a specific sign. Oedema is the most common presenting feature, but presentation with complications is also common. Typically these are infections, notably spontaneous peritonitis in children with ascites; venous thrombosis or thromboembolism; or manifestations of hyperlipidaemia.

Primary features

Nephrotic oedema

The five attributes of nephrotic oedema are gradually increasing, gravitational, generalized, pitting, and softness.

Nephrotic oedema is noticeable first only around the eyes in the morning, and the ankles in the evening, but with increasing fluid retention there is sustained swelling of ankles and face (Fig. 52.1) which can lead to a misdiagnosis of allergy. If patients are in bed, fluid accumulates as a sacral pad and oedematous elbows. The effects of gravity are less evident in children: children and sometimes young adults may suffer considerable ascites and facial oedema without ankle oedema. Younger patients also seem to tolerate lower levels of serum albumin before forming detectable oedema.

In adults, retention of up to 4 L of salt and water remains undetectable, revealed only by weighing. With increasing oedema, ascites may appear followed by pleural effusions, which are usually bilateral, occasionally unilateral, and usually limpid, but sometimes opaque and chylous. Genital oedema may be distressing, especially in males. The oedema remains soft and pitting even when profound (Fig. 52.2), but if it remains untreated for long periods it may become indurated and pit only with difficulty especially around the ankles. Ankle swelling may be asymmetrical if deep venous thrombosis supervenes. Striae may appear even if no corticosteroids are being given, and the skin may actually split and weep spontaneously. Needlestick punctures may also weep profusely. The liver may be painlessly enlarged, especially in children. Patients with increased portal vein pressure will form ascites.

General features

Patients with severe nephrotic syndrome often feel tired and lacking in energy, even if they do not have substantial oedema. The explanation for this is not clear.

The jugular venous pressure is usually normal or low, but if raised in association with a low or normal blood pressure in an older adult with nephrotic syndrome this raises suspicion of cardiac amyloidosis as a cause. The nails may show white bands corresponding to periods of hypoalbuminaemia (Fig. 52.3).

Where nephrotic syndrome is caused by a systemic disease or is otherwise secondary (e.g. to drugs or malignancy), history and examination may hint at or reveal these.

Many nephrotic patients lose muscle and flesh weight as well as oedema. This is probably a mixture of reduced appetite and activity, compounded by the effects of corticosteroid therapy in some.

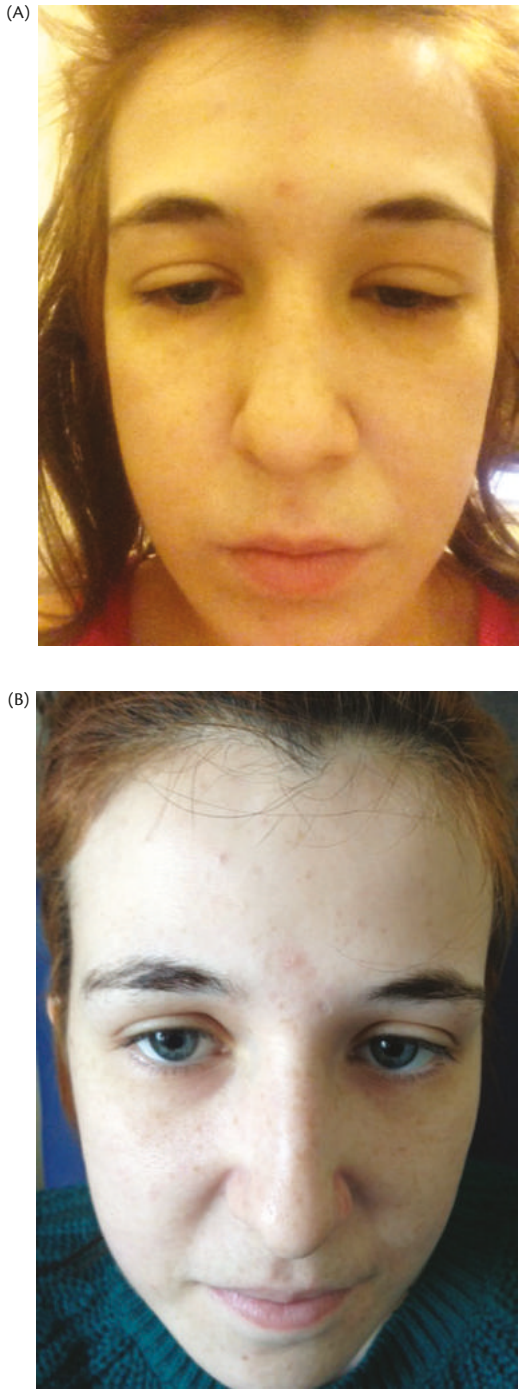

Fig. 52.1 Face of a patient with severe nephrotic syndrome (A) before and (B) after diuretics.

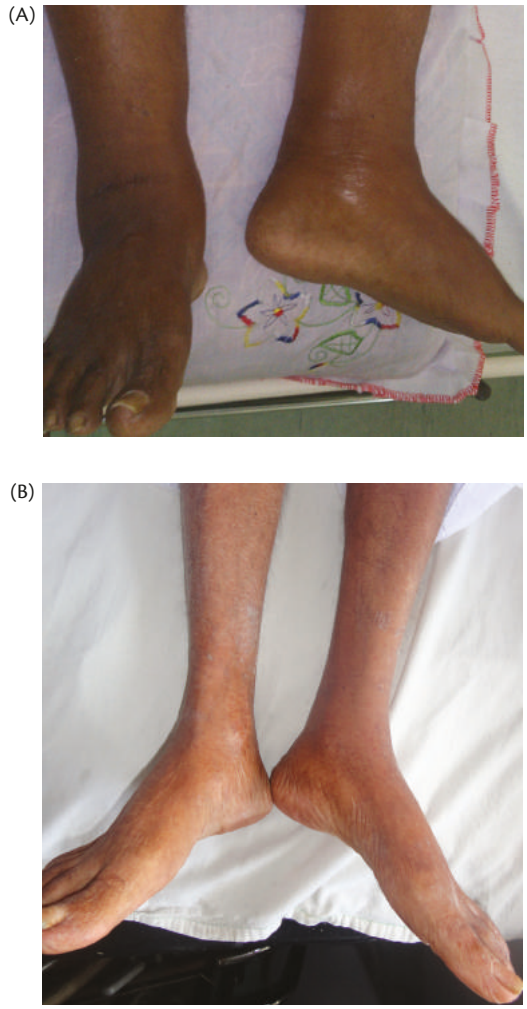

Fig. 52.2 Legs of an adult patient with minimal change disease (A) before and (B) after treatment with prednisolone.

Complications

Thrombosis

Presentation with a thrombotic complication is rare in children compared to adults, but more serious if it occurs (Deshpande and Griffiths, 2005). Both arterial thrombosis and venous thrombosis are reported but venous thrombosis is much more common in most series (Cameron et al., 1988).

Deep vein thrombosis is the commonest. The tendency for thrombosis is approximately related to the severity of nephrotic syndrome, but events are not evenly distributed. Most thrombotic events are reported within the first 6 months of the diagnosis (Mahmoodi et al., 2008, Lionaki et al., 2012). Membranous nephropathy is usually more commonly associated with thrombosis than other causes (Bellomo and Atkins, 1993), though the incidence was relatively

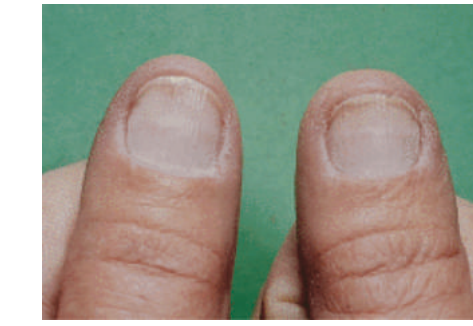

Fig. 52.3 The white-banded nail of an adult nephrotic patient with membranous nephropathy, representing a period of relapse when there was a severe nephrotic syndrome and profound hypoalbuminaemia. The exact pathogenesis of this type of nail appearance is not known; in some patients the nail becomes diffusely white from the lunula outwards as the disease continues (sometimes called a 'half and half' nail).

low in 898 patients studied in the Netherlands (Lionaki et al., 2012) at 7% over 3 years.

Pulmonary embolism should be suspected in cases of sudden shortness of breath, although chest infection, pleural effusion, acidosis with renal insufficiency, abdominal distension with ascites, or anaemia may be the cause.

Thrombosis of the deep calf veins is common, overt thrombosis occurring in 3–12% of adults in older series (Llach, 1982; Cameron, 1984). Mahmoodi et al. (2008) studied 298 consecutive adults with different diagnoses and found an annualized incidence of thrombosis of about 1%, but heavily biased to the first 6 months when the incidence was 10%. The rate of arterial events was also high in this study.

Thrombosis is less frequent in children; < 1% of 4158 paediatric patients in published series showed clinically evident deep vein thrombosis (Andrew and Brooker, 1996).

Studies seeking asymptomatic thrombosis find higher rates of occult thrombi. However, active approaches to seeking and preventing thrombosis beyond routine prophylactic measures are controversial (see 'Management').

Renal vein thrombosis

Clinically apparent renal vein thrombosis is rare, though the literature contains many reports suggesting a high but also extraordinarily variable rate of occult thrombi, 5–50% (Rostoker et al., 1992; Singhal and Birmble, 2006). The higher figures surprise clinicians who have looked with modern techniques and rarely identified renal vein thrombosis. Chronic renal vein thrombosis is usually asymptomatic, but may be detected after a pulmonary embolus is identified.

Acute renal vein thrombosis presents with loin pain, haematuria, and elevated lactate dehydrogenase levels. If the patient has associated renal failure, bilateral involvement should be suspected. The latter is rare and often seen when additional risk factors for thrombosis (severe dehydration, antiphospholipids, protein C or S deficiency, etc.) are present. It may be a presenting feature of nephrotic syndrome, with or without pulmonary embolism, when the complex acute clinical picture often creates diagnostic difficulty.

Selective renal venography remains the gold standard test for the diagnosis, but it is invasive and rarely undertaken (Singhal and Brimble, 2006). Renal ultrasound may show enlarged kidneys and Doppler ultrasound can identify thrombosis but its sensitivity is not clear. Contrast-enhanced spiral computed tomography is probably now the most commonly employed technique. It does, however, add to risk of AKI (see Chapter 14). It has not been established that seeking symptomless renal venous thrombi is useful (Rostocker et al., 1992), since their prognosis appears to be benign.

Infections

Infections remain a significant cause of morbidity and sometimes mortality in nephrotic syndrome, particularly in the developing world. Six of 10 deaths in 389 children with minimal change nephrotic syndrome were from sepsis (International Study of Kidney Disease in Children, 1984). Children with nephrotic syndrome appear more vulnerable to infections than adults but they can be serious in both. Pneumococcal infections are particularly prevalent. In a series of studies of peritonitis in nephrotic children, *Streptococcus pneumoniae* and *Escherichia coli* were the most common pathogens (Krensky et al., 1982). Increased incidences of urinary, respiratory, and central nervous system infections are also reported (Uncu et al., 2010).

The exact mechanisms of immune defects are not well understood, but urinary losses of immunoglobulins and other immune mediator molecules are presumed to contribute. Immunoglobulin levels are lowered but not to levels associated with infection in inherited hypogammaglobulinaemia. Sites of abnormal fluid collection are common infection sites, patients can present with peritonitis, cellulitis, or empyema. Spontaneous peritonitis may occur at presentation or as a later complication.

Varicella infection is also reported to be increased, but steroid treatment is likely to be a major contributor to this propensity.

Hyperlipidaemia

Dyslipidaemia is a universal finding in nephrotic syndrome, and characterized by often very high plasma cholesterol (> 10 mmol/L), low-density lipoprotein (LDL), triglyceride, and low high-density lipoprotein levels (Crew et al., 2004). The major clinical impact is seen in those with chronic nephrotic syndrome, and those on long-term steroid therapy complicated by additional risk factors such as hypertension.

Lipid abnormalities are proportionate to proteinuria, and remit as nephrotic state remits (Shearer et al., 2001). Treatments that lower proteinuria correct lipid abnormalities as well.

Hyperlipidaemia is thought to be due to a compensatory liver response to reduced plasma oncotic pressure mediated by hepatic apoprotein B gene transcription, but there may be a contribution from defective lipid catabolism as well (Shearer et al., 2001).

Acute kidney injury

AKI is unusual but well recognized. It may be related to the nephrotic state, iatrogenic, or related to the underlying disease process.

AKI at presentation

Apparently 'idiopathic' AKI is an occasional but important complication, and can be distinguished from AKI from identifiable causes such as interstitial nephritis, thrombosis, sepsis, or contrast media. Bilateral acute renal vein thrombosis is a rare explanation which needs to be considered (see above).

AKI at presentation occurs mostly in older patients of either gender, overwhelmingly (81%) in those with minimal change/focal segmental glomerulosclerosis (FSGS) histology (Smith and Hayslett, 1992; Waldman et al., 2007). In children with the same diagnosis, AKI is rare and usually follows either sepsis or thrombosis (Cameron et al., 1988; Cavangnaro and Lagomarsino, 2000).

Most adult patients present already in AKI; some develop it subsequent to diagnosis or in relapse. One-sixth were judged to be seriously hypovolaemic or in shock, and all had a very low serum albumin. Urine volume is low, containing < 5 mmol/L of sodium and unresponsive to diuretics and/or volume repletion, loaded with protein, and containing red cells and often red cell casts. Thus, renal biopsy is almost always necessary to establish a diagnosis, as this pattern of sediment suggests a proliferative nephritis rather than minimal changes.

The role of hypovolaemia and reduced renal perfusion is not clear as AKI is often present before diuretic therapy is initiated, and by the time of diagnosis many patients have a full circulation and hypertension was common in early series. However, renal circulatory disturbance may be a common feature in severe nephrotic syndrome and could predispose to AKI (Koomans et al., 2001; Vande Walle et al., 2004) (see Chapter 53).

Renal biopsy usually shows moderate to severe tubular changes (Venkataseshan et al., 1993). Interstitial oedema is usually present, perhaps indicating increased interstitial pressure (Lowenstein et al., 1970) and it has been suggested this could contribute to pathogenesis.

Management of these often elderly and severely ill patients follows usual principles (Chapters 228 and 233) but is difficult. They continue to pass large amounts of protein in tiny amounts of urine, have very low serum albumin and sometimes unstable circulation, and are of course uraemic. If not already malnourished, they rapidly become so.

Secondary AKI

Delayed AKI is commonly related to over-diuresis or a secondary complication such as vomiting or diarrhoea. Otherwise unexplained low blood pressure, tachycardia, cold extremities, restlessness, renal dysfunction should point to this (Vande Walle et al., 1995).

The commoner form is a transient mild rise in creatinine levels secondary to intravascular volume depletion due to over-diuresis or severe hypoalbuminaemia. Children and adults with minimal change disease are more vulnerable and correction of volume status reverses it.

Endocrine dysfunction

Endocrine dysfunctions such as hypothyroidism and vitamin D deficiencies are noted in nephrotics, but steroid-induced endocrine abnormalities are more commonly encountered (Crew et al., 2004).

Diagnosis

Proteinuria and serum albumin

There should be heavy proteinuria, for example, PCR > 300 mg/mmol, or 24-hour protein > 3.5 g. This odd value corresponds to an extension of the criterion for nephrotic proteinuria being > 40 mg/m^2/hour of albumin, applied to a 70-kg man. So 3.5 g should not be used as a diagnostic level for a 50-kg man or woman. The limits only apply to proteinuria that is composed predominantly of albumin, but in clinical practice almost all nephrotic range proteinuria is predominantly albumin. Unusually high levels of overflow protein such as immunoglobulin light chains (overflow proteinuria), or of tubular proteinuria, do not cause nephrotic syndrome (see Chapter 50).

These exact limits of even albuminuria should not be over-interpreted. The manifestations of nephrotic syndrome do not start at a given value of albumin or proteinuria, they are graded. However, it is clinically useful that at a certain level of proteinuria a characteristic set of renal diagnoses becomes most likely.

Whether or not there is associated haematuria is important in narrowing down the possible underlying cause (see Chapter 45).

Serum albumin will be low, usually < 30 g/L and sometimes very low, < 10 g/L. Correlation with the degree of oedema is imperfect, another indication that changes in Starling pressures alone cannot explain the pathogenesis of oedema in nephrotic syndrome (see Chapter 53).

Differential diagnosis

Demonstrating nephrotic range proteinuria and hypoalbuminaemia in an oedematous patient confirms the diagnosis of nephrotic syndrome. But in an outpatient setting and in complicated patients the diagnosis may be challenging. Findings in oedema of different causes are discussed in Chapter 30.

Patients with advanced renal impairment commonly present with fluid retention with significant proteinuria and oedema, mimicking nephrotic syndrome at first glance. These patients are typically characterized by excess intravascular fluid with elevated jugular venous pressure and hypertension, not simply peripheral oedema (see Chapter 53). They may show features of uraemia. Estimation of creatinine and albumin and renal ultrasound can quickly prove this.

Nephrotic syndrome implies dysfunction of the glomerular filtration barrier affecting the podocyte (see Chapter 45). 'Pure' nephrotic syndrome, in which there is no or minimal haematuria, has a characteristic set of causes. If there is significant haematuria the differential diagnosis broadens. Haematuria suggests that the glomerular basement membrane is being breached, sometimes by genetic cause (Alport syndrome), but most commonly by inflammation within the glomerulus. Many inflammatory diseases cause proteinuria, some to nephrotic levels, via podocyte damage which either occurs directly, or through alterations in the glomerular matrix and milieu that lead to podocyte dysfunction (see Chapter 45, Fig. 45.1).

The range of causes in a UK centre across several decades is shown in Fig. 52.4. In pregnancy, pre-eclampsia (see Chapter 296) must be added to the list of common causes of nephrotic syndrome.

Urinary sediment

Clues given by urine sediment examination under light microscopy can be time saving and save cost, particularly in resource-poor settings. Non-proliferative glomerulopathies (minimal change, membranous, and FSGS) are more likely to have a bland urinary sediment showing hyaline casts and oval fat bodies but few red cell or other cellular casts (see Chapter 6). The term 'nephrosis', now abandoned, was used to describe this type of kidney disease. More red cells and cellular casts in urine is indicative of proliferative

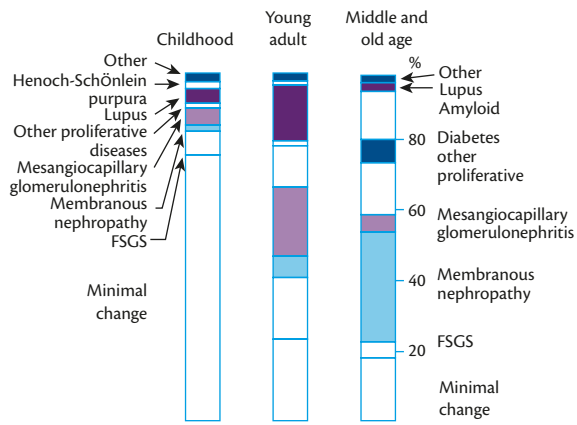

Fig. 52.4 Underlying histological appearances found in renal biopsies from more than 1000 nephrotic patients of all ages seen at Guy's Hospital, London, 1963–1990. Note that the majority of children under the age of 15 years have minimal change disease, the proportion falling steadily from 2 to 15 years of age. However, minimal change disease remains an important cause of the nephrotic syndrome in adult nephrotics, and overall is the commonest form. Membranous nephropathy, in contrast, becomes steadily more common with age and is the commonest form of nephrotic syndrome in elderly patients.

glomerulonephritis, (International Study of Kidney Disease in Children, 1978) (see Chapter 45).

Other blood tests

Hyponatraemia is common during diuretic therapy. Hypovolaemia may provoke AKI. Blood count may show haemoconcentration.

Immunological investigations (Table 52.1) should be used selectively, based on probability. Few of these tests prove a cause alone, or can completely replace all the information that may come from a renal biopsy.

Renal biopsy

Renal biopsy examination with light microscopy and immunofluorescence will allocate patients to a particular histological category, but the final interpretation often needs correlation with clinical and serological data.

Table 52.1 Tests to consider for main causes of nephrotic syndrome (Howard et al., 1990; Hofstra et al., 2011)

Nephropathy	Test	Comment
Minimal change/FSGS		No tests available. Age and race influence likelihood
Membranous	Hepatitis B and C and HIV serology (Antibodies to phospholipase A2 receptor (PLA2R)? Other?)	PLA2R not yet proven as a diagnostic test (Chapter 61) Consider skin-lightening creams—mercury level?
Diabetes mellitus	HbA1c	Check clinical likelihood—duration of diabetes, course (Chapter 149)
Amyloidosis	C-reactive protein (for chronic inflammation) Urinary immunofixation for light chains (Bence Jones protein) Serum free light chains; serum protein electrophoresis	Serum amyloid A component may be appropriate in monitoring for suppression of AA amyloid formation (Chapter 152)
Lupus nephritis	Complement (C3, C4, CH50) ANA, anti-dsDNA	May cause 'pure' nephrotic syndrome or mixed nephritic/nephrotic (see Chapter 162)
Membranoproliferative glomerulonephritis	Complement (C3, C4, CH50) Hepatitis B and C and HIV serology	More detailed complement studies may be indicated depending on subtype (Chapter 80) Consider cryoglobulins
Fibrillary and immunotactoid	Serum free light chains; serum protein electrophoresis; urine for Bence Jones protein	(Chapter 81)

Common exceptions to the need for a renal biopsy are

- Diabetes—long-standing diabetes with entirely typical progression from microalbuminuria to proteinuria over many years, with evidence of microvascular complications affecting other organs, particularly retinopathy or neuropathy. In these circumstances many clinicians do not undertake a renal biopsy.
- Children—if a child presents with nephrotic syndrome between the ages of 1 and 10 years, with normal renal function, normal complement levels without hypertension or haematuria, then the diagnosis is highly likely to be minimal change disease (International Study of Kidney Disease in Children, 1978). If these clinical criteria are met, treatment with steroids may be initiated avoiding a renal biopsy. At a later point, renal biopsy may be indicated if there is a poor response to steroids or clinical profile changes. The evidence behind this approach comes mostly from series in Caucasian populations, in which minimal change disease is the dominant cause. It caused 76% of primary disease as reported by the International Study of Kidney Disease in Children (1978).
- Frail elderly or others with severe comorbid conditions where a pathological diagnosis is very unlikely to alter best management.

Epidemiology

Incidence

In general, there is male predominance in the occurrence of nephrotic syndrome with a ratio of approximately 2:1. Lupus is the main condition with a contrary ratio, and if pre-eclampsia was included the distribution would look very different.

In children, a series of prospective studies in Caucasians give incidence rates between 1.2 and 2.0 cases per 100,000 per year, though the incidence is much higher in retrospective studies of African or Asian patients (Eddy and Symons, 2003; Wong et al., 2007).

Causative disease

Primary causes are more common than secondary in both children and adults. The diseases most commonly causing nephrotic syndrome vary with age and by race and/or geographical region. Fig. 52.4 shows the age effect in one centre over nearly three decades.

In a US study in adults, FSGS (35%) and membranous glomerulonephritis (33%) were the major primary histological pattern followed by minimal change disease (15%) and membranoproliferative glomerulonephritis (14%). This report is also one of several to have highlighted a trend towards increasing incidence of FSGS over membranous glomerulonephritis (Korbet et al., 1996).

Out of the secondary causes of nephrotic syndrome in adults, diabetic nephropathy (50 cases per 100,000 population) leads, followed by lupus and amyloid (Haas et al., 1997).

Ethnicity-related demographics

Asian children are at six times higher risk of developing primary nephrotic syndrome than Europeans, while black and Hispanic children report a higher rate of FSGS and steroid-resistant nephrotic syndrome (Niaudet, 2004; Boyer et al., 2007). Africa reports a strikingly low rate of primary steroid-sensitive nephrotic syndrome in

children (e.g. Pakasa and Sumaili, 2009). The pattern of histology from nephrotic children in India, Pakistan, and Turkey seems similar to that from European and North American studies (Kumar et al., 2003; Ozkaya et al., 2004; Kazi and Mubarak, 2007; El Bakkali et al., 2011). However, the wide variability in biopsy indications, time frames, and sample sizes of various studies makes it difficult to draw solid conclusions.

Management of nephrotic syndrome

Management of nephrotic oedema

Diuretics and salt restriction

Oedema is caused by sodium retention (see Chapter 53) so salt restriction is rational. Patients are advised to avoid salty food, use no added salt (using pepper or chilli or substitute tastes instead), to restrict salt intake below 50–70 mmol/day (about 4 g). Few patients can achieve lower on a modern diet, and given the effectiveness of current diuretics this is usually enough.

Diuretics are almost always required. Diuresis initially depletes intravascular volume and promotes gradual fluid shifts from tissue spaces to vascular compartment. Usually, adult nephrotics tolerate up to 2–3 L of fluid loss per day for short periods without critical depletion of intravascular volume. Acute depletion of effective circulatory volume may be detected clinically by tachycardia, reduced peripheral perfusion, postural drop, and restlessness. Ongoing subacute volume depletion may be picked up by noticing otherwise unexplained gradual increase in serum creatinine levels in the absence of the above-mentioned clinical features (Geers et al., 1985). Those with severe hypoalbuminaemia are more prone to this complication (see Chapter 51). Daily weights and fluid balance charts are helpful to titrate the diuretic dosage in severe nephrotic syndrome, avoiding this complication, but most patients can be managed as outpatients, particularly if able to weigh themselves and allowed to adjust diuretic dose responsively.

Loop diuretics are usually first line in nephrotic syndrome as there is relative diuretic resistance. Higher doses are needed than used in other oedematous patients with the same level of kidney function. Often up-titration of the loop diuretic dose is required which can be done by doubling the doses at short intervals, even daily. At each up-titration, caution has to be exercised to avoid critical plasma volume depletion (Wilcox, 2002).

Resistant oedema

Thiazide diuretics and aldosterone antagonists (spironolactone, epleronone) or amiloride may be used as second-line add-on diuretics. Amiloride has theoretical advantages that have not been systemically explored (see Chapter 53). The value of albumin-furosemide infusion to enhance diuretic action and reduce oedema has not been shown to provide additional benefit on average (Fliser et al., 1999) (see Chapter 30). Albumin infusion greatly increases proteinuria. Patients with ascites may better respond to intravenous diuretics if oral absorption is suspected to be poor due to gut oedema; but moving to intravenous diuretic therapy is in any case the next step up in therapy for patients not responding to maximum doses of combined oral diuretics. Sodium excretion should be checked in those who appear to be diuretic resistant, as some patients find sodium restriction very difficult. (See also Chapters 30 and 33.)

Reducing proteinuria

Treatment that induces disease remission rapidly mobilizes oedema and diuretics are quickly no longer required. This is best illustrated in minimal change disease with steroids (Fig. 52.5).

Where the cause of nephrotic syndrome cannot be reversed, other drugs that reduce proteinuria without altering the natural history of the disease may make oedema easier to control, and reduce risk of some of the general complications of nephrotic syndrome. Least controversially, angiotensin-converting enzyme inhibitors (ACEIs) reduce proteinuria in all glomerular proteinuric diseases. They are indicated in all patients with substantial proteinuria, whether they have nephrotic syndrome or not (see Chapter 50)

Second-line therapies

Agents in addition to ACEIs/angiotensin receptor blockers (ARBs) that might further reduce proteinuria have a less certain role in improving outcomes in renal disease (Chapters 45 and 99), but may have a place in the control of severe uncontrolled nephrotic syndrome. Dual therapy with ACEIs and ARBs is one approach, but does increase risk of hyperkalaemia and AKI. Some additional proteinuria lowering has been claimed for a variety of other drugs. Many of these may act at the podocyte. Candidates include aldosterone antagonists, vitamin D, endothelin antagonists, peroxisome proliferator-activated receptor (PPAR)-γ agonists, the PPAR-α agonist fenofibrate, and others. Most of these have relatively small potential incremental benefit, however. Clinically much more effective are the calcineurin inhibitors, tacrolimus or ciclosporin. While

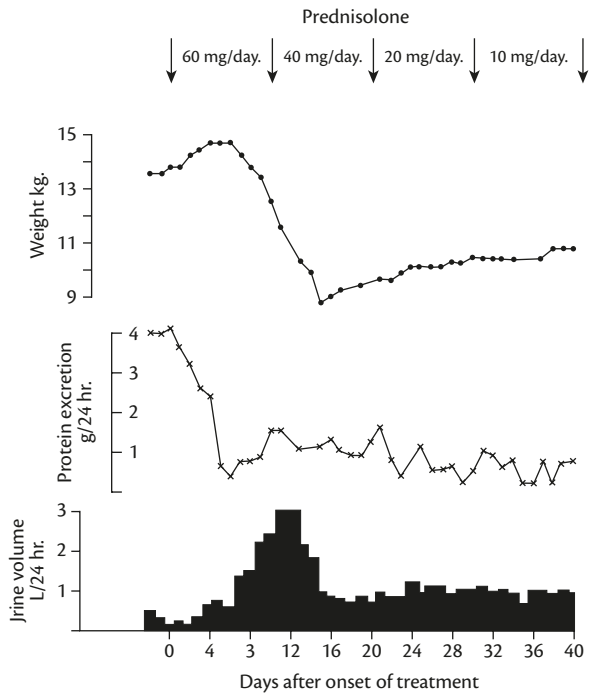

Fig. 52.5 Treatment of nephrotic syndrome in a child in the 1950s, before effective diuretics were available and soon after the discovery of the effect of corticosteroids in childhood nephrotic syndrome. Weight falls from 15 to 10 kg over about a week as the protein leak responds to steroid therapy. Note that the spontaneous diuresis commences as soon as the level of proteinuria drops below 1 g and before albumin levels could have recovered.
From DeWardener (1958), with permission.

some of their effect may be related to lowering glomerular filtration rate (GFR), it is possible that they have an additional direct effect on podocytes (see Chapter 136). Their ability to produce a complete remission in minimal change disease may be a pointer to this (see Chapter 45). Progressively increasing the dose will usually achieve some reduction in proteinuria regardless of the cause.

Physical therapies are possibly under-used. Head-out water immersion can improve fluid loss in generalized oedema of various causes (see Chapter 30).

Chemical or actual nephrectomy

Some patients with very severe nephrotic syndrome cannot achieve adequate quality of life despite all these therapies, requiring diuretic therapy that causes severe postural symptoms or AKI. This is rare, but particularly likely in congenital Finnish nephrotic syndrome, and is sometimes seen in amyloidosis. Even more rarely it may occur in other causes of nephrotic syndrome. Chemical nephrectomy usually refers to adding high dose non-steroidal therapy, usually with indomethacin, to the measures above, including high-dose calcineurin inhibition. This combination reduces GFR significantly.

As a last resort, renal tissue can be destroyed by embolization (which will provoke infarction that is likely to be painful and will be pro-inflammatory) or by physical nephrectomy.

General management

The optimal dietary protein intake for patients with a persisting nephrotic syndrome remains controversial. Although recommended in the past, it has long been known that a high protein intake (> 1.5 g/kg/24 hours) leads to an increase in urinary protein excretion but without any increase in serum albumin or total plasma protein concentrations. In contrast, reduction in protein intake to 0.8 g/kg/24 hours reduces proteinuria, although with controversial effects on serum albumin concentration (Mansy et al., 1989; Kaysen, 1991) and a risk of protein malnutrition (Guarneiri et al., 1989). Therefore, a dietary protein intake of 0.8 g of protein of high biological value/kg/24 hours, plus 1 g protein per gram of proteinuria, has been advocated. In fact 0.8 g/kg/24 hours represents an average protein intake in Europe, although less than American norms.

Management of hyperlipidaemia

Hyperlipidaemia is correlated with proteinuria, and patients with frequent relapses over longer periods, or with persistently poorly controlled proteinuria, are at a high risk of cardiovascular disease (Joven et al., 1990). Lipid control in renal disease is discussed in Chapter 102. It has been established that lipid-lowering therapy is safe in patients with chronic kidney disease in general. There is not good evidence specifically in nephrotic syndrome, but cholesterol and LDL are so high in many patients with persistent nephrotic syndrome, and tolerability of therapy seems high, so few nephrologists disagree with prescribing at least HMG-CoA inhibitors (statins) in these circumstances. These are the only current agents that make a substantial impact on the hyperlipidaemia of nephrotic syndrome, at least as a single agent. They reduce total cholesterol and LDL levels by 20–45% (Massy et al., 1995).

Other anti-lipidaemic drugs such as fibrates and nicotinic acids can lower triglycerides effectively but their clinical benefits are less certain. Although dietary modification seems good general advice, its role is unproven.

Thrombosis and thrombotic risk

Treatment of thrombosis

Overt thromboembolic events such as pulmonary embolism and deep venous thrombosis are treated the same way as in non-nephrotics; starting with unfractionated or (if renal function is good) low-molecular-weight heparin (Wu et al., 2006) and then warfarin to maintain an international normalized ratio of 2–3. The duration of therapy may be 6–12 months but arguably should be continued as long as the nephrotic state persists. Glassock (2007) describes this as a 'conundrum'. Most of the risk of nephrotic syndrome is concentrated around the time of diagnosis (see 'Complications' above). The risks of anticoagulation are increased in patients with renal disease and there is a clinical impression that this may be particularly true in those with nephrotic syndrome. Warfarin is bound to albumin, the concentration of which may change (Ganeval et al., 1986), and there may be other interfering factors.

Higher doses of heparin may be required as it activates antithrombin III, the concentration of which may be diminished in nephrotic patients (Kerlin et al., 2012), although the complex effects on other proteins are likely to interact too.

Asymptomatic renal vein or other thrombosis found incidentally is usually treated with anticoagulation, although there are no controlled randomized data available to support this approach.

Bilateral acute renal vein thrombosis is treated with standard anticoagulation. For severe cases local thrombolytic therapy rather than systemic fibrinolytic therapy may be used, given the high risk of bleeding with the latter. Thrombectomy may be considered with local therapy.

Management of long-term thrombotic risk

There is agreement on the importance of usual prophylactic measures in patients at temporary extra risk, for instance, in hospital, but views on full prophylactic anticoagulation vary widely. Data is lacking to support routine anticoagulation of all nephrotics with low serum proteins. Patients at greatest risk are within 6 months of diagnosis, and have the most severe nephrotic syndrome. Several studies observe that patients with membranous nephropathy are at higher risk. After this early period, risk remains increased, and may be of the order of 1% per annum, approximately eight times higher than the rate in matched controls (Mahmoodi et al., 2008, Lionaki et al., 2012). Very few of these late events are lethal.

Lee et al. (2014) applied a decision analysis strategy to Lionaki et al.'s (2012) series of 898 patients from two North American centres with membranous nephropathy. The model assumed no extra risk for anticoagulation in nephrotic patients, and could have overestimated thrombosis rate as a constant level of risk was assumed was assumed without adjustment for peak onset at the time of diagnosis and lower rate thereafter. With these assumptions it suggested that for patients at low risk of bleeding, benefits seemed certain for those with albumin < 20 g/L. For those at intermediate bleeding risk and albumin < 20 g/L benefits were lower, but still positive.

It is not possible to recommend a universal approach. The risks of thrombosis must be balanced with the bleeding risks of anticoagulation, which may be increased in this group. Individual decisions are appropriate.

Statins have been associated with reduced thrombotic risk in the general population, and a retrospective study in nephrotic syndrome (Resh et al., 2011) provided equivocal evidence for such an effect.

Management of infection risk

Prompt induction of remission of oedema or proteinuria are the most important goals and the decline in death rate from infection in nephrotic children is probably mainly the result of this, and the availability of effective antibiotics.

The need for supplementary corticosteroids in those taking these drugs, or in those who have recently stopped them, should be remembered. Any severe infection should prompt discontinuation of cytotoxic therapy.

Immunization against pneumococci is recommended. There is a high rate of seroconversion even in children taking high-dose prednisolone (Ulinski et al., 2008). The use of prophylactic penicillin or intravenous immunoglobulin administration is not supported by evidence (Wu et al., 2012) and most guidance does not recommend either of these.

In nephrotic children with active disease, varicella/chicken pox is a threat. Those taking high-dose corticosteroids or other immunosuppressive agents within the previous 3 months are at risk of severe progressive disseminated disease. Prophylactic therapy should be given to any non-immune contacts of cases. Where an effective varicella vaccine is available it should be administered to non-immune patients. It is a live vaccine so cannot be given during high-dose steroid or immunosuppressive therapy, but it has been shown to be safe to administer to children in a study that accepted those taking up to 2 mg/kg prednisone (maximum 40 mg) on alternate days (Furth et al., 2003).

Loss of hormones, vitamins, and other molecules

A number of plasma proteins important in the transport of metals, hormones, and drugs are of relatively small molecular weight and thus are lost easily into the urine of nephrotic patients. Free protein hormones, especially of low molecular weight, are also lost. Many of these have been studied, but remarkably few have substantial clinical impact.

A number of abnormalities of calcium and vitamin D metabolism have been described, in part the result of losses of vitamin D binding protein (molecular weight 59 kDa) and its associated vitamin in the urine (Vaziri, 1993; Harris and Ismail, 1994). Nephrotics with reduced renal function do more readily develop bone disease (Tessitore et al., 1984), and earlier treatment than usual with vitamin D may have a place.

A Fanconi syndrome has been described in a small number of nephrotic patients. Some of these had reversible tubular defects suggesting tubular damage from proteinuria (Shioji et al., 1974).

Proteinuria and progression of renal failure

This is discussed in Chapter 50 and Chapter 136.

References

Andrew, M. and Brooker, L. A. (1996). Hemostatic complications in renal disorders of the young. *Pediatr Nephrol*, 10, 88–99.

Bellomo, R. and Atkins, R. C. (1993). Membranous nephropathy and thromboembolism: is prophylactic anticoagulation warranted? *Nephron*, 63, 249–54.

Boyer, O., Moulder, J. K., and Somers, M. J. (2007). Focal and segmental glomerulosclerosis in children: a longitudinal assessment. *Pediatr Nephrol*, 22, 1159–66.

Cameron, J.S. (1984). Coagulation and thromboembolic complications in the nephrotic syndrome. *Adv Nephrol Necker Hosp*, 13, 75–114.

Cameron, J. S. and Hicks, J. A. (2002). The origins and development of the concept of a 'nephrotic syndrome'. *Am J Nephrol*, 22, 240–7.

Cameron, J. S., Ogg, C. S., and Wass, V. J. (1988). Complications of the nephrotic syndrome. In J. S. Cameron and R. J. Glassock (eds.) *The Nephrotic Syndrome*, pp. 849–920. New York: Marcel Dekker.

Crew, R. J., Radhakrishnan, J., and Appel, G. (2004). Complications of the nephrotic syndrome and their treatment. *Clin Nephrol*, 62, 245–59.

Deshpande, P. V. and Griffiths, M. (2005). Pulmonary thrombosis in steroid-sensitive nephrotic syndrome. *Pediatr Nephrol*, 20, 665–9.

De Wardener, H. E. (1958). *The Kidney* (1st ed.). London: Churchill.

Eddy, A. A. and Symons, J. M. (2003). Nephrotic syndrome in childhood. *Lancet*, 362, 629–39.

El Bakkali, L., Rodrigues Pereira, R., Kuik, D. J., *et al.* (2011). Nephrotic syndrome in The Netherlands: a population-based cohort study and a review of the literature. *Pediatr Nephrol*, 26, 1241.

Fliser, D., Zurbrüggen, I., Mutschler, E., *et al.* (1999). Coadministration of albumin and furosemide in patients with the nephrotic syndrome. *Kidney Int*, 55, 629–34.

Furth, S. L., Arbus, G. S., Hogg, R., *et al.* (2003). Varicella vaccination in children with nephrotic syndrome: a report of the Southwest Pediatric Nephrology Study Group. *J Pediatr*, 142, 145–8.

Ganeval, D., Fischer, A. M., Barre, J., *et al.* (1986). Pharmacokinetics of warfarin in the nephrotic syndrome and effect on vitamin K dependent clotting factors. *Clin Nephrol*, 25, 75–80.

Geers, A. B., Koomans, H. A., Roos, J. C., *et al.* (1985). Preservation of blood volume during edema removal in nephrotic subjects. *Kidney Int*, 28, 652–7.

Glassock, R. J. (2007). Prophylactic anticoagulation in nephrotic syndrome: a clinical conundrum. *J Am Soc Nephrol*, 18, 2221–5.

Glassock, R. J., Fervenza, F. C., Hebert, L., *et al.* (2015). Nephrotic syndrome redux, a clinical perspective. *Nephrol Dial Transplant*, 30, 12–17.

Guarneiri, G. F., Toigo, G., Situlin, R., *et al.* (1989). Nutritional status in patients on long-term low-protein diet or with nephrotic syndrome. *Kidney Int*, 36 (Suppl. 27), S195–200.

Haas, M., Meehan, S. M., Karrison, T. G., *et al.* (1997). Changing etiologies of unexplained adult nephrotic syndrome: a comparison of renal biopsy findings from 1976–1979 and 1995–1997. *Am J Kidney Dis*, 30, 621–31.

Harris, R. C. and Ismail, N. (1994). Extra renal complications of the nephrotic syndrome. *Am J Kidney Dis*, 23, 477–497.

Hofstra, J. M., Beck, L. H., Jr., Beck, D. M., *et al.* (2011). Anti-phospholipase A2 receptor antibodies correlate with clinical status in idiopathic membranous nephropathy. *Clin J Am Soc Nephrol*, 6, 1286–91.

Howard, A. D., Moore, J., Jr., Gouge, S. F., *et al.* (1990). Routine serologic tests in the differential diagnosis of the adult nephrotic syndrome. *Am J Kidney Dis*, 15, 24–30.

International Study of Kidney Disease in Children (1978). Nephrotic syndrome in children: prediction of histopathology from clinical and laboratory characteristics at time of diagnosis. A report of the International Study of Kidney Disease in Children. *Kidney Int*, 13, 159–65.

International Study of Kidney disease in Children (1984). Minimal change nephrotic syndrome in children: deaths during the first 5 to 15 years' observation. Report of the International Study of Kidney Disease in Children. *Pediatrics*, 73(4), 497–501.

Joven, J., Villabona, C., Vilella, E., *et al.* (1990). Abnormalities of lipoprotein metabolism in patients with the nephrotic syndrome. *N Engl J Med*, 323, 579–84.

Kazi, J. I. and Mubarak, M. (2007). Pattern of glomerulonephritides in adult nephrotic patients—report from SIUT. *J Pak Med Assoc*, 57, 574.

Kerlin, B. A., Ayoob, R., and Smoyer, W. E. (2012) Epidemiology and pathophysiology of nephrotic syndrome-associated thromboembolic disease. *Clin J Am Soc Nephrol*, 7, 513–20.

Koomans, H. A. (2001). Pathophysiology of acute renal failure in idiopathic nephrotic syndrome. *Nephrol Dial Transplant*, 16, 221–4.

Korbet, S. M., Genchi, R. M., Borok, R. Z., *et al.* (1996). The racial prevalence of glomerular lesions in nephrotic adults. *Am J Kidney Dis*, 27, 647–51.

Krensky, A. M., Ingelfinger, J. R., and Grupe, W. E. (1982). Peritonitis in childhood nephrotic syndrome: 1970–1980. *Am J Dis Child*, 136, 732–6.

Kumar, J., Gulati, S., Sharma, A. P., *et al.* (2003). Histopathological spectrum of childhood nephrotic syndrome in Indian children. *Pediatr Nephrol*, 18, 657–60.

Lionaki, S., Derebail, V. K., Hogan, S. L., *et al.* (2012). Venous thromboembolism in patients with membranous nephropathy. *Clin J Am Soc Nephrol*, 7, 43–51.

Lowenstein, J., Schacht, R. G., and Baldwin, D. S. (1970). Renal failure in minimal change nephrotic syndrome. *Am J Med*, 70, 227–33.

Mahmoodi, B. K., ten Kate, M. K., Waanders, F., *et al.* (2008). High absolute risks and predictors of venous and arterial thromboembolic events in patients with nephrotic syndrome: results from a large retrospective cohort study. *Circulation*, 117, 224–30.

Massy, Z. A., Ma, J. Z, Louis, T. A., *et al.* (1995). Lipid-lowering therapy in patients with renal disease. *Kidney Int*, 48, 188–98.

Kaysen, G. A. (1991). Hyperlipidemia of the nephrotic syndrome. *Kidney Int*, 39 (Suppl. 31), S8–15.

Lee, T., Biddle, A. K., and Lionaki, S. (2014). Personalized prophylactic anticoagulation decision analysis in patients with membranous nephropathy. *Kidney Int*, 85(6), 1412–20.

Llach, F. (1982). Nephrotic syndrome: hypercoagulability, renal vein thrombosis and other thromboembolic complications. In B. M. Brenner and J. H. Stein (eds.) *The Nephrotic Syndrome*, pp. 121–44. New York: Churchill- Livingstone.

Mansy, H., Goodship, T. H., Tapson, J. S., *et al.* (1989). Effect of a high protein diet in patients with the nephrotic syndrome. *Clin Sci*, 77, 445–51.

Niaudet, P. (2004). Steroid-sensitive idiopathic nephrotic syndrome in children. In E. Avner, W. Harmon, and P. Niaudet (eds.) *Pediatric Nephrology* (5th ed.), pp. 557–73. Philadelphia, PA: Lippincott, Williams & Wilkins.

Ozkaya, N., Cakar, N., Ekim, M., *et al.* (2004). Primary nephrotic syndrome during childhood in Turkey. *Pediatr Int*, 46, 436–8.

Pakasa, N. M. and Sumaili, E. K. (2009). The nephrotic syndrome in the Democratic Republic of Congo. *N Engl J Med*, 354, 1085–6.

Resh, M., Mahmoodi, B. K., Navis, G. J., *et al.* (2011). Statin use in patients with nephrotic syndrome is associated with a lower risk of venous thromboembolism. *Thromb Res*, 127, 395–9.

Rostoker, G., Texier, J. P., Jeandel, B., *et al.* (1992). Asymptomatic renal-vein thrombosis in adult nephrotic syndrome. Ultrasonography and urinary fibrin–fibrinogen degradation products: a prospective study. *Eur J Med*, 1, 19–22.

Schrier, R. W. and Fassett, R. G. (1998). A critique of the overfill hypothesis of sodium and water retention in the nephrotic syndrome. *Kidney Int*, 53, 1111–17.

Shearer, G. C., Stevenson, F. T., Atkinson, D. N., *et al.* (2001). Hypoalbuminemia and proteinuria contribute separately to reduced lipoprotein catabolism in the nephrotic syndrome. *Kidney Int*, 59, 179–89.

Shioji, R., Sasaki, Y., Saito, H., *et al.* (1974). Reversible tubular dysfunction associated with chronic renal failure in an adult patient with the nephrotic syndrome. *Clin Nephrol*, 2, 76–80.

Singhal, R. and Brimble, K. S. (2006). Thromboembolic complications in the nephrotic syndrome: pathophysiology and clinical management. *Thromb Res*, 118, 397–407.

Smith, J. D. and Hayslett, J. P. (1992). Reversible renal failure in the nephrotic syndrome. *Am J Kidney Dis*, 19, 201–3.

Tessitore, N., Bonucci, E., D'Angelo, A., *et al.* (1984). Bone histology and calcium metabolism in patients with nephrotic syndrome and normal or reduced renal function. *Nephron*, 37, 153–9.

Turner, A. N. (2010). *Dropsy, Nephrosis, Nephrotic Syndrome*. [Online] <http://historyofnephrology.blogspot.co.uk/2010/08/dropsy-nephrosis-nephrotic-syndrome.html>

Ulinski, T., Leroy, S., Dubrel, M., *et al.* (2008). High serological response to pneumococcal vaccine in nephrotic children at disease onset on high-dose prednisone. *Pediatr Nephrol*, 23, 1107–13.

Uncu, N., Bülbül, M., Yildiz, N., *et al.* (2010). Primary peritonitis in children with nephrotic syndrome: results of a 5-year multicenter study. *Eur J Pediatr*, 169, 73–6.

Vande Walle, J. G., Donckerwolcke, R. A., van Isselt, J. W., *et al.* (1995). Volume regulation in children with early relapse of minimal-change nephrosis with or without hypovolaemic symptoms. *Lancet*, 346, 148–52.

Vande Walle, J. G., Mauel, R., Raes, A., *et al.* (2004). ARF in children with minimal change nephrotic syndrome may be related to functional changes of the glomerular basal membrane. *Am J Kidney Dis*, 43, 399–404.

Vaziri, N. D. (1993). Endocrinological consequences of the nephrotic syndrome. *Am J Nephrol*, 13, 360–4.

Venkataseshan, V. S., Faraggiana, T., Grishman, E., *et al.* (1993). Renal failure due to tubular obstruction by large protein casts in patients with massive proteinuria. *Clin Nephrol*, 39, 321–6.

Waldman, M., Crew, R. J., Valeri, A., *et al.* (2007). Adult minimal-change disease: clinical characteristics, treatment, and outcomes. *Clin J Am Soc Nephrol*, 2, 445–53.

Wilcox, C. S. (2002). New insights into diuretic use in patients with chronic renal disease. *J Am Soc Nephrol*, 13, 798–805.

Wong, W. (2007). Idiopathic nephrotic syndrome in New Zealand children, demographic, clinical features, initial management and outcome after twelve-month follow-up: results of a three-year national surveillance study. *J Paediatr Child Health*, 43(5), 337–41.

Wu, C. H., Ko, S. F., Lee, C. H., *et al.* (2006). Successful outpatient treatment of renal vein thrombosis by low-molecular weight heparins in 3 patients with nephrotic syndrome. *Clin Nephrol*, 65, 433–40.

Wu, H. M., Tang, J. -L., Cao, L., *et al.* (2012). Interventions for preventing infection in nephrotic syndrome. *Cochrane Database Syst Rev*, 4, CD003964.

CHAPTER 53

Pathophysiology of oedema in nephrotic syndrome

Neil Turner and Premil Rajakrishna

Introduction

For many decades it was taught that the drop in colloid osmotic pressure was the primary driver to sodium retention in nephrotic syndrome. This was presumed to lead to fluid egress into the interstitial space, and thus to a reduction of intravascular fluid volume—the 'underfill' hypothesis. Problems with this model have been highlighted over a long period. Notably there is not consistent evidence that the circulation is actually underfilled. The contrary 'overfill' hypothesis proposes that sodium retention is the primary problem. A major problem with this hypothesis was lack of a mechanism to explain it. There is now a plausible mechanism, but it does not on its own fully explain findings in nephrotic syndrome.

Key observations on sodium retention

Evidence for a primary renal origin of sodium retention has been accumulating for over three decades, until in 2008/2009 evidence for a convincing mechanism was presented. Key points are summarized here. For more detailed bibliography see comprehensive reviews by Siddall and Radhakrishnan (2012) and Svenningsen et al. (2013).

Renal sodium retention is a local consequence of proteinuria

A landmark finding was made by Ichikawa et al. (1983) when they created a unilateral nephrotic lesion in a rat by treating a single kidney with puromycin aminonucleoside (PAN). PAN produces a podocyte injury mimicking focal segmental glomerulosclerosis (FSGS) (see Chapter 45), including a severe nephrotic syndrome followed later by loss of glomerular filtration rate. In their experiments the kidney with proteinuria also retained sodium, whereas the undamaged kidney handled both protein and sodium normally. They went on to show by *in vivo* micropuncture studies that the location of increased sodium reabsorption was distal to the distal convoluted tubule. This suggested that the mechanism of sodium retention was local, not related to circulating hormones, intimately related to proteinuria, and that the abnormality was probably located in the collecting duct. A number of subsequent experimental studies using different rodent models of nephrotic syndrome (e.g. Adriamycin® (doxorubicin) in mice, mercuric chloride in rats) confirmed the localization of sodium retention to the collecting duct.

ENaC is overactive in nephrotic syndrome

The epithelial sodium channel (ENaC), the amiloride-sensitive sodium channel, is the major route by which sodium is reabsorbed in the collecting duct. In animal models, amiloride inhibited sodium retention in nephrotic syndrome, but mRNA levels for the channel were not affected suggesting that the overactivity was not due to increased production. In fact the mRNA levels of other sodium transporters in the nephron (NHE3, Na/K-ATPase, NCC, NKCC2; see Chapter 21) tended to be reduced, a fact which might help to explain diuretic resistance in nephrotic syndrome (summarized by Svenningsen et al., 2013, 2015).

ENaC activity is increased by proteolytic activation

ENaC activity may be controlled by moving preformed channel molecules to the apical cell membrane, a process influenced by aldosterone and vasopressin; or by proteolytic activation of the channel which increases the proportion of time it spends in the 'open' configuration. Proteolytic activation occurs in the Golgi apparatus intracellularly, but it emerged that serine proteases such as plasmin could activate the channels on the cell surface, and that plasmin activity could be found in nephrotic urine. Nephrotic urine was shown to contain urokinase-type plasminogen activator which could explain the activation of filtered plasminogen (Passero et al., 2008; Svenningsen et al., 2009).

Sodium retention commences before serum albumin drops

This explanation helps to explain the sequence of events long observed in acute nephrotic syndrome (Koomans, 2003; Siddall and Radhakrishnan, 2012), which is (1) proteinuria, (2) sodium retention, then (3) serum protein levels fall. In other words, sodium retention commences before albumin levels have changed substantially. When remission occurs, the abnormalities switch off in the same sequence, sodium excretion rising before serum albumin levels have shown a rise (Fig. 53.1).

Activation of ENaC alone does not replicate nephrotic syndrome

Patients with Liddle syndrome (see Chapter 21) have mutations leading to constitutive activation of ENaC. They retain sodium but become hypertensive, not oedematous. Additional hypotheses to

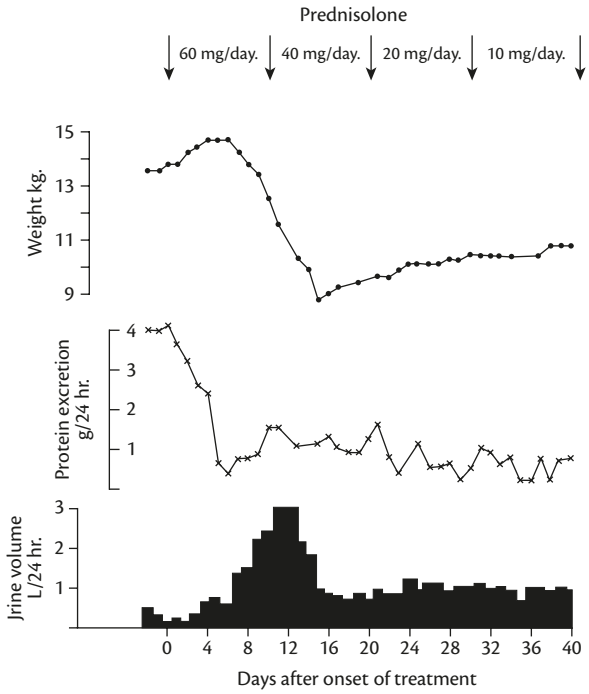

Fig. 53.1 Treatment of nephrotic syndrome in a child in the 1950s, before effective diuretics were available and soon after the discovery of the effect of corticosteroids in childhood nephrotic syndrome. Weight falls from 15 to 10 kg over about a week as the protein leak responds to steroid therapy. Note that the spontaneous diuresis commences as soon as the level of proteinuria drops below 1 g and before albumin levels could have recovered.
From DeWardener (1958), with permission.

explain this at present centre on capillary permeability or deranged osmotic forces (see below).

Inhibition of ENaC as a therapeutic strategy

The effect of amiloride alone in human proteinuric conditions has not been the subject of much published research, although it is known to potentiate the action of loop diuretics. Interestingly it may also have inhibitory effects on urokinase-like plasminogen activator (Svenningsen et al., 2013). But there may be serine protease inhibitors which could inhibit plasmin production in nephrotic urine, and that will be a fascinating drug target.

Key observations on the circulation

According to the 'underfill' hypothesis, reduced circulatory volume is a drive to sodium retention. Circulatory volume can be low but this is not a general feature of the syndrome; indeed, hypertension can be a feature. This begs the question, why is circulating volume not low, surely Starling forces will lead to egress of fluid into the interstitial space if serum proteins are decreased?

Interstitial colloid osmotic pressure is also low

For fluid to leave the circulation by osmotic pressure in nephrotic syndrome, the osmotic gradient between circulation and the interstitium must change. However observations in animal models and in man suggest that interstitial colloid osmotic pressure (COP) falls in parallel with the fall in serum COP, preserving the gradient. Interstitial albumin concentrations fall virtually to zero if serum albumin falls very low (Koomans, 2003; Siddall and Radhakrishnan, 2012).

However, there is a limit to how low interstitial COP can go, so that in very severe nephrotic syndrome, there may indeed be an adverse gradient, and fluid may be lost from the circulation by this mechanism. But in mild and moderate nephrotic syndrome, oedema occurs without reduced circulating volume. The homeostatic mechanisms maintaining the COP gradient are not fully understood.

There is a second exception to this. If serum protein concentration falls very rapidly, there is a period of increased movement of fluid into the interstitial space, until homeostatic mechanisms have restored the COP gradient. This is most likely in the very acute, very severe proteinuria that can occur in minimal change disease.

Blood volume is not generally low in nephrotic syndrome

Neither direct measurements of blood volume, nor levels of renin, aldosterone, or other hormones suggest that hypovolaemia is a general feature of nephrotic syndrome. When hypovolaemia is identified it is in patients with the most severe hypoproteinaemia. Modest hypervolaemia is a common finding. Blood pressure is normal or increased and tends to fall after steroid-induced recovery in minimal change disease in both adults and children (Koomans, 2003). These observations may help to explain why albumin infusions do not generally improve natriuresis substantially in patients with nephrotic syndrome (Fliser et al., 1999; see Chapter 52).

But blood volume can be low in some circumstances

The above paragraphs pointed out that low circulating volume can occur in patients with very severe nephrotic syndrome, particularly if it is of recent and rapid onset. This combination is most frequently found in minimal change disease, and matches the clinical observation that hypovolaemia is clinically most likely to be encountered in this group. It is most likely to cause acute kidney injury in adult patients with minimal change disease (see Chapter 52). The important implication of these observations are that individual patients must be assessed individually, a point made long ago (Schrier and Fassett, 1998).

Extracellular fluid distributes differently in nephrotic syndrome

Koomans et al. (1986) made a revealing comparison between patients with nephrotic syndrome and patients with chronic kidney disease (CKD) who each had extracellular fluid (ECF) volume expansion. Those with CKD had hypertension and increased blood volume with ECF volume about double normal. Those with nephrotic syndrome had greater (threefold) expansion of ECF volume but did not have increased blood pressure. So both groups had ECF volume expansion, but the fluid tended to be distributed more towards the intravascular compartment in patients with CKD, versus the interstitial compartment in patients with nephrotic syndrome. This explains how patients with nephrotic syndrome can tolerate greater ECF accumulation than patients with reduced cardiac or renal function.

Vascular permeability

A generalized abnormality of vascular permeability is one of the explanations that could help to explain the differences between nephrotic syndrome and other causes of increased extracellular fluid volume. There are some observations to support this (Rostoker et al., 2000; Siddall and Radhakrishnan, 2012).

Other abnormalities

Atrial natriuretic peptide resistance is reported in nephrotic syndrome, and levels are often low or normal. Arginine vasopressin (antidiuretic hormone) levels are high and may contribute to water retention. Increased sympathetic activity is described in animal models, but these tend to create severe nephrotic syndrome.

Mechanisms in specific diseases

There is no reason to think that the mechanism of oedema is different in different diseases that cause nephrotic syndrome. In the case of FSGS there is some evidence for a circulating factor affecting glomerular permeability (though it may simply be a podocyte-toxic factor) (Chapter 57). It has been suggested that this factor might also affect permeability of other membranes, for example, leading to increased protein loss through peritoneal dialysis.

Comparison with other causes of oedema

Chapter 30 discusses and compares oedema in other contexts.

References

De Wardener, H. E. (1958). *The Kidney* (1st ed.). London: Churchill.

Fliser, D., Zurbrüggen, I., Mutschler, E., *et al.* (1999). Coadministration of albumin and furosemide in patients with the nephrotic syndrome. *Kidney Int*, 55, 629.

Ichikawa, I., Rennke, H. G., Hoyer, J. R., *et al.* (1983). Role for intrarenal mechanisms in the impaired salt excretion of experimental nephrotic syndrome. *J Clin Invest*, 71, 91–103.

Koomans, H. A. (2003). Pathophysiology of oedema in idiopathic nephrotic syndrome. *Nephrol Dial Transplant*, 18 Suppl 6, vi30–2.

Koomans, H. A., Braam, B., Geers, A. B., *et al.* (1986). The importance of plasma protein for blood volume and blood pressure homeostasis. *Kidney Int*, 30, 730–5.

Passero, C. J., Mueller, G. M., Rondon-Berrios, H., *et al.* (2008). Plasmin activates epithelial Na^+ channels by cleaving the gamma sub-unit. *J Biol Chem*, 283, 36586–91.

Rostoker, G., Behar, A., and Lagrue, G. (2000). Vascular hyperpermeability in nephrotic edema. *Nephron*, 85, 194–200.

Schrier, R. W. and Fassett, R. G. (1998). A critique of the overfill hypothesis of sodium and water retention in the nephrotic syndrome. *Kidney Int*, 53, 1111–7.

Siddall, E. C. and Radhakrishnan, J. (2012). The pathophysiology of edema formation in the nephrotic syndrome. *Kidney Int*, 82, 635–42.

Svenningsen, P., Andersen, H., Nielsen, L. H., *et al.* (2015). Urinary serine proteases and activation of EnaC in kidney – implications for physiological renal salt handling and hypertensive disorders with albuminuria. *Pflugers Arch*, 467, 531–42.

Svenningsen, P., Bistrup, C., Friis, U. G., *et al.* (2009). Plasmin in nephrotic urine activates the epithelial sodium channel. *J Am Soc Nephrol*, 20, 299–310.

Svenningsen, P., Friis, U. G., Versland, J. B., *et al.* (2013). Mechanisms of renal NaCl retention in proteinuric disease. *Acta Physiol*, 207, 536–45.

CHAPTER 54

Idiopathic nephrotic syndrome: overview

Patrick Niaudet and Alain Meyrier

Introduction

Idiopathic nephrotic syndrome is defined by the combination of massive proteinuria, hypoalbuminaemia, hyperlipidaemia, and oedema and of non-specific histological abnormalities of the glomeruli. Light microscopy may disclose minimal changes (Chapter 55), diffuse mesangial proliferation, or focal and segmental glomerular sclerosis (FSGS) (Chapter 57). On electron microscopy the glomerular capillaries show a fusion of visceral epithelial cell (podocyte) foot processes and with the exception of some variants no significant deposits of immunoglobulins or complement by immunofluorescence. This excludes other idiopathic glomerulopathies, such as membranous glomerulopathy, that are in some publications lumped together with MCD and FSGS under the umbrella denomination of 'INS'. In a majority of children only minimal changes are seen on light microscopy. These children are referred to as having 'minimal-change disease'. In adults with idiopathic nephrotic syndrome, lesions of FSGS are more frequent.

A question remains: do minimal change disease (MCD) and FSGS represent two facets of the same disease, or distinct pathophysiologic entities?

The unitary view

The unitary view is the most appropriate regarding treatment options. This is especially true of paediatricians. The unitary view is compatible with varied causes and/or pathophysiological mechanisms. Rather than distinguishing FSGS from MCD on a kidney biopsy, the best guide to prognosis and to subsequent response to other drugs is the initial response to glucocorticoids. Patients with FSGS generally suffer a more severe disease, are often resistant to corticosteroids, and are prone to progressing to renal failure. In the early stages FSGS and MCD are histologically indistinguishable. The best illustration of this is the appearance of a kidney biopsy carried out shortly after relapse of nephrotic syndrome following transplantation in a patient whose primary renal disease was FSGS. Despite heavy proteinuria, the glomeruli initially show minimal changes.

A number of patients with FSGS respond to steroids whilst some steroid-resistant patients have no sclerotic changes on adequate biopsies. Therefore, some nephrologists believe that, although histological variants of the idiopathic nephrotic syndrome carry prognostic significance, they cannot at present be considered as separate entities. In fact, considering that corticosteroid (CS) responsiveness has a bearing on the response to other treatments, such as the efficacy of rituximab, one cannot exclude the hypothesis that CS-sensitive as opposed to CS-resistant MCD and FSGS might be due to different pathophysiologic mechanisms.

The pluralistic view

More recent data have tended to reinforce the concept that MCD and FSGS are different entities. MCD appears to be a *functional* podocyte disease whereas FSGS clearly appears to be a *structural* podocytopathy (Kriz et al., 1994; Barisoni et al., 2007) that develops, amongst other subsets, to the cell and the scar variants of the glomerular lesion and less frequently to a highly cellular and shrinking appearance of the glomerular tuft, 'collapsing glomerulopathy'. The notion of 'podocyte dysregulation' (Barisoni et al., 1999; Bariety et al., 2001), the different expression of cyclin-dependent kinase inhibitors in MCD and in FSGS, the role of these cell cycle disturbances leading to podocyte proliferation and maturation (Shankland et al., 2000), are in favour of distinct entities. The finding that intrarenal transcription of cytotoxic T-lymphocyte effectors and transforming growth factor beta 1 is increased in a majority of children with FSGS, contrasting with the rare occurrence of this phenomenon in children with MCD is in favour of the pluralistic view (Strehlau et al., 2002). Moreover the fact that overexpression of interleukin 13 induces minimal change nephropathy and no FSGS in rats (Lai et al., 2007), whereas in the human high circulating levels of the soluble urokinase receptor are found in FSGS but not in MCD (Wei et al., 2011; Jefferson and Alpers, 2013) lend support to interpreting MCD and FSGS as podocytopathies originating from different glomerular permeability factors. On these grounds minimal change disease and focal segmental glomerulosclerosis are covered separately.

Minimal change disease is considered in chapters 55 and 56, FSGS in chapters 57 and 58, and pathogenesis of these conditions in chapter 59. Matters more specifically paediatric as opposed to those related to adults will be as much as possible distinguished, which does not avoid some repetitions.

References

Bariety, J., Bruneval, P., Hill, G., *et al.* (2001). Posttransplantation relapse of FSGS is characterized by glomerular epithelial cell transdifferentiation. *J Am Soc Nephrol*, 12, 261–74.

Barisoni, L., Kriz, W., Mundel, P., *et al.* (1999). The dysregulated podocyte phenotype: a novel concept in the pathogenesis of collapsing idiopathic focal segmental glomerulosclerosis and HIV-associated nephropathy. *J Am Soc Nephrol*, 10, 51–61.

Barisoni, L., Schnaper, H. W., and Kopp, J. B. (2007). A proposed taxonomy for the podocytopathies: a reassessment of the primary nephrotic diseases. *Clin J Am Soc Nephrol*, 2, 529–42.

Jefferson, J. A. and Alpers, C. E. (2013). Glomerular disease: 'suPAR'-exciting times for FSGS. *Nat Rev Nephrol*, 9, 127–8.

Kriz, W., Elger, M., Nagata, M., *et al.* (1994). The role of podocytes in the development of glomerular sclerosis. *Kidney Int Suppl*, 45, S64–72.

Lai, K. W., Wei, C. L., Tan, L. K., *et al.* (2007). Overexpression of interleukin-13 induces minimal-change-like nephropathy in rats. *J Am Soc Nephrol*, 18, 1476–85.

Shankland, S. J., Eitner, F., Hudkins, K. L., *et al.* (2000). Differential expression of cyclin-dependent kinase inhibitors in human glomerular disease: role in podocyte proliferation and maturation. *Kidney Int*, 58, 674–83.

Strehlau, J., Schachter, A. D., Pavlakis, M., *et al.* (2002). Activated intrarenal transcription of CTL-effectors and TGF-beta1 in children with focal segmental glomerulosclerosis. *Kidney Int*, 61, 90–5.

Wei, C., El Hindi, S., Li, J., *et al.* (2011). Circulating urokinase receptor as a cause of focal segmental glomerulosclerosis. *Nat Med*, 17, 952–60.

CHAPTER 55

Minimal change disease: clinical features and diagnosis

Patrick Niaudet and Alain Meyrier

Epidemiology

The incidence of idiopathic nephrotic syndrome varies with age, race, and geography. The annual incidence in children in the United States has been estimated to be 2–2.7/100,000 (McEnery and Strife, 1982) with a cumulative prevalence of 16/100,000. In the United Kingdom, the incidence of idiopathic nephrotic syndrome is sixfold greater in Asian than in European children (Sharples et al., 1985). Whereas idiopathic nephrotic syndrome accounts for only 25% of adult cases (Cameron et al., 1974b), it is by far the most common cause of nephrotic syndrome in children. The International Study of Kidney Disease in Children found minimal change disease (MCD) in 76.6% of children with primary nephrotic syndrome, with a male/female ratio of 2/1 (International Study of Kidney Disease in Children, 1978).

Conditions with a possible aetiologic role

Many factors are commonly cited as possible 'causes' or temporally associated conditions for MCD (Glassock, 2003). They include viral diseases, allergies, drugs, vaccinations, and some malignancies. The prevalence of positive Epstein–Barr virus DNA detection and recent infection or reactivation is higher in children at onset of idiopathic nephrotic syndrome compared to a population matched for age, gender, and time of sampling (Dossier et al., 2014).

It is not easy to understand what final common pathway permits these differing factors to result in the common clinical and pathological outcome of MCD, or how this relates to pathogenesis as outlined below.

Allergy

Allergy is associated with up to 30% of cases, which suggests some involvement of type IV reactions in the pathogenesis of MCD. An allergic episode is often followed by a relapse of the nephrotic syndrome.

Amongst a list of anecdotal cases, the allergens reported include fungi, poison ivy, ragweed and timothy grass pollen, house dust, medusa stings, bee stings, and cat fur.

A food allergen may be responsible for relapsing episodes of steroid-sensitive nephrosis, such as cows' milk and eggs. Laurent et al. evaluated the effect of an oligoantigenic diet given for 10–15 days to 13 patients with an unsatisfactory response to corticosteroids (Laurent et al., 1987). This diet coincided with an improvement of proteinuria in nine, including complete remission in five.

Drugs

A number of drugs may induce a nephrotic syndrome with the histopathological appearance of MCD. The list (see Chapter 82) comprises unrelated drugs such as non-steroidal anti-inflammatory agents, including sulfasalazine and mesalazine, D-penicillamine, lithium, rifampicin, heavy metals (gold, mercury), and trimethadione.

Malignancy

The association with malignancies mainly concerns lymphomatous disorders and rarely solid tumours.

MCD has been associated with haematologic malignancies, such as Hodgkin lymphoma, non-Hodgkin lymphoma, and leukaemia (Alpers and Cotran, 1986; Dabbs et al., 1986; Audard et al., 2006). MCD has been reported in 0.4% of patients with Hodgkin lymphoma (Dabbs et al., 1986). Audard et al. reported that MCD appeared prior to the discovery of the lymphoma in 8 out of 21 patients (Audard et al., 2006). In such cases, MCD was either steroid dependent or steroid resistant. The haemopathy is already apparent at the time of onset of MCD or is diagnosed simultaneously in the other cases. In most but not all patients, remission of proteinuria is obtained with the cure of lymphoma.

Solid tumours are more commonly associated with membranous nephropathy (Alpers and Cotran, 1986). However, some patients with solid tumours may develop MCD. These include thymoma, renal cell carcinoma, mesothelioma, and bronchogenic, colon, bladder, lung, breast, pancreatic, duodenal, and prostate cancer (Meyrier et al., 1992; Auguet et al., 1998; Glassock, 2003). Removal of the tumour may be followed by remission of the nephrotic syndrome.

Inheritance

The familial occurrence of MCD is well known. White found that 3.3% of 1877 patients with idiopathic nephrotic syndrome had affected family members mainly siblings (White, 1973). This inherited trend is different from the genetic forms of focal segmental glomerulosclerosis (FSGS) that are described in Chapter 327.

Histocompatibility antigens

A three- to fourfold increased incidence of human leucocyte antigen (HLA)-DR7 in nephrotic children has been reported (Alfiler et al., 1980; de Mouzon-Cambon et al., 1981). Clark et al. found a strong association between HLA-DR7 and the *DQB1* gene of HLA-DQW2 and steroid-sensitive nephrosis, and suggested that DR7 and

DQW2 contribute to disease susceptibility (Clark et al., 1990). HLA-DR3 has been associated with steroid-resistant nephrosis in children, with a relative risk of 3. The incidence of HLA-DR3-DR7 is increased in steroid-resistant patients with a relative risk of 9.3. An association with HLA-B8 was reported in Europe. Children with atopy and HLA-B12 have a 13-fold increased risk of developing nephrosis.

Clinical features in children

The disease is rarely discovered on routine urine analysis, and oedema is the most frequent presenting symptom (see Chapter 52). The onset is usually rapid and even 'explosive'. Oedema increases gradually and becomes clinically detectable when fluid retention exceeds 3–5% of body weight. It is often initially apparent around the eyes and can be misdiagnosed as allergy. During the day, periorbital oedema decreases whilst it localizes at the lower extremities. In the reclining position, it localizes on the back. It is white, soft, and pitting. Oedema of the scrotum and penis, or labia, may also be observed. Anasarca (severe generalized oedema) may develop. Abdominal pain may result from ascites, severe hypovolaemia, peritonitis, pancreatitis, thrombosis, or steroid-induced gastritis. Shock with abdominal pain and peripheral circulatory failure may follow a sudden fall of plasma albumin and requires emergency treatment. Blood pressure is usually normal, but sometimes elevated. The disease may also be revealed by a complication. Peritonitis due to *Streptococcus pneumoniae* is a classical mode of onset. Other infections include meningitis, cellulitis, and pneumonia. Deep vein or arterial thromboses and pulmonary embolism may also occur during the first attack or during a relapse (see Chapter 52).

Clinical features in adults

The clinical picture in adults is similarly characterized by generalized oedema of sudden onset. Contrary to FSGS in which proteinuria apparently precedes oedema for a period of time of uncertain duration (with the exception of the 'tip lesion' variant (Stokes et al., 2004)), oedema sets in in a matter of days. However, hypovolaemic shock and abdominal pain are quite unusual. Blood pressure is moderately elevated in about half of the cases.

Box 55.1 Definitions with regard to the nephrotic syndrome and its response to treatment

- Nephrotic range proteinuria:
  - Adults: proteinuria > 3.5 g per day
  - Children: > 40 mg/m^2 per hour; urinary protein:creatinine ratio > 2 mg/mg or > 200 mg/mmol
- Complete remission:
  - Adults: proteinuria < 0.3–1.0 g per day, normal serum albumin (> 30 g/L), and stable renal function
  - Children: urinary protein:creatinine ratio < 0.2–0.3 mg/mg or < 30 mg/mmol and normal serum albumin (> 30 g/L)
- Partial remission:
  - Adults: proteinuria 0.3–3.5 g per day and/or ≥ 50% decrease in proteinuria from baseline, and stable renal function
  - Children: urinary protein:creatinine ratio 0.2–2.0 mg/mg or 30–350 mg/mmol; and serum albumin > 30 g/L
- Steroid-dependent nephrotic syndrome:
  - Two consecutive relapses whilst receiving predniso(lo)ne on alternate days, or within 15 days of its discontinuation
- Steroid-resistant nephrotic syndrome:
  - Children: lack of remission despite 4–8 weeks of therapy with daily predniso(lo)ne at a dose of 60 mg/m^2 or 2 mg/kg (maximum 60 mg) per day
  - Adults: lack of remission despite 4 months of therapy with daily prednisone at a dose of 1 mg/kg/day (maximum 80 mg/day)
- Calcineurin-inhibitor (CNI) dependent nephrotic syndrome:
  - Remission of steroid-dependent nephrotic syndrome is achieved during therapy with CNIs (ciclosporin or tacrolimus)
- CNI-resistant and steroid-resistant nephrotic syndrome:
  - No response to therapy with predniso(lo)ne as defined above, or to CNI therapy.

Laboratory abnormalities

Urine analysis

Nephrotic-range proteinuria is defined as > 50 mg/kg/day or 40 mg/hour/ m^2 in children and > 3.5 g/24 hours in adults (see Chapter 50). In children, the urinary protein:creatinine ratio or urinary albumin:creatinine ratio are useful (Box 55.1). For these two indices, the nephrotic range is 200–400 mg/mmol.

In minimal change, steroid-sensitive nephrotic syndrome, proteinuria consists mainly of albumin and low-molecular-weight proteins, whilst in severe nephrotic syndrome with glomerular lesions and steroid resistance the urine also contains globulins. This can be quantified by means of the selectivity index, that is, the ratio of immunoglobulin (Ig)-G to albumin or transferrin clearance (see Chapter 52). A favourable index would be < 0.05; a poor index > 0.15–0.20. There is a considerable overlap in results and the test has a limited value, especially in adults, and is rarely performed. Some children with severe steroid-resistant nephrotic syndrome have both glomerular and tubular proteinuria. Macroscopic haematuria is rare, occurring in 1% of steroid responders and in 3% of non-responders. Persistent microscopic haematuria is more common, and may be observed in up to 30% of patients, with no particular histopathologic or prognostic significance.

Blood chemistries

Serum proteins are markedly reduced, to < 50 g/L. Albumin concentration is usually < 20 g/L and may be < 10 g/L. Electrophoresis of plasma proteins shows a typical pattern with low albumin, increased alpha-2 globulins and, to a lesser extent, beta globulins whilst gamma globulins are decreased. IgG is considerably decreased, IgA slightly, and IgM is increased. Amongst other proteins, fibrinogen, and lipoproteins are increased, whilst small molecules such as antithrombin III are lost in the urine and their concentration in plasma is decreased. A detailed analysis of the

hyperlipidaemia of nephrotic syndrome and other complications is to be found in Chapter 52.

Serum electrolytes are usually within the normal range. Low plasma sodium may be related to dilution from inappropriate renal water retention. Mild hyponatraemia may be an artefact related to hyperlipidaemia. Serum calcium is low as a result of hypoalbuminaemia. Ionized calcium may be decreased in persistent nephrotic syndrome, due to urinary loss of 25-hydroxyvitamin D_3. Blood urea and serum creatinine are often within the normal range, or increased in relation to functional renal insufficiency.

Haematology

Haemoglobin and the haematocrit may be increased in patients with a reduced plasma volume. Microcytic anaemia may be observed in chronic, steroid-resistant nephrotic syndrome, in some cases following urinary loss of transferrin (see Chapter 57). Thrombocytosis is common.

Hypercoagulability

Hypercoagulability is a common feature of all forms of severe nephrotic syndrome. It is worth recalling here that before the first effective therapeutic measures became available, that is, adrenocorticotrophic hormone and glucocorticoids, thromboembolic events represented a major cause of mortality in children with nephrosis.

Renal function

Renal function is usually normal, but some patients have a reduction of the glomerular filtration rate (GFR) attributed to hypovolaemia, with return to normal after remission. A reduced GFR may also be found despite normal effective plasma flow (Dorhout et al., 1979; Bohlin and Berg, 1984) with a rapid return to normal after remission. Tubular functions are occasionally altered with glycosuria, aminoaciduria, hypokalaemia, and acidosis.

Acute kidney injury complicating nephrosis

Marked oliguria occurs mainly in adults, particularly in middle-aged or older patients (Cameron et al., 1974b). It may also occur in children (Sakarcan et al., 1994). Bilateral renal vein thrombosis may be recognized by sonography. Interstitial nephritis has been reported, usually allergic in response to drugs (see Chapter 101). Acute kidney injury (see Chapter 52) is usually reversible, it may be one of those rare justifications for intravenous infusion of albumin (Fliser et al., 1999; and see Chapter 52). In some cases where glomerular structure is close to normal on initial histology, acute kidney injury may last for as long as a year (Sakarcan et al., 1994) and sometimes be irreversible (Raij et al., 1976).

Kidney biopsy

Indications

Kidney biopsy is not indicated at onset in a child 1–10 years old with typical symptoms and complete remission obtained by corticosteroids. Biopsy is, however, indicated at onset in circumstances suggesting another type of glomerular disease, including moderate nephrotic syndrome or a long previous course of minor proteinuria, macroscopic haematuria, marked hypertension, and/or persistent renal insufficiency. A decreased plasma C3 fraction is also an indication for performing a biopsy. Age < 12 months and > 11 years is another indication, even in patients with a typical picture. However, the main indication is failure to respond to a 4-week course of prednisone given and taken in adequate dosage. A biopsy may be necessary to allow assessment of nephrotoxicity in patients receiving ciclosporin.

Since MCD is much less common in adults, a kidney biopsy is in most cases performed before any treatment and should be repeated in case of resistance to a 4-month course of steroids. In such a case, this repeat biopsy usually discloses lesions of FSGS that had either been overlooked on initial histology, or have appeared since, explaining steroid resistance.

Histopathology

Light microscopy shows three patterns: minimal changes, diffuse mesangial proliferation, or FSGS. Their relative incidence is difficult to determine. Minimal changes are found in the majority of children, and FSGS in only 5–7% of them (Southwest Pediatric Nephrology Study Group, 1985). Mesangial proliferation is reported in a small number (3–5%) of patients. These proportions are different in adults, less than one-third of whom have MCD and a majority focal segmental sclerosis (see Chapter 52).

Light microscopy

Minimal glomerular changes

Under light microscopy the glomeruli are mostly normal. Mild changes, including podocyte swelling and vacuolation, a slight increase in mesangial matrix, and mild, focal, mesangial hypercellularity may be seen (Churg et al., 1970; Cameron et al., 1974b). Lipid vacuoles and degenerative changes of proximal tubules are rare. Scattered foci of tubular lesions and interstitial fibrosis may be observed, such as obstruction by hyaline casts, dilatation with epithelial cell thinning, tubular basement membrane thickening, interstitial foam cells, and calcium deposits. Vascular changes are absent in children. In adults they are age related (Cameron et al., 1974b).

Diffuse mesangial proliferation

It is not always easy to draw the line between 'mild' and 'marked' mesangial hypercellularity. A subset of patients shows a marked increase in mesangial matrix associated with hypercellularity (Churg et al., 1970; Waldherr et al., 1978). However, peripheral capillary walls are normal, and immunofluorescence does not show humps. Electron microscopy shows foot-process effacement (Fig. 55.1). Mesangial hypercellularity has been attributed a prognostic significance. Waldherr et al. found a higher rate of initial steroid resistance and of progression to renal failure (Waldherr et al., 1978). Other studies failed to confirm these findings (Southwest Pediatric Nephrology Study Group, 1985).

Electron microscopy

Ultrastructural changes are constant, mainly involving podocytes and mesangial stalks. Podocyte foot-process effacement is generalized (Fig. 55.2) and closely related to the degree of proteinuria. This flattening of foot processes is due to a reversible rearrangement of the podocyte actin cytoskeleton that affects an elongated disposition. Immunoelectron microscopy has shown that the expression of nephrin is lower than normal in regions where the foot processes are effaced (Huh et al., 2002). Other epithelial changes consist of microvilli formation and numerous protein reabsorption droplets. The glomerular basement membranes are normal. The endothelial cells are often swollen.

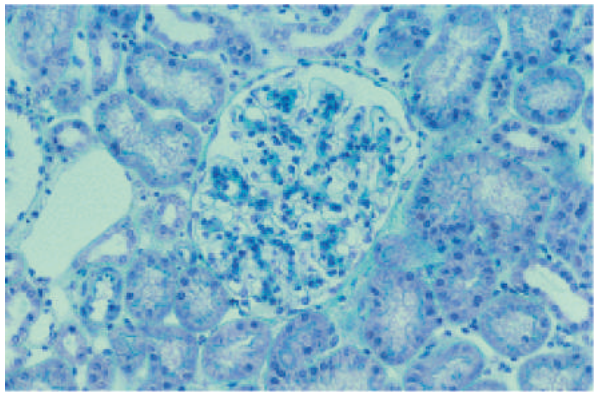

Fig. 55.1 Diffuse mesangial proliferation: the glomerular basement membrane is normal but there is an increased number of mesangial cells and amount of mesangial matrix. (Masson's trichrome, 250×.)

Immunofluorescence patterns

IgM-associated nephropathy

Cohen et al. proposed that mesangial IgM be considered a separate entity 'IgM-associated nephropathy' (Cohen et al., 1978). Patients with IgM nephropathy are less likely to respond to immunosuppressive agents than those with MCD and a higher probability of developing end-stage renal disease (Border, 1988; O'Donoghue et al., 1991). However, Habib et al. found that even if IgM was the immunoglobulin most frequently found in the glomeruli, there was no relationship between these deposits and initial response to therapy or final outcome (Habib et al., 1988). In fact, IgM is a large molecule that can be non-specifically trapped in an injured glomerulus and it is likely that mesangial IgM deposits represent an epiphenomenon.

IgA and minimal change disease

Some patients with nephrosis show mesangial deposits of IgA (Lai et al., 1986). It is likely that mesangial IgA in patients with minimal changes without cellular proliferation is coincidental (Barbiano di Belgiojoso et al., 1986; Habib et al., 1988). These patients have a favourable response to steroids, which would not be the case in true Berger's disease.

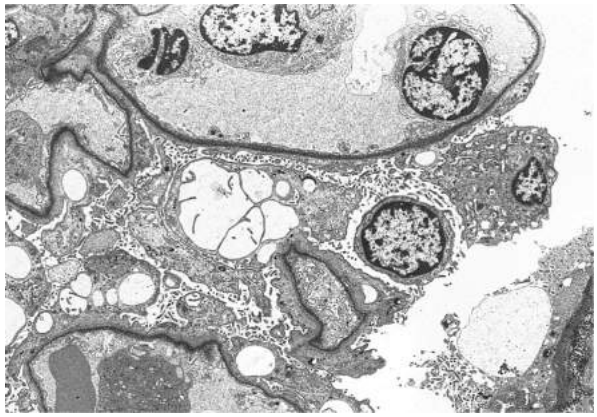

Fig. 55.2 Minimal change disease (electron microscopy). The glomerular basement membrane is normal; the cytoplasm of the podocytes is vacuolated, with effacement of foot processes and microvilli. (Methenamine silver, 2800×.)

C1q glomerulopathy

C1q glomerulopathy refers to a disorder in which mesangial proliferation is associated with mesangial deposits on electron microscopy and prominent C1q deposits on immunofluorescence microscopy (Jennette and Hipp, 1985; Iskandar et al., 1991; Markowitz et al., 2003; Kersnik Levart et al., 2005) and no clinical and laboratory evidence of systemic lupus erythematosus.

C1q nephropathy has been thought to be a subgroup of primary FSGS. However, many reports describe different symptoms, histopathologies, therapeutic responses, and prognoses, suggesting that C1q glomerulopathy may be a combination of several disease groups rather than a single disease entity (Mii et al., 2009). C1q glomerulopathy may be associated with either MCD, FSGS, or proliferative glomerulonephritis.

References

Alfiler, C. A., Roy, L. P., Doran, T., *et al.* (1980). HLA-DRw7 and steroid-responsive nephrotic syndrome of childhood. *Clin Nephrol*, 14, 71–4.

Alpers, C. E., and Cotran, R. S. (1986). Neoplasia and glomerular injury. *Kidney Int*, 30, 465–73.

Audard, V., Larousserie, F., Grimbert, P., *et al.* (2006). Minimal change nephrotic syndrome and classical Hodgkin's lymphoma: report of 21 cases and review of the literature. *Kidney Int*, 69, 2251–60.

Auguet, T., Lorenzo, A., Colomer, E., *et al.* (1998). Recovery of minimal change nephrotic syndrome and acute renal failure in a patient with renal cell carcinoma. *Am J Nephrol*, 18, 433–5.

Barbiano Di Belgiojoso, G., Mazzucco, G., Casanova, S., *et al.* (1986). Steroid-sensitive nephrotic syndrome with mesangial IgA deposits: a separate entity? Observation of two cases. *Am J Nephrol*, 6, 141–5.

Bohlin, A. B., and Berg, U. (1984). Renal water handling in minimal change nephrotic syndrome. *Int J Pediatr Nephrol*, 5, 93–8.

Border, W. A. (1988). Distinguishing minimal-change disease from mesangial disorders. *Kidney Int*, 34, 419–34.

Cameron, J. S., Turner, D. R., Ogg, C. S., *et al.* (1974b). The nephrotic syndrome in adults with 'minimal change' glomerular lesions. *QJM*, 43, 461–88.

Churg, J., Habib, R., and White, R. H. (1970). Pathology of the nephrotic syndrome in children: a report for the International Study of Kidney Disease in Children. *Lancet*, 760, 1299–302.

Clark, A. G., Vaughan, R. W., Stephens, H. A., *et al.* (1990). Genes encoding the beta-chains of HLA-DR7 and HLA-DQw2 define major susceptibility determinants for idiopathic nephrotic syndrome. *Clin Sci (Lond)*, 78, 391–7.

Cohen, A. H., Border, W. A., and Glassock, R. J. (1978). Nehprotic syndrome with glomerular mesangial IgM deposits. *Lab Invest*, 38, 610–19.

Dabbs, D. J., Striker, L. M., Mignon, F., *et al.* (1986). Glomerular lesions in lymphomas and leukemias. *Am J Med*, 80, 63–70.

De Mouzon-Cambon, A., Bouissou, F., Dutau, G., *et al.* (1981). HLA-DR7 in children with idiopathic nephrotic syndrome. Correlation with atopy. *Tissue Antigens*, 17, 518–24.

Dorhout, E. J., Roos, J. C., Boer, P., *et al.* (1979). Observations on edema formation in the nephrotic syndrome in adults with minimal lesions. *Am J Med*, 67, 378–84.

Dossier, C., Sellier-Leclerc, A. L., Rousseau, A., *et al.* (2014). Prevalence of herpesviruses at onset of idiopathic nephrotic syndrome. *Pediatr Nephrol*, 29(12), 2325–31.

Fliser, D., Zurbruggen, I., Mutschler, E., *et al.* (1999). Coadministration of albumin and furosemide in patients with the nephrotic syndrome. *Kidney Int*, 55, 629–34.

Glassock, R. J. (2003). Secondary minimal change disease. *Nephrol Dial Transplant*, 18 Suppl 6, vi52–8.

Habib, R., Girardin, E., Gagnadoux, M. F., *et al.* (1988). Immunopathological findings in idiopathic nephrosis: clinical significance of glomerular "immune deposits". *Pediatr Nephrol*, 2, 402–8.

Huh, W., Kim, D. J., Kim, M. K., *et al.* (2002). Expression of nephrin in acquired human glomerular disease. *Nephrol Dial Transplant*, 17, 478–84.

International Study of Kidney Disease in Children (1981). The primary nephrotic syndrome in children. Identification of patients with minimal change nephrotic syndrome from initial response to prednisone. A report of the International Study of Kidney Disease in Children. *J Pediatr*, 98, 561–4.

Iskandar, S. S., Browning, M. C., and Lorentz, W. B. (1991). C1q nephropathy: a pediatric clinicopathologic study. *Am J Kidney Dis*, 18, 459–65.

Jennette, J. C., and Hipp, C. G. (1985). C1q nephropathy: a distinct pathologic entity usually causing nephrotic syndrome. *Am J Kidney Dis*, 6, 103–10.

Kersnik Levart, T., Kenda, R. B., Avgustin Cavic, M., *et al.* (2005). C1Q nephropathy in children. *Pediatr Nephrol*, 20, 1756–61.

Lai, K. N., Lai, F. M., Chan, K. W., *et al.* (1986). An overlapping syndrome of IgA nephropathy and lipoid nephrosis. *Am J Clin Pathol*, 86, 716–23.

Laurent, J., Rostoker, G., Robeva, R., *et al.* (1987). Is adult idiopathic nephrotic syndrome food allergy? Value of oligoantigenic diets. *Nephron*, 47, 7–11.

Markowitz, G. S., Schwimmer, J. A., Stokes, M. B., *et al.* (2003). C1q nephropathy: a variant of focal segmental glomerulosclerosis. *Kidney Int*, 64, 1232–40.

McEnery, P. T., and Strife, C. F. (1982). Nephrotic syndrome in childhood. Management and treatment in patients with minimal change disease, mesangial proliferation, or focal glomerulosclerosis. *Pediatr Clin North Am*, 29, 875–94.

Meyrier, A., Delahousse, M., Callard, P., *et al.* (1992). Minimal change nephrotic syndrome revealing solid tumors. *Nephron*, 61, 220–3.

Mii, A., Shimizu, A., Masuda, Y., *et al.* (2009). Current status and issues of C1q nephropathy. *Clin Exp Nephrol*, 13, 263–74.

O'Donoghue, D. J., Lawler, W., Hunt, L. P., *et al.* (1991). IgM-associated primary diffuse mesangial proliferative glomerulonephritis: natural history and prognostic indicators. *QJM*, 79, 333–50.

Raij, L., Keane, W. F., Leonard, A., *et al.* (1976). Irreversible acute renal failure in idiopathic nephrotic syndrome. *Am J Med*, 61, 207–14.

Sakarcan, A., Timmons, C., and Seikaly, M. G. (1994). Reversible idiopathic acute renal failure in children with primary nephrotic syndrome. *J Pediatr*, 125, 723–7.

Sharples, P. M., Poulton, J., and White, R. H. (1985). Steroid responsive nephrotic syndrome is more common in Asians. *Arch Dis Child*, 60, 1014–7.

Southwest Pediatric Nephrology Study Group (1985). Focal segmental glomerulosclerosis in children with idiopathic nephrotic syndrome. A report of the Southwest Pediatric Nephrology Study Group. *Kidney Int*, 27, 442–9.

Stokes, M. B., Markowitz, G. S., Lin, J., *et al.* (2004). Glomerular tip lesion: a distinct entity within the minimal change disease/focal segmental glomerulosclerosis spectrum. *Kidney Int*, 65, 1690–702.

Waldherr, R., Gubler, M. C., Levy, M., *et al.* (1978). The significance of pure diffuse mesangial proliferation in idiopathic nephrotic syndrome. *Clin Nephrol*, 10, 171–9.

White, R. H. (1973). The familial nephrotic syndrome. I. A European survey. *Clin Nephrol*, 1, 215–19.

CHAPTER 56

Minimal change disease: treatment and outcome

Patrick Niaudet and Alain Meyrier

Introduction

The principles of management of minimal change disease (MCD) are similar in children and adults with a key difference being whether or when to undertake a renal biopsy:

- Children with nephrotic syndrome are usually treated with steroids first, and a renal biopsy only undertaken if there is no response to corticosteroids. However, a biopsy should be undertaken if there are atypical features such as macroscopic haematuria, hypertension, or lasting renal function impairment, all of which argue against a diagnosis of minimal change disease.
- In adults, the pre-treatment probability of MCD is lower, and the response to treatment often slower. A kidney biopsy is indicated to decide on treatment.

General management

The general management of nephrotic syndrome is described in Chapter 52.

Treatment and outcome in children

Specific treatment

The majority of children with minimal changes are steroid responsive (White et al., 1970). Steroid responders may relapse, but the majority still responds over the subsequent course (Pollak et al., 1968; Habib and Kleinknecht, 1971). Only 1–3% of patients who are initially steroid sensitive subsequently become steroid resistant and are defined as 'late non-responders' (Habib and Kleinknecht, 1971; Siegel et al., 1974; Trainin et al., 1975). Conversely, patients who do not respond to an initial steroid regimen given at an adequate dosage usually remain non-responders although they may respond to a combination of prednisone and calcineurin inhibitors (Niaudet, 1994). Response to corticosteroids is therefore the best prognostic index, not only since non-responders are more exposed to complications of persistent nephrotic syndrome, but also (and mainly) because they may develop end-stage renal disease (ESRD) after several years.

Initial treatment: corticosteroids

Steroids should not be started too early as spontaneous remission may occur in 5% of cases within the first 8–15 days. Some of these early spontaneous remissions are definitive, others are not. Infection must be sought and treated before starting steroids, not only to prevent the risk of overwhelming sepsis during treatment, but also because occult infection may be responsible for steroid resistance (McEnery and Strife, 1982). Metronidazole must not be forgotten for preventing malignant *Strongyloides stercoralis* infestation in patients from countries where carriage of this worm is endemic.

Steroid therapy is started when the diagnosis of idiopathic nephrotic syndrome is most likely in a child or after renal biopsy has been performed. Prednisone remains the reference drug. Prednisolone has the advantage of being soluble in water, making treatment easier in young children, and is the standard drug in many countries.

The International Study of Kidney Disease in Children (ISKDC) regimen consists of prednisone, 60 mg/m^2/day with a maximum of 80 mg/day, in divided doses for 4 weeks followed by 40 mg/m^2/day with a maximum of 60 mg/day, on alternate days, for 4 weeks. A response occurs in most cases within 10–15 days (median 11 days). According to the ISKDC (1981), approximately 90% of responders enter into remission within 4 weeks after starting steroids whilst < 10% enter remission after 2–4 more weeks of a daily regimen. Prolongation of daily steroid treatment beyond 4 or 5 weeks increases the risk of side effects. An alternative for patients who are not in remission after 4 weeks consists of three to four pulses of methylprednisolone (1 g/1.73 m^2). This additional regimen seems to be associated with few side effects, and probably produces remission more rapidly in the few patients who would have entered the second month of daily therapy (Murnaghan et al., 1984).

A working committee recently developed the following Kidney Disease: Improving Global Outcomes (KDIGO) guidelines (Lombel et al., 2013). Initial prednisone therapy consists of 60 mg/m^{2}or 2 mg/kg per day for 6 weeks (maximum dose of 60 mg/day), followed by alternate-day prednisone of 40 mg/m^2 or 1.5 mg/kg (maximum dose of 40 mg/day) for an additional 6 weeks.

A longer duration of the initial course of steroids appears to reduce the risk of relapse. A meta-analysis concluded that initial steroid therapy should be of at least 3 months duration in children with idiopathic nephrotic syndrome to reduce the risk of subsequent relapse.

In a pooled analysis from six trials, treatment with prednisone for 3–7 months versus a 2-month regimen reduced the risk of relapse at 12–24 months post-therapy (relative risk (RR) 0.70; 95%

confidence interval (CI) 0.58–0.84). There was no difference in cumulative steroid dose. Similarly, in a pooled analysis of four trials of 382 children, the risk of relapse was lower with 6 versus 3 months of therapy (RR 0.57; 95% CI 0.45–0.71). There was no difference in cumulative steroid dose. There was an inverse linear relationship between treatment duration and the risk of relapse (Hodson et al., 2000, 2007).

A longer duration is more important than the cumulative dose of prednisone in reducing the risk of relapse. This RR decreases by 0.133 (13%) for every additional month of treatment up to 7 months (Hodson et al., 2000). There are no data showing that treating for > 7 months is beneficial.

However, two recent studies have shown no benefit of a prolonged initial course of prednisolone therapy in reducing the rate of relapse. In one trial, 181 children received an initial treatment with a 6-week course of prednisolone 2 mg/kg per day followed by 1.5 mg/kg on alternate days for 6 weeks and were randomized to receive either placebo or decreasing doses of prednisolone for an additional duration of 3 months (Sinha et al., 2015). There was no difference in the mean number of relapses between both groups or in the rate of relapses, although the mean time to first relapse was later in the group receiving prednisolone for 6 months. The second study from Japan included 255 children who received in the control group a daily dose of 60 mg/m^2 for 4 weeks followed by 40 mg/m^2 on alternate days for 4 weeks or the same initial treatment followed by a progressive decrease for a total duration of 6 months. There were no differences in the mean number of relapses per patient-year between both groups, or in the rate of relapse after 2 years of follow-up (Yoshikawa et al., 2015).

Corticosteroid-responsive minimal change disease in children

Most children with idiopathic nephrotic syndrome respond to steroid therapy but a majority of them experience relapses. A prospective cohort study showed that boys are more likely to respond initially, more likely to relapse, and to be classified as having frequently relapsing nephrotic syndrome (Sureshkumar et al., 2014).

About 30% of patients experience only one attack and are definitively cured after a single course of steroids. Persistent remission for 18–24 months after stopping treatment is likely to reflect definitive cure, and the risk of later relapses is low. About 10–20% of patients relapse several months after stopping treatment and are apparently free of disease after three or four episodes, which respond to a standard course of corticosteroids. The remaining 50–60% experience frequent relapses as soon as steroid therapy is stopped or when dosage is decreased. The risk of relapse is greater in children aged < 5 years at onset and in males. These steroid-dependent patients often raise difficult therapeutic problems.

Steroid-dependent patients may be treated with repeated courses of prednisone, 60 mg/m^2/day, continued 3 days after the urine has become protein free, followed by alternate-day prednisone, 40 mg/m^2, for 4 weeks as proposed by the ISKDC (1981). Another option is based on treating relapses with daily prednisone, 40–60 mg/m^2, until proteinuria has disappeared for 4–5 days. Thereafter, prednisone is switched to alternate days and the dosage is tapered to 15–20 mg/m^2 every other day, according to the steroid threshold, that is, the dosage at which the relapse has occurred. Treatment is then continued for 12–18 months. The first approach allows better definition in terms of relapses but is associated with more relapses. The latter regimen is associated with fewer steroid side effects as the cumulative dosage is lower. Prolonged courses of alternate-day steroid therapy are often well tolerated by young children and growth velocity is not affected. However, prednisone dosage must be as low as possible in order to reduce side effects. The role of upper respiratory tract infections in exacerbating nephrotic syndrome has been highlighted in all series: 71% of relapses were preceded by such an event in a prospective study, although only 45% of respiratory infections were followed by an exacerbation of proteinuria (MacDonald et al., 1986). Gulati et al. performed a randomized controlled trial and found that the risk of relapse was significantly decreased during upper respiratory tract infections when prednisone was given daily for 7 days rather than on alternate days (Gulati et al., 2011).

Leisti and Koskimies studied the degree of adrenocortical suppression and found that adrenocortical suppression increased the risk of relapse (Leisti and Koskimies, 1983). Severe suppression was always associated with a relapse, the longest remission time being 6 months. In patients with moderate suppression, several long, relapse-free intervals were observed, but the risk of relapse was still higher than in episodes with normal adrenocortical function. Cortisol substitution possibly decreased the risk of a relapse after severe adrenocortical suppression. These findings might incite to add adrenocortical substitution to a long-term corticosteroid regimen for entertaining remission in patients with idiopathic nephrotic syndrome.

Alternative treatments

An alternative treatment is required in children who relapse on alternate-day prednisone therapy and suffer severe side effects such as growth retardation, behaviour disturbances, cushingoid features, hypertension, cataract, or osteopenia. Such treatment is also indicated in children at risk of toxicity such as diabetes or during puberty, in children with severe relapses accompanied by thrombotic complications or severe hypovolaemia, and in those with poor compliance. Alternative treatments include levamisole, alkylating agents, mycophenolate mofetil (MMF), ciclosporin, and rituximab (RTX).

Levamisole

The beneficial effect of levamisole was first described by Tanphaichitr et al. (1980) and was subsequently reported to reduce the risk of relapse in steroid-dependent patients. A significant steroid-sparing effect at a dose of 2.5 mg/kg every other day was demonstrated in two controlled trials (British Association for Paediatric Nephrology, 1991; Dayal et al., 1994). However, the beneficial effect of levamisole is not sustained after stopping treatment.

Side effects occasionally include neutropenia, agranulocytosis, vomiting, cutaneous rash, neurological symptoms including insomnia, hyperactivity, and seizures. Levamisole is well tolerated in most children but is not widely available.

Alkylating agents

Alkylating agents, such as cyclophosphamide and chlorambucil, have been used for > 50 years to achieve long-lasting remission. Unfortunately they are toxic, and in the long term, remissions may not seem long enough.

The efficacy of alkylating agents is illustrated by a meta-analysis that compared alkylating agents with prednisone in maintaining remission. In three trials including 102 patients, oral

cyclophosphamide reduced the risk of relapse at 6–12 months (RR 0.44; 95% CI 0.26–0.73). Similarly, chlorambucil compared with prednisone alone reduced the risk of relapse at 12 months in 32 children (RR 0.13; 95% CI 0.03–0.57) (Hodson et al., 2008). Several studies involving patients with frequently relapsing or steroid-dependent nephrotic syndrome showed that cyclophosphamide resulted in sustained remission in 57–93% of patients at 1 year, 31–66% at 5 years, and 25% at 10 years (Cameron et al., 1974a; Chiu and Drummond, 1974; McDonald et al., 1974; Vester et al., 2005). However, a more recent series reported lower remission rates of 44%, 27%, and 13% at 1, 2, and 5 years after cyclophosphamide therapy (Cammas et al., 2011). In another study of 90 children with a steroid-dependent course, sustained remissions were observed in 31% of patients at 5-year follow-up (Azib et al., 2011). These variations are probably due to differences in the patient populations as steroid-dependent patients have a lower response rate than frequently relapsing patients. The degree of steroid dependency also affects remission rates (Zagury et al., 2011).

The Arbeitsgemeinschaft für pädiatrische Nephrologie (1987) reported that treatment for 12 weeks at a daily dose of 2 mg/kg was more effective than an 8-week course, with 67% as compared to 22% remaining in remission after 2 years. However, a randomized trial showed that prolonging the course of cyclophosphamide from 8 to 12 weeks did not further reduce the proportion of children experiencing relapses (Ueda et al., 1990).

Cyclophosphamide toxicity includes bone marrow depression, haemorrhagic cystitis, gastrointestinal disturbances, alopecia, and infection (Latta et al., 2001). Leucopoenia is frequently observed, but weekly haematological monitoring may limit its severity and concomitant steroids help blunt marrow depression. Haemorrhagic cystitis rarely occurs. Alopecia, which is variably pronounced, remits a few weeks after stopping treatment. Viral infections can be overwhelming if cyclophosphamide is not stopped in due time.

Long-term toxicity includes malignancy, even more rarely pulmonary fibrosis. Gonadal toxicity is well established and the risk of sterility is greater in boys than in girls. The cumulative threshold dose above which oligo/azoospermia may be feared lies between 150 and 250 mg/kg (Penso et al., 1974; Hsu et al., 1979; Trompeter et al., 1981). In females, the cumulative dose associated with sterility is greater, but not well defined. In this and other contexts, early menopause may be a late consequence of alkylating agents.

Most authors would prescribe a 12-week course of oral cyclophosphamide at a daily dose of 2 mg/kg.

Beneficial results have also been achieved with chlorambucil in steroid-responsive nephrosis (Grupe et al., 1976; Baluarte et al., 1978; Williams et al., 1980). Acute and long-term toxic effects are similar to those observed with cyclophosphamide.

Calcineurin inhibitors

Ciclosporin

Ciclosporin has been shown in a number of studies to reduce the incidence of relapses in 75–90% of patients with steroid-dependent nephrotic syndrome thereby allowing withdrawal of prednisone (Kitano et al., 1990; Niaudet and Habib, 1994; Gregory et al., 1996; Inoue et al., 1999; El-Husseini et al., 2005; Tanaka et al., 2006; Cattran et al., 2007; Ishikura et al., 2008, 2010). However, most patients relapse when the drug is withdrawn (Ishikura et al., 2012). Therefore a prolonged treatment is necessary with an increased risk of nephrotoxicity (Niaudet and Habib, 1994; Hulton et al., 1994).

Ciclosporin has been compared to alkylating agents in two randomized trials (Niaudet, 1992; Ponticelli et al., 1993a). The effect of ciclosporin was initially the same as chlorambucil and cyclophosphamide in maintaining remission. However, after ciclosporin was discontinued, it was less effective in maintaining remission at 12 months compared with either alkylating agents and at 24 months for chlorambucil.

Because of the concern for nephrotoxicity, the serum creatinine concentration should be monitored regularly in patients who are maintained on a long-term course of ciclosporin. However, serial renal biopsies demonstrate histologic lesions of nephrotoxicity without clinical evidence of renal function impairment (Habib and Niaudet, 1994; Iijima et al., 2002; Kengne-Wafo et al., 2009). Histological lesions most often consist of tubulointerstitial injury, characterized by stripes of interstitial fibrosis containing clusters of atrophic tubules and by lesions of arteriolopathy. Thus, some authors propose to routinely perform a kidney biopsy in asymptomatic patients after 18 months of ciclosporin therapy.

Other side effects include hypertension, hyperkalaemia, hypertrichosis, gum hypertrophy, and hypomagnesaemia.

The recommended starting ciclosporin dose is 150 mg/m^2 per day divided into two oral doses. The dose should be adjusted to maintain trough whole blood levels between 100 and 200 ng/mL, and the level should not exceed 200 ng/mL. In order to limit the risk of nephrotoxicity, once remission is achieved, we recommend decreasing the dose to < 5 mg/kg, if possible. Low-dose alternate-day prednisone in combination with ciclosporin may be a good approach to maintain remission with lower doses of ciclosporin.

Tacrolimus

Though data is not so comprehensive, tacrolimus is probably as effective as ciclosporin in maintaining remission in children with steroid-sensitive nephrotic syndrome, Transplantation experience (see Chapter 281) suggests that it is less nephrotoxic but more likely to be associated with diabetes (Dotsch et al., 2006; Sinha et al., 2006). In a series of five children treated with tacrolimus, two developed type 1 diabetes mellitus, which resolved after stopping tacrolimus therapy (Dittrich et al., 2006). However, one advantage of tacrolimus over ciclosporin is reduced cosmetic side effects (hypertrichosis, gum hypertrophy).

Mycophenolate mofetil

MMF inhibits T- and B-cell proliferation. Small studies suggest that MMF is effective in increasing the duration of remission in children with idiopathic nephrotic syndrome; however, relapses often occur after the treatment is discontinued in steroid-dependent children (Novak et al., 2005; Hogg et al., 2006; Afzal et al., 2007).

In a small randomized trial comparing MMF to ciclosporin, sustained complete remission was achieved in 7 of 12 patients who received MMF and in 11 of 12 patients treated with ciclosporin suggesting that ciclosporin is more effective than MMF (Dorresteijn et al., 2008). Side effects of MMF include gastrointestinal disturbances (abdominal pain and diarrhoea) and haematological abnormalities.

A 'Bayesian study' was conducted in 23 children with steroid-dependent idiopathic nephrotic syndrome having received prior alkylating-agent treatment and two-thirds of them levamisole (Baudouin et al., 2012). They were treated with MMF and prednisone according to a defined schedule (reduction of alternate-day dose to 50% of pre-MMF dose at 3 months, 25% at 6 months). Twenty-three children completed the study. Four relapsed during the first 6 months and two at months 8 and 11.5. In the 19 patients free of relapse during the first 6 months, median prednisone maintenance dose decreased from 25 (10–44) to 9 (7.5–11.2) mg/m^2 and cumulative dose from 459 (382–689) to 264 (196–306) mg/m^2 before and on MMF respectively. The authors concluded that MMF reduces relapse rate and steroid dose in children with steroid-dependent nephrotic syndrome and should be proposed before ciclosporin.

Whilst awaiting further information, MMF appears to be reasonable choice to treat children with steroid-dependent nephrotic syndrome with steroid toxicity. Although two trials comparing ciclosporin and MMF suggest that ciclosporin is more effective in preventing relapses (Dorresteijn et al., 2008; Gellermann et al., 2013), MMF may still be more appropriate as it is not nephrotoxic

Rituximab

Several reports originally suggested that RTX, a chimeric anti-CD20 monoclonal antibody that depletes B-cell lymphocytes, may be effective in steroid-dependent or calcineurin inhibitor-dependent patients (Guigonis et al., 2008; Fujinaga et al., 2010; Ravani et al., 2011; Sellier-Leclerc et al., 2011). Larger and now randomised studies have confirmed this.

In a case series of 54 children with severe steroid or ciclosporin-dependent nephrotic syndrome, the administration of RTX allowed the discontinuation of one or more immunosuppressive agents (Guigonis et al., 2008). The response to RTX appears to be better if the patient is in remission at the time of the infusion. Seven patients were nephrotic at the time of RTX treatment. Remission was induced in three of the seven proteinuric patients. RTX was effective in all patients when administered during a proteinuria-free period in association with other immunosuppressive agents. Therefore, RTX should not be administered during a relapse but after remission has been induced by increased doses of steroids. One or more immunosuppressive treatments could be withdrawn in 19 patients, with no relapse of proteinuria and without increasing other drug dosage. When relapses occurred, they were associated with an increase in CD19 cell count. Adverse effects were observed in 45% of cases, but most of them were mild and transient.

Ravani et al. randomized 54 children (mean age 11 ± 4 years) with idiopathic nephrotic syndrome dependent on prednisone and calcineurin inhibitors for > 12 months (Ravani et al., 2011). RTX with lower doses of prednisone and calcineurin inhibitors was compared to current therapy alone. Three-month proteinuria was 70% lower in the RTX arm as compared with standard therapy arm (intention-to-treat); relapse rates were 18.5% (intervention) and 48.1% (standard arm) ($P = 0.029$). Probabilities of being drug-free at 3 months were 62.9% and 3.7%, respectively ($P < 0.001$); 50% of RTX cases were in stable remission without drugs after 9 months. The authors concluded that RTX and lower doses of prednisone and calcineurin inhibitors are non-inferior to standard therapy in maintaining short-term remission in children with idiopathic nephrotic syndrome dependent on both drugs and allow their temporary withdrawal.

Iijama et al. reported the results of a clinical trial involving 48 children with frequently relapsing steroid-dependent nephritic syndrome who were randomly assigned to receive rituximab or placebo once a week for 4 weeks whilst in remission (Iijima et al., 2014). Patients received standard steroid therapy and immunosuppressive agents were stopped 6 months after randomization. At 1 year, median relapse-free duration was longer in the rituximab group compared with controls (267 vs 101 days). Moreover, the relapse rate and the daily prednisone dose were lower although by 19 months, all patients had relapsed.

A significant proportion of patients relapse after RTX administration. Most relapses occur after recovery of B lymphocyte counts. Maintenance therapy with MMF has been shown to be effective in preventing relapse after treatment with RTX (Ito et al., 2011). Fujinaga et al. found that ciclosporin was even more effective than MMF for maintaining remission after a single infusion of RTX (Fujinaga et al., 2013).

However, RTX may be associated with adverse effects including infusion-related reactions (hypotension, fever, and rigors), serious infections due to leukopenia and/or hypogammaglobulinaemia (Delbe-Bertin et al., 2013; Sellier-Leclerc et al., 2013; Kamei et al., 2015), and progressive multifocal leucoencephalopathy. In addition, there is one published case report of death due to lung fibrosis (Chaumais et al., 2009) associated with RTX therapy in a child with nephrotic syndrome and another one of severe myocarditis (Sellier-Leclerc et al., 2013) in two children with nephrotic syndrome treated with rituximab. Additional severe adverse effects reported in childhood nephrotic syndrome include *Pneumocystis jirovecii* pneumonia requiring heart transplantation (Guigonis et al., 2008; Sato et al., 2013) and severe immune-mediated ulcerative gastrointestinal disease (Ardelean et al., 2010).

The KDIGO Glomerulonephritis Workgroup recommended that rituximab be considered only in children who have continuing relapses despite optimal combinations of prednisone and steroid-sparing agents or in patients who had developed calcineurin inhibitor nephrotoxicity (Lombel et al., 2013). Well-defined randomized controlled studies are mandatory to examine which drug has the best risk/benefit profile and to better define the place of rituximab in the treatment of childhood steroid-sensitive nephrotic syndrome (Boyer and Niaudet, 2013), knowing that rituximab does not cure the disease and repeated courses may be necessary (Razzak, 2014).

Corticosteroid-resistant minimal change disease in children

Less than 5% of children with MCD are steroid resistant, either primary non-responders or late non-responders. Hammad et al. found that patients who are primary non-responders have a low expression of glucocorticoid receptors on peripheral blood mononuclear cells before starting therapy, and they suggest that this low expression may be one of the pathophysiological mechanisms of steroid resistance in these children (Hammad et al., 2013). Patients with steroid-resistant MCD may develop in the long-term lesions of focal segmental glomerular sclerosis and progress to ESRD. Some of these children have a genetic form of idiopathic nephrotic syndrome and are at high risk of progression to ESRD.

The treatment of these patients remains difficult. Several therapeutic options have been proposed. The results of these treatments are difficult to analyse, as a significant proportion of the patients

progress to cure gradually, with a slow decrease of proteinuria and in such cases, relapses are rare.

Intravenous cyclophosphamide pulses were found to be more effective than oral cyclophosphamide. In this trial, patients receiving cyclophosphamide pulses had more sustained remissions despite lower cumulative dose (Elhence et al., 1994). Another study found that sustained remission is more likely to occur in patients with late steroid résistance (Bajpai et al., 2003). The French Society of Pediatric Nephrology reported the results of a prospective study where 45 children with minimal change steroid-resistant nephrotic syndrome received a combination of ciclosporin and prednisone (Niaudet, 1994). Complete remission was observed in 21 patients and none of them progressed to ESRD. Six patients relapsed after ciclosporin was stopped but they responded to a second course of prednisone. Gulati et al. found that tacrolimus and prednisone are more effective than cyclophosphamide pulses in children with steroid-resistant nephrotic syndrome, including those with MCD (Gulati et al., 2012).

Treatment in adults

There are hints that MCD is not the same illness in adults and in children. This is especially true in terms of progression and treatment.

Corticosteroid therapy

The first-line treatment of MCD in adults is based on glucocorticoids. However considerable differences in treatment modes between adults and children have been reported in the literature (Wang et al., 1982; Nolasco et al., 1986; Nair et al., 1987; Korbet et al., 1988a; Fujimoto et al., 1991; Mak et al., 1996; Nakayama et al., 2002). Definitions of steroid sensitivity, dependency, resistance, and multiple relapses vary among papers dealing with adult idiopathic nephrotic syndrome, owing to lack of unified agreement regarding treatment protocol.

The response to treatment is currently defined as disclosed in Box 56.1.

Initial response to corticosteroids does not seem to differ greatly according to age, although it appears to be somewhat lower in adults. The response of MCD to corticosteroids in adults is much slower than in children (Nolasco et al., 1986; Fujimoto et al., 1991; Korbet et al., 1994, 1995, 1999; Rydel et al., 1995; Mak et al., 1996; Nakayama et al., 2002) (Fig. 56.1).

Fujimoto et al. treated 33 patients having adult-onset MCD with prednisolone at 1 mg/kg per day for 4–8 weeks, followed by slow tailing off, for a total duration of > 6 months (Fujimoto et al., 1991). Seventy-six per cent of patients were pushed into remission within 8 weeks, but in the extant cases the longest time to remission was 4 months. Five patients went into remission whilst the steroid dosage was being tapered. Mak et al. treated 40 patients with adult-onset minimal change nephropathy. The remission rate was 46% by the fourth week, 69% by the eighth week, 85% by the sixteenth week, and 87% by the twenty-first week (Mak et al., 1996). These figures clearly show that corticosteroid resistance in adult nephrosis should not be pronounced before 4 months and probably even 6 months of full-dose (1 mg/kg/day) treatment.

Another difference stems from the trend toward a diminished frequency of relapses with increasing age, provided idiopathic nephrotic syndrome did not start during childhood.

Box 56.1 Definitions

- Nephrotic range proteinuria:
  - Adults: proteinuria > 3.5 g per day
  - Children: > 40 mg/m^2 per hour; urinary protein:creatinine ratio >2 mg/mg or >200 mg/mmol
- Complete remission:
  - Adults: proteinuria < 0.3–1.0 g per day, normal serum albumin (> 30 g/L), and stable renal function
  - Children: urinary protein:creatinine ratio < 0.2–0.3 mg/mg or < 30 mg/mmol and normal serum albumin (> 30 g/L)
- Partial remission:
  - Adults: proteinuria 0.3–3.5 g per day and/or ≥ 50% decrease in proteinuria from baseline, and stable renal function
  - Children: urinary protein:creatinine ratio 0.2–2.0 mg/mg or 30–350 mg/mmol; and serum albumin >30 g/L
- Steroid-dependent nephrotic syndrome:
  - Two consecutive relapses whilst receiving predniso(lo)ne on alternate days, or within 15 days of its discontinuation
- Steroid-resistant nephrotic syndrome:
  - Children: lack of remission despite 4–8 weeks of therapy with daily predniso(lo)ne at a dose of 60 mg/m^2 or 2 mg/kg (maximum 60 mg) per day
  - Adults: lack of remission despite 4 months of therapy with daily prednisone at a dose of 1 mg/kg/day (maximum 80 mg/day)
- Calcineurin-inhibitor (CNI) dependent nephrotic syndrome:
  - Remission of steroid-dependent nephrotic syndrome is achieved during therapy with CNIs (ciclosporin or tacrolimus)
- CNI-resistant and steroid-resistant nephrotic syndrome:
  - No response to therapy with predniso(lo)ne as defined above, or to CNI therapy.

Nolasco et al. followed 89 patients with adult-onset MCD, 58 of whom responded to corticosteroid treatment: 24% never relapsed, 56% relapsed on a single occasion or infrequently, and only 21% were frequent relapsers (Nolasco et al., 1986). Korbet et al. followed 40 adults with MCD: 34 were treated with prednisone, and 31 (91%) achieved remission, of whom only three suffered multiple relapses (Korbet et al., 1988a). Relapses were infrequent in 99 adult nephrotics with MCD followed by Wang et al. for 3–102 months (Wang et al., 1982). In 85 patients whose urine was protein-free for at least 6 months, four relapsed; of 46 who were protein-free for 24 months, three relapsed; of 37 followed for 36–96 months, only three relapsed. Nair et al. treated 54 adults with MCD. Only 17 had relapses, a rate of 31% at 3 years of follow-up (Nair et al., 1987). Fujimoto et al. also found an incidence of relapse significantly lower in patients > 30 years (Fujimoto et al., 1991). Nakayama et al. analysed retrospectively 62 Japanese adults with minimal change nephrotic syndrome.

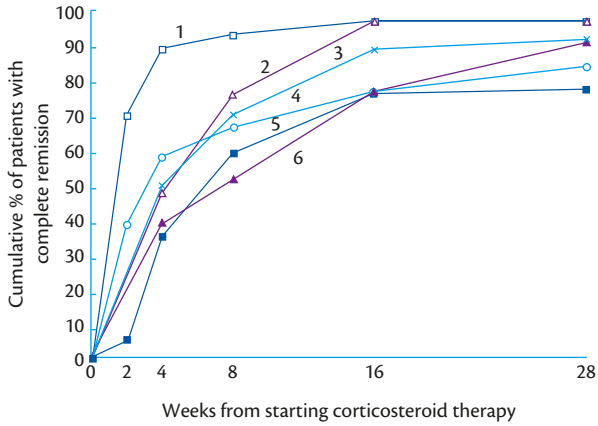

Fig. 56.1 Comparing the time of response to corticosteroid treatment in children (1) shows that the definition of corticosteroid resistance in adults (2–6) is in the order of 4 months. Many articles in the literature on the treatment of adult nephrosis are biased, as they adopt the same definition of 'steroid resistance' as for children, that is, 6–8 weeks of this regimen without remission. (1) International Study of Kidney Disease in Children (1981); (2) Fujimoto et al. (1991); (3) Mak et al. (1996); (4) Nakayama et al. (2002); (5) Nolasco et al. (1986); (6) Korbet et al. (1981). From Nakayama et al. (2002).

Five experienced remission spontaneously. Fifty-three entered complete remission, three partial remission, and one patient showed no response to corticosteroids (Nakayama et al., 2002). Fifty-three patients with complete remission were divided into two groups: 38 early responders who experienced remission completely within 8 weeks after starting treatment and 15 late responders who experienced remission after 8 weeks. Thirty-three patients experienced a relapse; 13 experienced multiple relapses. Fifty-three patients with remission were divided into three groups: 16 patients who experienced relapse within 6 months after the initial response (early relapsers), 17 who experienced relapse after 6 months (late-relapsers), and 20 non-relapsers. Mean age at onset was younger in early relapsers than in late or non-relapsers. Age at onset correlated inversely with relapse rate in 53 patients with remission and correlated positively with timing of the first relapse in 33 relapsers.

Thus, the experience of nephrologists treating adult MCD is comparable in Europe, America, and the Far East, with a multiple relapse rate in the order of 10–20%, as opposed to a greater percentage in children.

Apart from age, insufficient treatment might also explain some of the relapses observed in adults. In reports published between 1971 and 1988, patients were treated with a short course of steroids. The initial remission rate was comparable with a short versus a long treatment mode, but duration of remission was superior when patients received > 8 weeks of prednisone. A long initial alternate-day corticosteroid regimen followed by slow tapering is effective in obtaining sustained remission in adult MCD.

Other treatment modes

Contraindications to corticosteroid therapy

In some patients, high-dose corticosteroids are hazardous. This is the occasional case of a patient with morbid obesity and glucose intolerance, of a patient with psychiatric disorders or of a patient with a haemorrhagic gastrointestinal ulcer. In such cases first-line treatment may be based on a calcineurin inhibitor (Meyrier, 1997).

Corticosteroid dependency and multiple relapses

Steroid dependency is defined by relapses occurring at the time when tapering treatment reaches a threshold dose. When this dose is high, for example, > 20 mg/day of prednisone the patient is exposed to all the long-term complications of steroids. Multiple relapses defined by three or four attacks per year despite an adequate initial treatment put the nephrologist in a similar quandary with respect to the tolerability of available drugs. There are no randomized series to determine the best approach for suppressing or spacing out the nephrotic episodes. A first option is based on a continuous course of the smallest dose of prednisone that maintains remission, either on a daily or on an alternate-day treatment. The cumulative dosage of steroids, however, portends a risk of toxicity in the long run, in particular of hip osteonecrosis.

Calcineurin inhibitors (ciclosporin or tacrolimus), alone or with a very small dose of prednisone, are quite effective but even with the small risk shown by serial biopsies the long term implications for kidney function are concerning minimal risk of renal toxicity as shown by repeat kidney biopsies (Meyrier et al., 1994; Cattran et al., 2007).

A third option consists of a 4-month course of cyclophosphamide. This alkylating agent is credited with long-lasting remissions with a protracted 'treatment holiday'. However, this alkylating agent is characterized by a narrow margin between efficacy and toxicity and in a young male entails a risk of definitive infertility. The efficacy of MMF in multirelapsing or steroid-dependent adult minimal change nephrotic syndrome has been documented in small series (Waldman et al., 2007; Siu et al., 2008; Hogan and Radhakrishnan, 2013).

RTX offers a new hope to suppress the relapses or control steroid dependency in adults, although long term studies are still lacking to determine outcome and predictors of response and long term toxicity (Sinha and Bagga, 2013). Munyentwali et al. analysed the efficacy and safety of RTX in 17 adult patients with steroid-dependent MCD over a mean follow-up of 29.5 months (range 5.1–82 months) (Munyentwali et al., 2013). Seventeen patients with steroid-dependent or frequently relapsing minimal change nephrotic syndrome, unresponsive to several immunosuppressive medications, were treated with RTX. Eleven patients had no relapses after RTX infusion (mean follow-up 26.7 months, range 5.1–82 months) and nine of them were able to come off all other immunosuppressive drugs and steroids during follow-up. Six patients relapsed at least once after a mean time of 11.9 months (mean follow-up 34.5 months, range 16.9–50.1 months), but their immunosuppressive drug treatment could be stopped or markedly reduced during this time. No adverse events were recorded. The authors concluded that RTX is efficient and safe in adult patients suffering from severe steroid-dependent MCD.

Late relapses

A relapse occurring years after a first episode of nephrotic syndrome followed by a long period of remission is not unusual. In such a case it is not mandatory to commence a 4-month course of high-dose steroids. A stable remission is often achieved with a short

(in the order of 1-month) treatment with rapid tapering of steroids to a stop over the following 4 weeks.

Corticosteroid resistance

An adult with an initial diagnosis of MCD and resistance to a 4-month course of glucocorticoids represents an indication to perform a repeat kidney biopsy. In most cases histology reveals lesions of focal segmental glomerulosclerosis that have been overlooked on the first biopsy or have appeared since. In such a case the treatment is as described for patients with focal segmental glomerulosclerosis. A possible cause of apparent steroid resistance in patients treated with prednisolone is a concomitant treatment with aluminium gels for gastric protection, as (contrary to prednisone) the intestinal absorption of prednisolone is significantly diminished or abolished by these antacids.

Long-term outcome in children

About one-third of patients experience only one attack and are definitively cured after the course of corticosteroids. Ten to 20% of patients experience relapses several months after stopping the treatment and a cure takes place after three or four episodes. The remaining 40–50% of patients have either frequent relapses or are steroid dependent. These steroid-dependent patients may have a prolonged course. However, if the patient continues to respond to steroids, the risk of progression to chronic renal failure is minimal.

Trompeter et al. reported the late outcome of 152 children steroid-responsive nephrotic syndrome after a follow-up of 14–19 years: 127 (83%) were in remission, four had hypertension, 10 were still relapsing, and 11 had died (Trompeter et al., 1985). The duration of the disease was longer in children who had started before the age of 6 years. Wynn et al. found that 15% of 132 patients had a persistent relapsing course with a mean follow-up of 27.5 years (Wynn et al., 1988). Lewis et al. reported on 26 patients over the age of 20 years, of whom five were still relapsing in adulthood (Lewis et al., 1989).

Koskimies et al. reported on 94 cases. Twenty-four per cent of steroid responders had no relapse, 22% had infrequent relapses and 54% frequent relapses. More than two-thirds were in remission at time of report (Koskimies et al., 1982). None of these patients developed renal insufficiency and none died from the disease. Lahdenkari et al. reported a 30-year follow-up of the patients reported previously by Koskimies et al. (Lahdenkari et al., 2005). Of 104 patients, 10% had further relapses in adulthood.

Fakhouri et al. reported on the outcome in adulthood of 102 patients born between 1970 and 1975 (Fakhouri et al., 2003). Forty-two per cent presented at least one relapse in adulthood. A young age at onset and a high number of relapses during childhood were associated with a higher risk of relapse in adulthood. Similarly, Ruth et al. in a study of 42 patients, found that 14 (33%) relapsed in adulthood (Ruth et al., 2005). The higher relapse rates in these two reports probably reflect patient selection with more steroid-dependent cases compared to Koskimies et al.'s series.

References

Afzal, K., Bagga, A., Menon, S., *et al.* (2007). Treatment with mycophenolate mofetil and prednisolone for steroid-dependent nephrotic syndrome. *Pediatr Nephrol*, 22, 2059–65.

Arbeitsgemeinschaft für Padiatrische Nephrologie (1987). Cyclophosphamide treatment of steroid dependent nephrotic syndrome: comparison of eight week with 12 week course. Report of Arbeitsgemeinschaft fur Padiatrische Nephrologie. *Arch Dis Child*, 62, 1102–6.

Ardelean, D. S., Gonska, T., Wires, S., *et al.* (2010). Severe ulcerative colitis after rituximab therapy. *Pediatrics*, 126, e243–6.

Azib, S., Macher, M. A., Kwon, T., *et al.* (2011). Cyclophosphamide in steroid-dependent nephrotic syndrome. *Pediatr Nephrol*, 26, 927–32.

Bajpai, A., Bagga, A., Hari, P., *et al.* (2003). Intravenous cyclophosphamide in steroid-resistant nephrotic syndrome. *Pediatr Nephrol*, 18, 351–6.

Baluarte, H. J., Hiner, L., and Gruskin, A. B. (1978). Chlorambucil dosage in frequently relapsing nephrotic syndrome: a controlled clinical trial. *J Pediatr*, 92, 295–8.

Baudouin, V., Alberti, C., Lapeyraque, A. L., *et al.* (2012). Mycophenolate mofetil for steroid-dependent nephrotic syndrome: a phase II Bayesian trial. *Pediatr Nephrol*, 27, 389–96.

Boyer, O. and Niaudet, P. (2013). Nephrotic syndrome: rituximab in childhood steroid-dependent nephrotic syndrome. *Nat Rev Nephrol*, 9, 562–3.

Cameron, J. S., Chantler, C., Ogg, C. S., *et al.* (1974a). Long-term stability of remission in nephrotic syndrome after treatment with cyclophosphamide. *Br Med J*, 4, 7–11.

Cammas, B., Harambat, J., Bertholet-Thomas, A., *et al.* (2011). Long-term effects of cyclophosphamide therapy in steroid-dependent or frequently relapsing idiopathic nephrotic syndrome. *Nephrol Dial Transplant*, 26, 178–84.

Cattran, D. C., Alexopoulos, E., Heering, P., *et al.* (2007). Cyclosporin in idiopathic glomerular disease associated with the nephrotic syndrome : workshop recommendations. *Kidney Int*, 72, 1429–47.

Chaumais, M. C., Garnier, A., Chalard, F., *et al.* (2009). Fatal pulmonary fibrosis after rituximab administration. *Pediatr Nephrol*, 24, 1753–5.

Chiu, J. and Drummond, K. N. (1974). Long-term follow-up of cyclophosphamide therapy in frequent relapsing minimal lesion nephrotic syndrome. *J Pediatr*, 84, 825–30.

Dayal, U., Dayal, A. K., Shastry, J. C., *et al.* (1994). Use of levamisole in maintaining remission in steroid-sensitive nephrotic syndrome in children. *Nephron*, 66, 408–12.

Delbe-Bertin, L., Aoun, B., Tudorache, E., *et al.* (2013). Does rituximab induce hypogammaglobulinemia in patients with pediatric idiopathic nephrotic syndrome? *Pediatr Nephrol*, 28, 447–51.

Dittrich, K., Knerr, I., Rascher, W., (2006). Transient insulin-dependent diabetes mellitus in children with steroid-dependent idiopathic nephrotic syndrome during tacrolimus treatment. *Pediatr Nephrol*, 21, 958–61.

Dorresteijn, E. M., Kist-Van Holthe, J. E., Levtchenko, E. N., *et al.* (2008). Mycophenolate mofetil versus cyclosporine for remission maintenance in nephrotic syndrome. *Pediatr Nephrol*, 23, 2013–20.

Dotsch, J., Dittrich, K., Plank, C., *et al.* (2006). Is tacrolimus for childhood steroid-dependent nephrotic syndrome better than ciclosporin A? *Nephrol Dial Transplant*, 21, 1761–3.

El-Husseini, A., El-Basuony, F., Mahmoud, I., *et al.* (2005). Long-term effects of cyclosporine in children with idiopathic nephrotic syndrome: a single-centre experience. *Nephrol Dial Transplant*, 20, 2433–8.

Elhence, R., Gulati, S., Kher, V., *et al.* (1994). Intravenous pulse cyclophosphamide--a new regime for steroid-resistant minimal change nephrotic syndrome. *Pediatr Nephrol*, 8, 1–3.

Fakhouri, F., Bocquet, N., Taupin, P., *et al.* (2003). Steroid-sensitive nephrotic syndrome: from childhood to adulthood. *Am J Kidney Dis*, 41, 550–7.

Fujimoto, S., Yamamoto, Y., Hisanaga, S., *et al.* (1991). Minimal change nephrotic syndrome in adults: response to corticosteroid therapy and frequency of relapse. *Am J Kidney Dis*, 17, 687–92.

Fujinaga, S., Hirano, D., Nishizaki, N., *et al.* (2010). Single infusion of rituximab for persistent steroid-dependent minimal-change nephrotic syndrome after long-term cyclosporine. *Pediatr Nephrol*, 25, 539–44.

Fujinaga, S., Someya, T., Watanabe, T., *et al.* (2013). Cyclosporine versus mycophenolate mofetil for maintenance of remission of steroid-dependent nephrotic syndrome after a single infusion of rituximab. *Eur J Pediatr*, 172, 513–8.

Gellermann, J., Weber, L., Pape, L., *et al.* (2013). Mycophenolate mofetil versus cyclosporin A in children with frequently relapsing nephrotic syndrome. *J Am Soc Nephrol*, 24, 1689–97.
Gregory, M. J., Smoyer, W. E., Sedman, A., *et al.* (1996). Long-term cyclosporine therapy for pediatric nephrotic syndrome: a clinical and histologic analysis. *J Am Soc Nephrol*, 7, 543–9.
Grupe, W. E., Makker, S. P., and Ingelfinger, J. R. (1976). Chlorambucil treatment of frequently relapsing nephrotic syndrome. *N Engl J Med*, 295, 746–9.
Guigonis, V., Dallocchio, A., Baudouin, V., *et al.* (2008). Rituximab treatment for severe steroid- or cyclosporine-dependent nephrotic syndrome: a multicentric series of 22 cases. *Pediatr Nephrol*, 23(8), 1269–79.
Gulati, A., Sinha, A., Gupta, A., *et al.* (2012). Treatment with tacrolimus and prednisolone is preferable to intravenous cyclophosphamide as the initial therapy for children with steroid-resistant nephrotic syndrome. *Kidney Int*, 82, 1130–5.
Gulati, A., Sinha, A., Sreenivas, V., *et al.* (2011). Daily corticosteroids reduce infection-associated relapses in frequently relapsing nephrotic syndrome: a randomized controlled trial. *Clin J Am Soc Nephrol*, 6, 63–9.
Habib, R. and Kleinknecht, C. (1971). The primary nephrotic syndrome of childhood. Classification and clinicopathologic study of 406 cases. *Pathol Annu*, 6, 417–74.
Habib, R. and Niaudet, P. (1994). Comparison between pre- and posttreatment renal biopsies in children receiving ciclosporine for idiopathic nephrosis. *Clin Nephrol*, 42, 141–6.
Hammad, A., Yahia, S., Gouida, M. S., *et al.* (2013). Low expression of glucocorticoid receptors in children with steroid-resistant nephrotic syndrome. *Pediatr Nephrol*, 28, 759–63.
Hodson, E. M., Knight, J. F., Willis, N. S., *et al.* (2000). Corticosteroid therapy in nephrotic syndrome: a meta-analysis of randomised controlled trials. *Arch Dis Child*, 83, 45–51.
Hodson, E. M., Willis, N. S., and Craig, J. C. (2007). Corticosteroid therapy for nephrotic syndrome in children. *Cochrane Database Syst Rev*, CD001533.
Hodson, E. M., Willis, N. S., and Craig, J. C. (2008). Non-corticosteroid treatment for nephrotic syndrome in children. *Cochrane Database Syst Rev*, CD002290.
Hogan, J. and Radhakrishnan, J. (2013). The Treatment of Minimal Change Disease in Adults. *J Am Soc Nephrol*.
Hogg, R. J., Fitzgibbons, L., Bruick, J., *et al.* (2006). Mycophenolate mofetil in children with frequently relapsing nephrotic syndrome: a report from the Southwest Pediatric Nephrology Study Group. *Clin J Am Soc Nephrol*, 1, 1173–8.
Hsu, A. C., Folami, A. O., Bain, J., *et al.* (1979). Gonadal function in males treated with cyclophosphamide for nephrotic syndrome. *Fertil Steril*, 31, 173–7.
Hulton, S. A., Neuhaus, T. J., Dillon, M. J., *et al.* (1994). Long-term cyclosporin A treatment of minimal-change nephrotic syndrome of childhood. *Pediatr Nephrol*, 8, 401–3.
Iijima, K., Hamahira, K., Tanaka, R., *et al.* (2002). Risk factors for cyclosporine-induced tubulointerstitial lesions in children with minimal change nephrotic syndrome. *Kidney Int*, 61, 1801–5.
Iijima, K., Sako, M., Nozu, K., *et al.* (2014). Rituximab for childhood-onset, complicated, frequently relapsing nephrotic syndrome or steroid-dependent nephrotic syndrome: a multicentre, double-blind, randomised, placebo-controlled trial. *Lancet*, 384(9950), 1273–81.
Inoue, Y., Iijima, K., Nakamura, H., *et al.* (1999). Two-year cyclosporin treatment in children with steroid-dependent nephrotic syndrome. *Pediatr Nephrol*, 13, 33–8.
International Study of Kidney Disease in Children. 1981. The primary nephrotic syndrome in children. Identification of patients with minimal change nephrotic syndrome from initial response to prednisone. A report of the International Study of Kidney Disease in Children. *J Pediatr*, 98, 561–4.
Ishikura, K., Ikeda, M., Hattori, S., *et al.* (2008). Effective and safe treatment with cyclosporine in nephrotic children: a prospective, randomized multicenter trial. *Kidney Int*, 73, 1167–73.
Ishikura, K., Yoshikawa, N., Hattori, S., *et al.* (2010). Treatment with microemulsified cyclosporine in children with frequently relapsing nephrotic syndrome. *Nephrol Dial Transplant*, 25, 3956–62.
Ishikura, K., Yoshikawa, N., Nakazato, H., *et al.* (2012). Two-year follow-up of a prospective clinical trial of cyclosporine for frequently relapsing nephrotic syndrome in children. *Clin J Am Soc Nephrol*, 7, 1576–83.
Ito, S., Kamei, K., Ogura, M., *et al.* (2011). Maintenance therapy with mycophenolate mofetil after rituximab in pediatric patients with steroid-dependent nephrotic syndrome. *Pediatr Nephrol*, 26, 1823–8.
Kamei, K., Takahashi, M., Fuyama, M., *et al.* (2015). Rituximab-associated agranulocytosis in children with refractory idiopathic nephrotic syndrome: case series and review of literature. *Nephrol Dial Transplant*, 30, 91–6.
Kengne-Wafo, S., Massella, L., Diomedi-Camassei, F., *et al.* (2009). Risk factors for cyclosporin A nephrotoxicity in children with steroid-dependant nephrotic syndrome. *Clin J Am Soc Nephrol*, 4, 1409–16.
Kitano, Y., Yoshikawa, N., Tanaka, R., *et al.* (1990). Ciclosporin treatment in children with steroid-dependent nephrotic syndrome. *Pediatr Nephrol*, 4, 474–7.
Korbet, S. M. (1995). Management of idiopathic nephrosis in adults, including steroid-resistant nephrosis. *Curr Opin Nephrol Hypertens*, 4, 169–76.
Korbet, S. M. (1999). Clinical picture and outcome of primary focal segmental glomerulosclerosis. *Nephrol Dial Transplant*, 14 Suppl 3, 68–73.
Korbet, S. M., Schwartz, M. M., and Lewis, E. J. (1988a). Minimal-change glomerulopathy of adulthood. *Am J Nephrol*, 8, 291–7.
Korbet, S. M., Schwartz, M. M., and Lewis, E. J. (1994). Primary focal segmental glomerulosclerosis: clinical course and response to therapy. *Am J Kidney Dis*, 23, 773–83.
Koskimies, O., Vilska, J., Rapola, J., *et al.* (1982). Long-term outcome of primary nephrotic syndrome. *Arch Dis Child*, 57, 544–8.
Lahdenkari, A. T., Suvanto, M., Kajantie, E., *et al.* (2005). Clinical features and outcome of childhood minimal change nephrotic syndrome: is genetics involved? *Pediatr Nephrol*, 20, 1073–80.
Latta, K., Von Schnakenburg, C., and Ehrich, J. H. (2001). A meta-analysis of cytotoxic treatment for frequently relapsing nephrotic syndrome in children. *Pediatr Nephrol*, 16, 271–82.
Lewis, M. A., Baildom, E. M., Davis, N., *et al.* (1989). Nephrotic syndrome: from toddlers to twenties. *Lancet*, 1, 255–9.
Lombel, R. M., Gipson, D. S., and Hodson, E. M. (2013). Treatment of steroid-sensitive nephrotic syndrome: new guidelines from KDIGO. *Pediatr Nephrol*, 28, 415–26.
Macdonald, N. E., Wolfish, N., McLaine, P., *et al.* (1986). Role of respiratory viruses in exacerbations of primary nephrotic syndrome. *J Pediatr*, 108, 378–82.
Mak, S. K., Short, C. D., and Mallick, N. P. (1996). Long-term outcome of adult-onset minimal-change nephropathy. *Nephrol Dial Transplant*, 11, 2192–201.
McDonald, J., Murphy, A. V., and Arneil, G. C. (1974). Long-term assessment of cyclophosphamide therapy for nephrosis in children. *Lancet*, 2, 980–2.
McEnery, P. T., and Strife, C. F. 1982. Nephrotic syndrome in childhood. Management and treatment in patients with minimal change disease, mesangial proliferation, or focal glomerulosclerosis. *Pediatr Clin North Am*, 29, 875–94.
Meyrier, A. (1997). Treatment of idiopathic nephrotic syndrome with cyclosporine A. *J Nephrol*, 10, 14–24.
Munyentwali, H., Bouachi, K., Audard, V., *et al.* (2013). Rituximab is an efficient and safe treatment in adults with steroid-dependent minimal change disease. *Kidney Int*, 83, 511–6.
Murnaghan, K., Vasmant, D., and Bensman A. (1984). Pulse methylprednisolone therapy in severe idiopathic childhood nephrotic syndrome. *Acta Paediatr Scand*, 73, 733–9.
Nair, R. B., Date, A., Kirubakaran, M. G., *et al.* (1987). Minimal-change nephrotic syndrome in adults treated with alternate-day steroids. *Nephron*, 47, 209–10.

Nakayama, M., Katafuchi, R., Yanase, T., *et al.* (2002). Steroid responsiveness and frequency of relapse in adult-onset minimal change nephrotic syndrome. *Am J Kidney Dis*, 39, 503–12.

Niaudet, P. (1992). Comparison of cyclosporin and chlorambucil in the treatment of steroid-dependent idiopathic nephrotic syndrome: a multicentre randomized controlled trial. The French Society of Paediatric Nephrology. *Pediatr Nephrol*, 6, 1–3.

Niaudet, P. (1994). Treatment of childhood steroid-resistant idiopathic nephrosis with a combination of cyclosporine and prednisone. French Society of Pediatric Nephrology. *J Pediatr*, 125, 981–6.

Niaudet, P., and Habib, R. (1994). Cyclosporine in the treatment of idiopathic nephrosis. *J Am Soc Nephrol*, 5, 1049–56.

Nolasco, F., Cameron, J. S., Heywood, E. F., *et al.* (1986). Adult-onset minimal change nephrotic syndrome: a long-term follow-up. *Kidney Int*, 29, 1215–23.

Novak, I., Frank, R., Vento, S., *et al.* (2005). Efficacy of mycophenolate mofetil in pediatric patients with steroid-dependent nephrotic syndrome. *Pediatr Nephrol*, 20, 1265–8.

Penso, J., Lippe, B., Ehrlich, R., *et al.* (1974). Testicular function in prepubertal and pubertal male patients treated with cyclophosphamide for nephrotic syndrome. *J Pediatr*, 84, 831–6.

Pollak, V. E., Rosen, S., Pirani, C. L., *et al.* 1968. Natural history of lipoid nephrosis and of membranous glomerulonephritis. *Ann Intern Med*, 69, 1171–96.

Ponticelli, C., Edefonti, A., Ghio, L., *et al.* (1993a). Cyclosporin versus cyclophosphamide for patients with steroid-dependent and frequently relapsing idiopathic nephrotic syndrome: a multicentre randomized controlled trial. *Nephrol Dial Transplant*, 8, 1326–32.

Ravani, P., Magnasco, A., Edefonti, A., *et al.* (2011). Short-term effects of rituximab in children with steroid- and calcineurin-dependent nephrotic syndrome: a randomized controlled trial. *Clin J Am Soc Nephrol*, 6, 1308–15.

Razzak, M. (2014). Nephrotic syndrome: rituximab is safe and effective in FRNS and SDNS-but where to go from here? *Nat Rev Nephrol*, 10, 421.

Ruth, E. M., Kemper, M. J., Leumann, E. P., *et al.* (2005). Children with steroid-sensitive nephrotic syndrome come of age: long-term outcome. *J Pediatr*, 147, 202–7.

Rydel, J. J., Korbet, S. M., Borok, R. Z., *et al.* (1995). Focal segmental glomerular sclerosis in adults: presentation, course, and response to treatment. *Am J Kidney Dis*, 25, 534–42.

Sato, M., Ito, S., Ogura, M., *et al.* (2013). Atypical Pneumocystis jiroveci pneumonia with multiple nodular granulomas after rituximab for refractory nephrotic syndrome. *Pediatr Nephrol*, 28, 145–9.

Sellier-Leclerc, A. L., Baudouin, V., Kwon, T., *et al.* (2011). Rituximab in steroid-dependent idiopathic nephrotic syndrome in childhood--follow-up after CD19 recovery. *Nephrol Dial Transplant*.

Sellier-Leclerc, A. L., Belli, E., Guerin, V., *et al.* (2013). Fulminant viral myocarditis after rituximab therapy in pediatric nephrotic syndrome. *Pediatr Nephrol*, 28(9), 1875–9.

Siegel, N. J., Kashgarian, M., Spargo, B. H., *et al.* (1974). Minimal change and focal sclerotic lesions in lipoid nephrosis. *Nephron*, 13, 125–37.

Sinha, A., and Bagga, A. (2013). Rituximab therapy in nephrotic syndrome: implications for patients' management. *Nat Rev Nephrol*, 9, 154–69.

Sinha, A., Saha, A., Kumar, M., *et al.* (2015). Extending initial prednisolone treatment in a randomized control trial from 3 to 6 months did not significantly influence the course of illness in children with steroid-sensitive nephrotic syndrome. *Kidney Int*, 87, 217–24.

Sinha, M. D., Macleod, R., Rigby, E., *et al.* (2006). Treatment of severe steroid-dependent nephrotic syndrome (SDNS) in children with tacrolimus. *Nephrol Dial Transplant*, 21, 1848–54.

Siu, Y. P., Tong, M. K., Leung, K., *et al.* (2008). The use of enteric-coated mycophenolate sodium in the treatment of relapsing and steroid-dependent minimal change disease. *J Nephrol*, 21, 127–31.

Sureshkumar, P., Hodson, E. M., Willis, N. S., *et al.* (2014). Predictors of remission and relapse in idiopathic nephrotic syndrome: a prospective cohort study. *Pediatr Nephrol*, 29, 1039–46.

Tanaka, H., Tsugawa, K., Tsuruga, K., *et al.* (2006). Single-dose daily treatment with cyclosporin A for relapsing nephrotic syndrome: report of a case showing poor response. *Clin Nephrol*, 66, 219–20.

Tanphaichitr, P., Tanphaichitr, D., Sureeratanan, J., *et al.* (1980). Treatment of nephrotic syndrome with levamisole. *J Pediatr*, 96, 490–3.

Trainin, E. B., Boichis, H., Spitzer, A., *et al.* (1975). Late nonresponsiveness to steroids in children with the nephrotic syndrome. *J Pediatr*, 87, 519–23.

Trompeter, R. S., Evans, P. R., and Barratt, T. M. (1981). Gonadal function in boys with steroid-responsive nephrotic syndrome treated with cyclophosphamide for short periods. *Lancet*, 1, 1177–9.

Trompeter, R. S., Lloyd, B. W., Hicks, J., *et al.* (1985). Long-term outcome for children with minimal-change nephrotic syndrome. *Lancet*, 1, 368–70.

Ueda, N., Kuno, K., and Ito, S. (1990). Eight and 12 week courses of cyclophosphamide in nephrotic syndrome. *Arch Dis Child*, 65, 1147–50.

Vester, U., Kranz, B., Zimmermann, S., *et al.* (2005). The response to cyclophosphamide in steroid-sensitive nephrotic syndrome is influenced by polymorphic expression of glutathion-S-transferases-M1 and -P1. *Pediatr Nephrol*, 20, 478–81.

Waldman, M., Crew, R. J., Valeri, A., *et al.* (2007). Adult minimal-change disease: clinical characteristics, treatment, and outcomes. *Clin J Am Soc Nephrol*, 2, 445–53.

Wang, F., Looi, L. M., and Chua, C. T. (1982). Minimal change glomerular disease in Malaysian adults and use of alternate day steroid therapy. *Q J Med*, 51, 312–28.

Watson, A. R., Taylor, J., Rance, C. P., *et al.* (1986). Gonadal function in women treated with cyclophosphamide for childhood nephrotic syndrome: a long-term follow-up study. *Fertil Steril*, 46, 331–3.

White, R. H., Glasgow, E. F., and Mills, R. J. (1970). Clinicopathological study of nephrotic syndrome in childhood. *Lancet*, 1, 1353–9.

Williams, S. A., Makker, S. P., Ingelfinger, J. R., *et al.* (1980). Long-term evaluation of chlorambucil plus prednisone in the idiopathic nephrotic syndrome of childhood. *N Engl J Med*, 302, 929–33.

Wynn, S. R., Stickler, G. B., and Burke, E. C. (1988). Long-term prognosis for children with nephrotic syndrome. *Clin Pediatr (Phila)*, 27, 63–8.

Yoshikawa, N., Nakanishi, K., Sako, M., *et al.* (2015). A multicenter randomized trial indicates initial prednisolone treatment for childhood nephrotic syndrome for two months is not inferior to six-month treatment. *Kidney Int*, 87, 225–32.

Zagury, A., De Oliveira, A. L., De Moraes, C. A., *et al.* (2011). Long-term follow-up after cyclophosphamide therapy in steroid-dependent nephrotic syndrome. *Pediatr Nephrol*, 26, 915–20.

CHAPTER 57

Primary focal segmental glomerulosclerosis: clinical features and diagnosis

Alain Meyrier and Patrick Niaudet

Introduction

Focal segmental glomerulosclerosis (FSGS) is the other histopathologic subset of idiopathic nephrotic syndrome. It accounts for 40% of cases in adults but < 20% of cases in children (Korbet, 2012). FSGS is often '*idiopathic*' but cannot be considered as '*primary*' without ruling out identified aetiologies of the podocytopathy that characterize it and leads to proteinuria, glomerular sclerosis, and end-stage renal failure. Therefore FSGS is not a disease but a clinicopathologic nephrotic condition, the description of which is primarily based on the lesions found on a kidney biopsy. Of note, the umbrella term FSGS is a misnomer as the lesions are not always focal, segmental, and/or sclerotic.

Demography

FSGS is considered 'primary' when all causes of 'secondary' FSGS have been excluded (Table 57.1). This subset of idiopathic nephrotic syndrome accounts for 20% of children and 40% of adults with an estimated incidence of 7 per million (D'Agati et al., 2011; Korbet, 2012). For unknown reasons the incidence of idiopathic FSGS has steadily increased over recent decades, in children (Filler et al., 2003) and in adults (Braden et al., 2000). This subset of idiopathic nephrotic syndrome represents a major cause of end-stage renal failure leading to renal replacement therapy with a prevalence of 4% in the United States.

There is a strong predominance in black patients of African ancestry that is explained by inherited risk factors. This population develop more severe forms of FSGS than their white and Asian fellow sufferers, are less prone to enjoy remission with treatment (Crook et al., 2005) and follow a more rapid course to end-stage renal disease (ESRD) when their kidney histology reveals cellular forms of FSGS (Schwartz et al., 1999).

This risk has been strongly associated with sequence variants (G1 and G2) in the gene encoding apolipoprotein L1 (*APOL1*) (Genovese et al., 2010; see Chapter 341). This association of both G1 and G2 seems to account for the excess risk of FSGS in African Americans, both in primary FSGS and in HIV-associated nephropathy (HIVAN). The same variants were also associated with hypertension-attributed end-stage kidney disease, raising the question of whether the hypertension in these patients was in fact a sign of kidney disease. Alternatively the genotype confers greater susceptibility to progression of different types of injury. The *APOL1* gene is very close to the *MYH9* gene and represents a 100-fold greater risk factor than had previously been associated with the latter (Kopp et al., 2008). APOL1 is a lysis factor for *Trypanosoma brucei brucei,* the parasite transmitted by the Tsetse fly and causing sleeping sickness. However, *T. brucei gambiense* resists *APOL1* lysis and this could have induced a selection of African subjects genetically protected from *T. brucei brucei* sleeping illness but with a trade-off propensity to APOL1 G1 and G2 induced podocyte injury, the mechanism of which is still unknown.

Histopathology

Distribution of lesions

FSGS is characterized by focal lesions affecting a variable proportion of glomeruli at any time during the course of the disease (Churg et al., 1970; Habib and Gubler, 1973). They predominate in the deeper cortex and juxtamedullary glomeruli are mainly affected (Rich, 1957). Focal changes are frequently limited to part of the tuft. Glomerular hypertrophy is common in FSGS, and glomerulomegaly found in minimal change disease (MCD) is somewhat predictive of further development to FSGS (Fogo et al., 1990; Muda et al., 1994).

By serial three-dimensional (3D) ultrathin sections the distribution of focal sclerosis is more widespread than what is seen by conventional microscopy. Fogo et al. studied kidney biopsies from 15 adults and six children (Fogo et al., 1995). Sclerosis assessed on a single section involved 31.5 ± 6.8% of glomeruli in adults, contrasting with only 11.7 ± 5.7% in children. After 3D screening, the percentage of glomeruli involved by sclerosis increased to 48.0 ± 6.6% in adults and 23.2 ± 7.4% in children. The greater increase in sclerosis after 3D analysis in children versus adults reflected the predominance of small peripheral, that is, more segmental, lesions in children than adults. Similar findings were described by Fuiano et al. (1996). They concur to indicate that FSGS is less segmental and probably less focal than previously thought. This may explain the fact that FSGS can be overlooked on an early initial kidney biopsy or in cases of sampling error with < 10 glomeruli studied by light microscopy.

Table 57.1 Differential expression of cyclin-dependent kinase inhibitors in human glomerular disease

Response (N)	All (187)	Cell (18)	CG (53)	GTL (33)	NOS (83)
CR-PR-F (N)	51-21-115	7-1-10	4-3-46	20-5-8	20-12-51
CR+PR (%)	38.5	44.4	13.2	75.8	38.6
ESRD (%)	30.7	27.8	65.3	5.7	34.5
Kaplan–Meier months survival to ESRD (median)	72 ± 10	35 ± 6	23 ± 11	85 ± 8	90 ± 10

Cell = cellular FSGS; CG = collapsing FSGS; CR = complete remission; ESRD = end-stage renal disease; F = no remission; GTL = glomerular tip lesion variant; NOS = FSGS not otherwise specified; PR = partial remission.

Part of Table 57.1 from Stokes et al. (2006).

The initial podocyte lesion

FSGS is a podocyte disease that starts with changes that are initially only detectable by electron microscopy, as shown by kidney biopsy carried out when proteinuria relapses shortly, sometimes within hours, after kidney transplantation (Schwartz and Korbet, 1993; Kriz et al., 1994; Rydel et al., 1995; Schwartz et al., 1995; Bariéty et al., 1998a; Elger and Kriz, 1998). Relapse of nephrotic primary FSGS on a renal transplant offers a privileged model to study the incipient lesion in man and to follow its progression (Verani and Hawkins, 1986; Korbet et al., 1988; Bariéty et al., 2001). In such a case the initial appearance is often limited to foot process fusion that can be restricted to some segments of the glomerular basement membrane. As noted in Chapter 56, this fusion, or 'flattening' of the podocyte foot processes is due to a rearrangement of the actin cytoskeleton fibres. However, contrary to MCD, this rearrangement is usually not reversible. In fact the podocyte cytoskeleton derangement is an essential factor inherent in the pathogenesis of FSGS (Mathieson, 2010).

Within weeks following recurrence of proteinuria, podocytes observed by electron microscopy appear swollen and vacuolated. Some vacuoles are round and by immunofluorescence appear to contain immunoglobulin (Ig)-A (Fig. 57.1). The podocytes exhibit strong mitotic activity, with multinucleation and expression of the PCNA and Ki-67 proliferation markers. The number of visceral epithelial cells seems to be increased, suggesting a mitotic activity and replication. Podocytes detached from the glomerular basement membranes assume a round shape and form grape-like clusters of cells on the outer aspect of the tuft. Their number may be such that they assume the appearance of a pseudocrescent. Some appear to drift free in the urinary chamber, and migrate into the tubular lumen (Oda et al., 1998) (Fig. 57.2). Other dysmorphic podocytes assume a 'cobblestone' pattern covering the outer aspect of the tuft. The parietal epithelial cells of Bowman's capsule facing this line of cuboid cells usually proliferate and form a pluricellular stratum (Fig. 57.3). Underlying endocapillary lesions comprise foam cells, macrophages, and mesangial cell proliferation along with capillary loop collapse. This subset of FSGS is the '*cellular lesion*' (Schwartz and Lewis, 1985). Further stages are characterized by extracellular matrix build-up, comprising ubiquitous collagen, leading to the *scar lesion*, variably hilar, peripheral, or central (Schwartz and Korbet, 1993; Schwartz et al., 1995; D'Agati et al., 2011).

The progression to segmental glomerular lesions

Following a progression that may take a few weeks, the lesion of FSGS affects a few capillary loops, which stick together either at the hilum or at the periphery of the tuft, often at both. Hyaline material is often present within sclerotic areas, appearing as a peripheral rim or as round deposits obstructing lumens. Foamy endothelial cells and lipid inclusions may be found. At the periphery of sclerotic segments there is, in most cases, a clear 'halo' (Fig. 57.4). The segmental lesion has a different appearance depending on whether it affects a group of capillary loops free in Bowman's space or is adherent to Bowman's capsule. The 'free' sclerotic segments are surrounded by a layer of cuboid podocytes ('cobblestones') in close apposition to the clear 'halo'. When the sclerotic lesion adheres to Bowman's capsule, podocytes are no longer identifiable and a synechia forms an adherence between the collapsed capillary loops and Bowman's capsule. The rest of the tuft and the non-sclerotic glomeruli show either 'minimal changes' or 'mesangial proliferation' with foot-process effacement (Figs 57.5, 57.6, and 57.7).

In some areas the tuft is separated from Bowman's capsule by a continuous layer of parietal epithelial cells progressing along the outer aspect of the tuft, thereby circumscribing an empty slit and

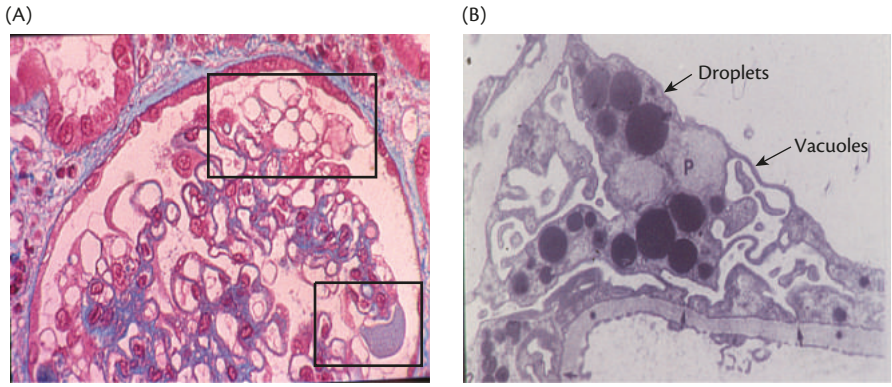

Fig. 57.1 Dysmorphic dedifferentiated podocytes.
(A) The upper part of this glomerulus (upper rectangle) shows a cluster of hypertrophied podocytes, each one forming a giant clear vacuole limited by a thin border of remaining cytoplasm. In the lower rectangle a grossly hypertrophied podocyte seems about to detach from the glomerular basement membrane. (Masson trichrome, ×350.)
(B) Electron microscopy discloses clear vacuoles (lower arrow) and proteinaceous droplets (upper arrow). (Uranyl-acetate lead citrate ×4500.)

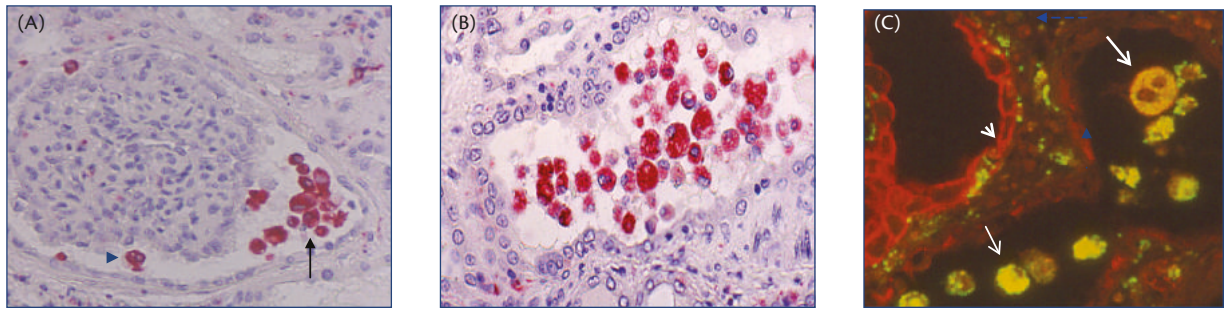

Fig. 57.2 Microscopic preparation using a monoclonal anti-CD68 antibody that labels macrophagic epitopes in red. Detachment and migration of transdifferentiated podocytes. (A) A cluster of detached podocytes (thin arrow) migrates into Bowman's chamber towards the glomerular outlet to the proximal tubule. An isolated macrophage-like cell (arrowhead) is bi-nucleated indicating mitosis. (B) Proximal tubule. The macrophagic-like cells drift along the tubules. The tubular epithelium is thinned and atrophic, limited to a single layer of tubular cells. The surrounding interstitium is fibrous and inflammatory.
(C) Confocal laser microscopy using an anticytokeratin (CK) monoclonal antibody (AE1/AE3 anti-CK mAb.) that labels the tubular basement basement membrane in bright red (arrowhead) and a monoclonal antibody (PGM1) that identifies macrophagic epitopes (CD68). The macrophage-like cells appear in a yellowish-green colour (dotted arrow). One of the cells (broken arrow) assumes a brownish colour indicating the presence of CD68 and cytokeratin. This indicates that a process of epithelial-mesenchymatous transition (EMT) is at work. This cell is bi-nucleated indicating mitosis.

assuming the appearance of a pseudo-tubule. This pseudo-tubule persists in obsolescent glomeruli, which allows retrospective diagnosis of terminal forms of FSGS (Fig. 57.8).

Glomerular obsolescence follows. FSGS is an irreversible scarring process, as demonstrated by repeat biopsies (Velosa et al., 1975; Nash et al., 1976). The whole tuft is sclerotic, often in association with conspicuous interstitial and tubular damage. In children, focal global sclerosis should be differentiated from 'congenital glomerulosclerosis', which is a developmental anomaly frequently found in kidneys of infants and young children (Kohaut et al., 1976) and is not associated with tubulointerstitial changes. In normal adults, the number of sclerotic glomeruli increases with age (Kaplan et al., 1975) and their interpretation on biopsies from patients > 40 years is difficult (Cameron et al., 1974).

Electron microscopy shows that capillary obliteration is mainly due to paramesangial and subendothelial, finely granular deposits (Velosa et al., 1975) with endothelial cell disappearance or swelling, and increased mesangial matrix. Fatty vacuoles may be seen, among the abnormal deposits or in the cytoplasm of endothelial and mesangial cells. Synechiae are formed by apposition of a cloudy, acellular material containing thin and irregular layers of newly formed basement membrane (Fig. 57.9). Sclerotic lesions result from a marked increase in glomerular basement menbrane-like material with capillary wall wrinkling and capillary collapse (Rumpelt and Thoenes, 1974). Collagen fibres are seen within some segmental areas. Studies in animals (Couser and Stilmant, 1975) and in nephrotic patients have shown that proteinuria precedes the development to focal sclerotic lesions. The same sequence was reported in patients with recurrence after transplantation.

Cell dedifferentiation and transdifferentiation in cellular forms of FSGS

Since 1998, a number of studies, especially but not exclusively regarding collapsing forms of FSGS, have shown that the process inducing the podocyte disease which characterizes this condition is accompanied by striking changes occurring in these visceral

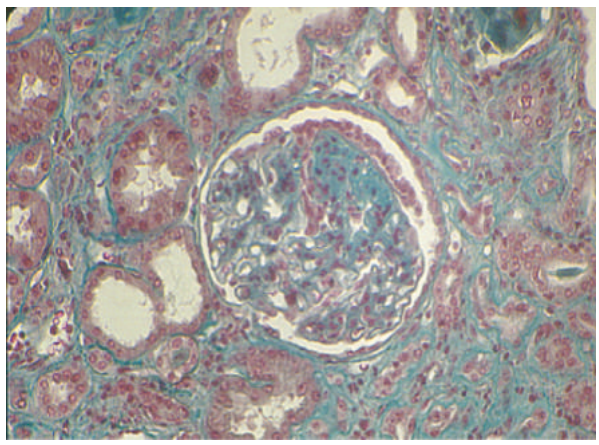

Fig. 57.3 'Cobblestones'. Masson trichrome. A row of dedifferentiated podocytes lines a large scar lesion. Parietal epithelial cells (not shown here) may participate to 'cobblestone' formation and form a pseudotubule as shown in Fig. 57.8.

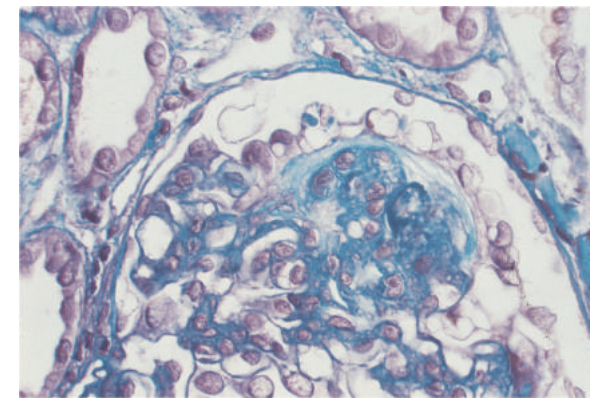

Fig. 57.4 Focal segmental sclerosis/hyalinosis: segmental lesion of the tuft characterized by the deposition of hyaline material in the inner side of glomerular basement membrane, and a ring of detached podocytes separated from the glomerular basement membrane by a clear 'halo'. (Masson's trichrome, ×520.)

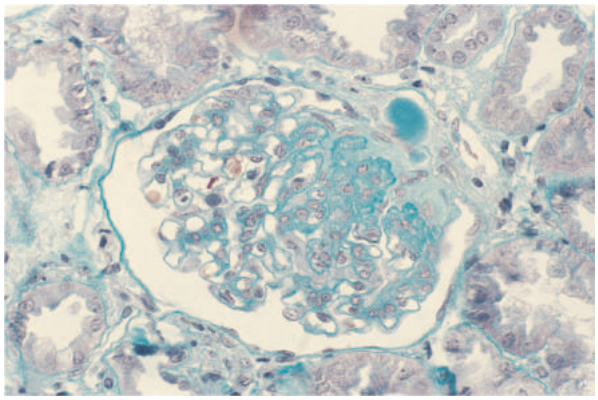

Fig. 57.5 Focal segmental sclerosis/hyalinosis: obliteration of capillary lumens at the vascular pole of the glomerulus by a combination of sclerosis and hyalinosis. (Masson's trichrome, ×320.)

epithelial cells. These changes have been attributed to a profound cell cycle derangement.

The normal mature podocyte is unable to replicate and does not express the proliferation markers PCNA and Ki-67. It is characterized by the expression of numerous epitopes comprising Wilms tumour protein-1 (WT-1), common acute lymphoblastic leukaemia antigen (CALLA), C3b receptor (CR1), glomerular epithelial protein-1 (GLEPP-1), podocalyxin, synaptopodin, and vimentin. The first stages of FSGS, as observed when the lesions relapse on a renal transplant (Bariéty et al., 2001) and in 'collapsing glomerulopathy' (Bariéty et al., 1998b; Barisoni et al., 1999, 2000), are characterized by loss of normal podocyte epitopes and acquisition of macrophagic and cytokeratin markers. Nuclear proliferation markers PCNA and Ki-67 are expressed indicating strong mitotic activity. Such 'podocyte dysregulation' is accompanied by podocyte detachment from the basement membranes, and migration into the urinary chamber and the tubular lumens where they assume a round shape and occasionally co-express macrophagic and cytokeratin epitopes. By laser confocal microscopy some of these round cells co-express CD68 or cytokeratin markers and original podocyte markers such as podocalyxin (Fig. 57.10). Shankland et al. showed that the expression of cyclins and cyclin inhibitors of the Cip/Kip family differ among patients with MCD or idiopathic membranous glomerulopathy and those with cellular focal segmental glomerulonephritis (Shankland et al., 2000). These remarkable findings are summarized in Table 57.2.

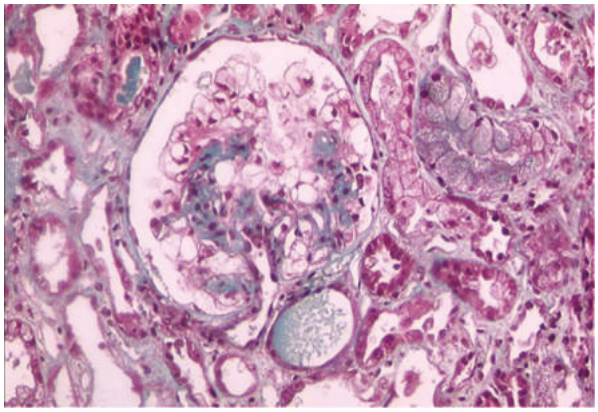

Fig. 57.6 Collapsing glomerulopathy. Masson trichrome, ×200. The glomerular tuft is shrunken and only a withered stalk remains (arrowhead). A great number of de-differentiated podocytes are completely vacuolated and occupy the urinary chamber (thin arrow). When more numerous they may assume the appearance of a pseudocrescent.
Courtesy of Laure-Hélène Noël MD, Necker Hospital, Paris, France.

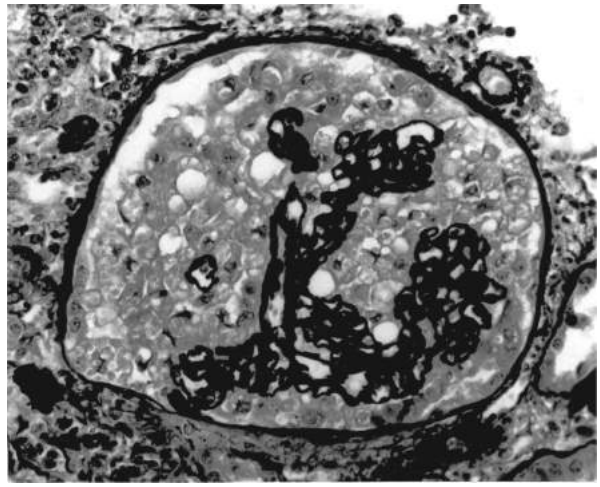

Fig. 57.7 Cell-FSGS. Methenamine silver–periodic acid–Schiff (Jones stain), ×185. This variant of FSGS was first described by Schwartz and Lewis in 1985 (Schwartz and Lewis, 1985).There is a collapse of all of the glomerular capillaries and diffuse proliferation and hypertrophy of the visceral epithelial cells. It is difficult to find a difference, if any, between this picture and severe forms of the 'Collapsing variant' of the Columbia classification.
From Schwartz et al. (1999).

Reversibility of the cellular FSGS lesion

That a circulating factor induces proteinuria followed by podocyte lesions, capillary collapse, and perpetuating to fibrosis is a well-established notion. The question at stake is to know if the initial podocytopathy is at some point reversible before sclerosis sets in. A few privileged clinical observations point to a

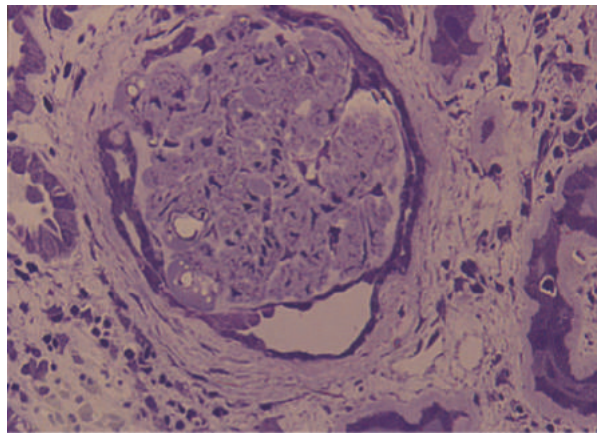

Fig. 57.8 Appearance of terminal FSGS. Toluidine blue stain. ×250.This glomerulus is totally sclerotic. However a pseudotubule remains at the lower pole, presumably formed by a continuous joining of visceral and parietal epithelial cells, a feature indicating retrospectively that the initial glomerulopathy was FSGS.

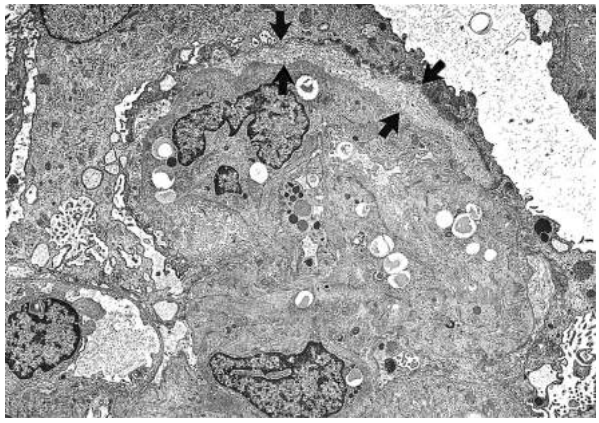

Fig. 57.9 Focal segmental sclerosis/hyalinosis (electron microscopy): note the presence around the collapsed capillary loops of multilayered basement-membrane material in a subepithelial location (arrows). (Uranyl acetate–lead citrate, ×2380.)

reversibility of both proteinuria and podocytopathy when a normal kidney is withdrawn from the 'nephrotic environment'. This was shown in cases of pregnancy when after delivery of a woman with nephrotic FSGS the children were proteinuric at birth and proteinuria progressively waned in a matter of weeks (Lagrue et al., 1989; Kemper et al., 2001). A paradigmatic case demonstrates the reversibility of proteinuria and of podocyte changes. A patient with nephrotic FSGS was transplanted with a living donor kidney. Proteinuria recurred within 2 days. The recipient was fully nephrotic with a decline in renal function. Postoperative biopsies showed recurrence of the podocytopathy. The kidney was removed and re-transplanted in a patient whose primary renal disease was diabetic glomerulopathy. The evolution was to disappearance of proteinuria, recovery of renal function, and kidney biopsies showed that podocytes had recovered a normal appearance within 2 weeks (Gallon et al., 2012). Such observations show that removing or counteracting the elusive glomerular permeability factor (GPF) should be the first goal of treatment, a goal poorly achieved with plasmapheresis (see Chapter 289). In fact, the nature of the GPF and its source are still virtually unknown. Neutralizing the GPF and/or acting on its source would represent the real treatment of FSGS and, as analysed below, this goal is not necessarily based on our current corticosteroid and immunosuppressive therapy.

Associated tubulointerstitial lesions

Tubular atrophy and interstitial fibrosis are common and proportional to the glomerular damage (Hyman and Burkholder, 1973; Newman et al., 1976). Overlooked FSGS should therefore be suspected when tubular and interstitial changes are associated with minimal glomerular changes. In rare instances tubular lesions

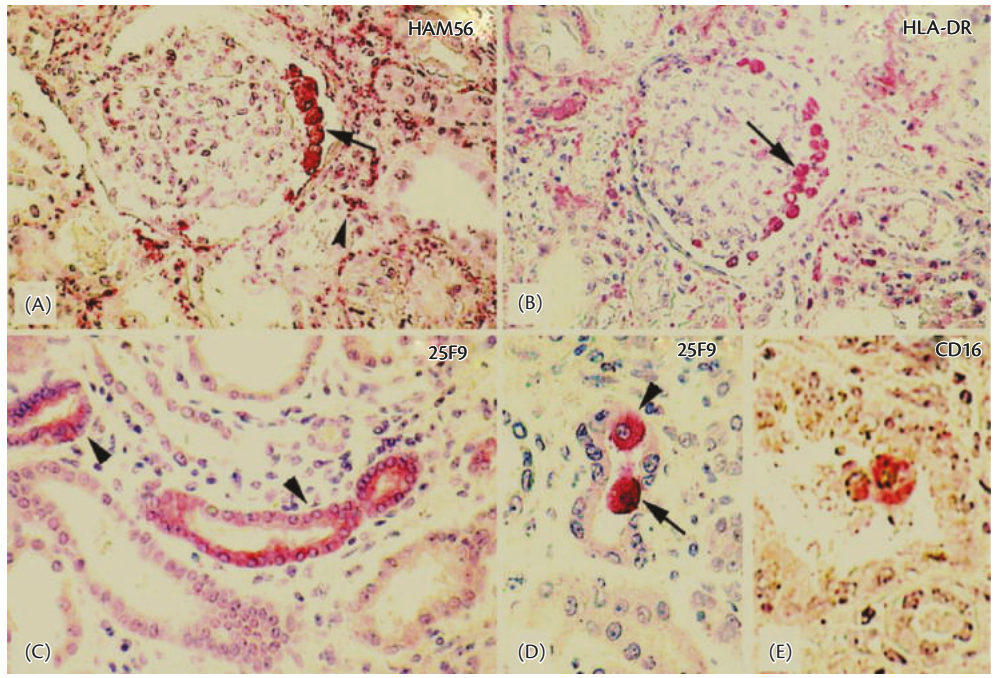

Fig. 57.10 Podocyte transdifferentiation in FSGS. (A) HAM56, a marker of the human macrophage, labels large round cells aligned at the periphery of the glomerular tuft (arrow). Note the numerous small spindle-shaped cells in the interstitium (arrowhead). (B) CRD/43, an mAb specific for HLA-DR, labels a row of large round cells at the periphery of the tuft (arrow), indicating that these cells can be considered activated. (C) 25F9 mAb, a marker of macrophage maturation, is detected on all cells of some tubules (arrowheads), whereas other tubules do not exhibit such transdifferentiation. Interstitial inflammatory cells are not tagged by 25F9. (D) 25F9 mAb labels two large round cells in a tubular lumen. One (arrow) is apparently free in the lumen. The other (arrowhead) seems to be inserted between tubular cells or, alternatively, could be a transdifferentiated tubular cell. (E) Anti-CD16 mAb, a marker of activated macrophages, tags large round cells within the tubular lumens. The interstitial inflammatory cells are CD16 negative. (Magnifications: ×120 in A and B; ×200 in C and D.)
From Bariéty et al. (2001).

Table 57.2 Differential expression of cyclin-dependent kinase inhibitors in human glomerular disease

	p57	p27	p21	Ki-67
Controls	+	+	–	–
Minimal change disease	+	+	–	–
Membranous glomerulopathy	+	+	–	–
FSGS, cellular variant	–	–	+	+
FSGS, collapsing variant	–	–	+	+
HIV-associated FSGS	–	–	+	+

The expression by podocytes of the cyclin-dependent kinase inhibitors p57, p27, and p21 and the proliferation marker Ki-67 differs among control renal tissue and glomerular disease tissue. Notably, the cellular and collapsing variants of idiopathic FSGS and HIV-associated FSGS share a similar podocyte phenotype.

Data summarizing Shankland et al. (2000).

predominate (Hayslett et al., 1969). Foam cells may be seen in the interstitium and occasionally in the glomeruli.

Detached podocytes that drift along the tubules and can be recovered in the urine might explain the tubulointerstitial lesions. These dysregulated podocytes express macrophagic markers and it is conceivable that along with heavy proteinuria they contribute to injuring the tubular cells.

Histological subtypes of FSGS

A consensus workshop elaborated a classification of FSGS that is widely used and has the merit of making pathologists speak the same language (D'Agati et al., 2004). This 'Columbia classification' applies both to idiopathic and secondary forms of FSGS. It sorts out five histologic subtypes shown in Figs 57.6, 57.7, 57.11, 57.12, and 57.13.

1. *FSGS not otherwise specified (NOS).* This common variant is a diagnosis of exclusion based on absent features of the following forms. These other forms may evolve into FSGS NOS.
2. *Perihilar FSGS.* This subtype consists of lesions located at the vascular pole of the glomerular tuft. It is considered as an 'adaptive' form of FSGS and is accompanied with glomerulomegaly. It is the common appearance in secondary forms with hyperfiltration.
3. *Cellular FSGS.* It is characterized by podocyte hyperplasia and endocapillary hypercellularity, foam cells and leucocyte infiltration.
4. *Collapsing glomerulopathy.* This 'implosive' type of FSGS is characterized by segmental or global wrinkling and collapse of the glomerular capillary walls with prominent hypertrophy and hyperplasia of the overlying podocytes that can form 'pseudocrescents'. Biopsies containing any glomeruli with global collapse and/or > 20% of glomeruli with segmental collapse are considered to be 'collapsing glomerulopathy'.

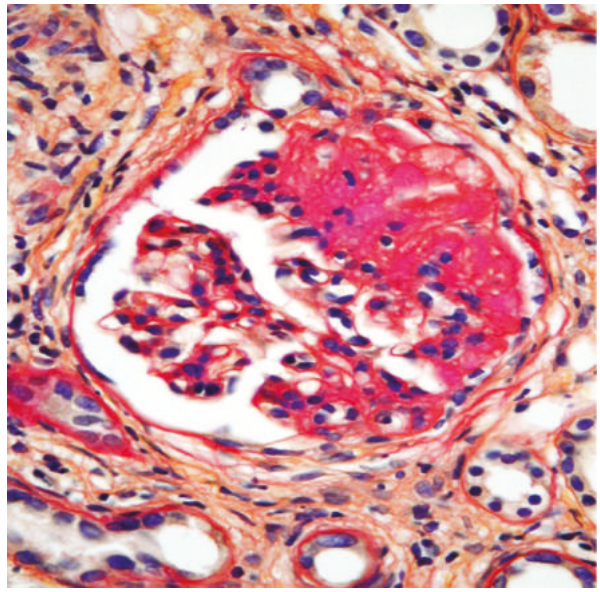

Fig. 57.11 FSGS 'NOS' ('not otherwise specified'). Counterstained periodic acid–Schiff staining. A large area of the glomerulus is fibrous (upper half, bright red) and still contains sparse podocyte remnants. Another lesion is found in the lower pole of this glomerulus and involves several lobules.

Courtesy of Ian Roberts MD, Pathology Department, John Radcliffe Hospital, Oxford, UK.

5. *Glomerular tip lesion (GTL).* The lesion involves the tubular pole. It might be due to an adaptive mechanism to protein-rich ultrafiltrate causing podocyte shear stress and prolapse of part of the tuft protruding into the initial segment of the tubule.

Clinicopathologic correlations

Three variants of the above classification deserve a particular attention with regard to their clinical implications.

Collapsing glomerulopathy

Collapsing glomerulopathy (CG) is a severe form of cellular FSGS. In 1986, Weiss et al. published on 'A new clinicopathologic entity' observed in a small group of patients with glomerular 'collapse' and nephrotic syndrome rapidly progressing to renal failure

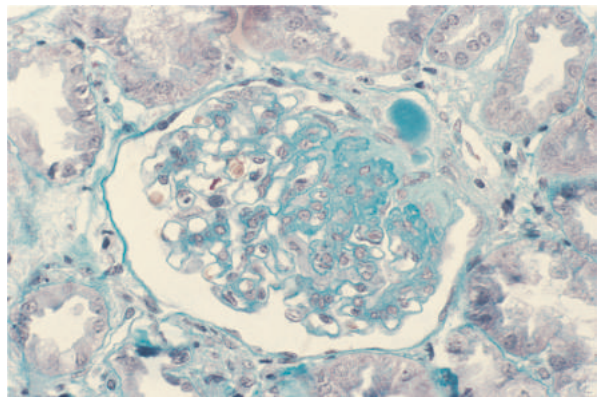

Fig. 57.12 Focal-segmental sclerosis/hyalinosis: obliteration of capillary lumens at the vascular pole of the glomerulus by a combination of sclerosis and hyalinosis. (Masson's trichrome, ×320.)

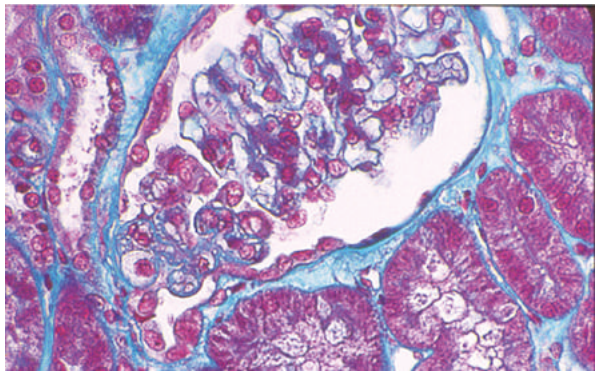

Fig. 57.13 Typical tip lesion. The photomicrograph is focused on the 'glomerular outlet'. Note the 'cell-type' of this form of focal–segmental glomerulonephritis, with abundant, swollen macrophages in the capillary lumens. (Masson's trichrome, ×600.)
Courtesy of Professor Jean Bariéty, Paris VI University, Broussais-Hôtel Dieu Medical School and INSERM U 430, Paris.

(Weiss et al., 1986). These features closely resembled those of the newly described HIVAN and in fact some of their patients were HIV-1 infected. Eight years later, Detwiler et al. reported on 16 HIV-negative patients with idiopathic FSGS, a rapid course, and collapsing glomerular features characterized by segmental or global wrinkling and collapse of the glomerular capillary walls with prominent hypertrophy and hyperplasia of the overlying podocytes (Detwiler et al., 1994). Biopsies containing any glomeruli with global collapse and/or > 20% of glomeruli with segmental collapse were considered to be 'collapsing glomerulopathy'. In fact, earlier descriptions of FSGS comprised a collapsing component of some capillary loops (Velosa et al., 1975). Korbet et al. studied two cases of recurrent FSGS on renal allografts. Focal glomerular lesions consisted of segmental epithelial cell proliferation with mitotic figures and collapse of glomerular capillaries (Korbet et al., 1988). This cellular and collapsing lesion was later followed by a scar lesion. The same was found by Bariéty et al. after relapse of FSGS on renal transplants (Bariéty et al., 2001).

Since then, numerous articles have appeared which all consider CG as a distinct form of FSGS with increasing incidence, distinct clinicopathologic features, black racial predominance, a relative steroid resistance, and a rapidly progressive course. Collapsing glomerulopathy may be 'idiopathic' (Nagata et al., 1998; Barisoni et al., 1999, 2000), recur on a renal transplant (Clarkson et al., 1998; Toth et al., 1998), represent a variety of *de novo* glomerular disease after renal transplantation (Meehan et al., 1998; Stokes et al., 1999), or be associated with Loa Loa (Pakasa et al., 1997), malaria (Niang et al., 2008), or viral infection other than HIVAN (see Chapter 187). Viral aetiologies are discussed below.

Cellular FSGS

'Cell-FSGS' described by Schwartz and Lewis is a common form of FSGS (Schwartz and Lewis, 1985). Schwartz et al. do not sort out 'cell-FSGS' from collapsing glomerulopathy and their histopathologic pictures of cell-FSGS are indistinguishable from CG (Schwartz et al., 1999) (Fig. 57.7). In the latter article, the prognosis of the cellular lesion was retrospectively studied in 100 patients with FSGS (43 had cell-FSGS and 57 had FSGS with the classic segmental scar (CS)). Patients with the cell-FSGS lesion were more often black and severely proteinuric and developed more ESRD. They were more proteinuric at presentation than patients with FSGS-CS. ESRD developed more frequently in patients with cell-FSGS and patients with extensive cell-FSGS (≥ 20% glomeruli) were mainly black (94%), severely nephrotic (67%, >10 g/day), and had a poor response to treatment (23% remission). The rates of remission in treated nephrotic patients with cell-FSGS and FSGS-CS were the same (53% vs 52%), and patients in both groups who achieved a remission had a 5-year survival of 100%. Steroid responsiveness was the only variable that predicted remission. These data are not worse than those of Stokes et al. (Table 57.1) who in 187 cases of FSGS (all types) found 72 cases (38.5%) of complete plus partial remission (Stokes et al., 2006).

Glomerular tip lesion

The GTL is the most benign form of FSGS. Stokes et al followed up 29 cases. The clinical onset of the nephrotic syndrome was rapid, as it is in MCD, there was no tubular disease and the response to treatment was favourable with 21 complete plus partial remissions and eight failures (Stokes et al., 2004). The authors concluded that 'Within the MCD/FSGS spectrum, GTL is a distinctive and prognostically favourable clinico-pathologic entity whose presenting features and outcome more closely approximate those of MCD'.

Differential diagnosis

Clinical

Heavy proteinuria with or without the full-blown picture of nephrotic syndrome may be the consequence of virtually all glomerulopathies. This implies that the diagnosis of FSGS rests on a kidney biopsy comprising a sufficient number of glomeruli adequately processed for light, electron, and immunofluorescence microscopy. However, it is worth recalling that *primary* FSGS does not comprise extrarenal abnormalities. This not the case for some forms of *secondary* FSGS that can be syndromic as briefly analysed below.

Histopathologic

FSGS is well defined histologically but some forms may still pose a diagnostic problem. This is true of elderly patients (although the definition of 'elderly' is subject to some interpretation), patients in whom other diseases may induce glomerular lesions (especially the hilar variant) that cannot be considered as true primary FSGS. A kidney biopsy disclosing only obsolescent glomeruli may also leave the diagnosis of the primary glomerulopathy pending, except when remnant pseudotubules (Fig. 57.8) suggest a diagnosis of terminal FSGS. This has practical implications in case of post-transplant relapse in a patient whose primary glomerulopathy had not been previously identified (see Chapter 283).

Aetiologic

The renal biopsy appearance of FSGS can be *secondary* to diverse mechanisms (see Box 57.1). This is an important distinction as these are unlikely to respond to the same therapies.

Box 57.1 Main conditions associated with 'secondary' focal segmental glomerulosclerosis[a]

- Hyperfiltration inducing shear stretch on podocytes[b] (Chapter 139)
- Unilateral renal agenesis or hypoplasia (Chapter 138)
- Renal ablation—remnant kidney
- Reflux interstitial nephropathy (chapter 355)
- Oligomeganephronia
- Morbid obesity
- Sickle cell disease (Chapter 167)
- Diabetic glomerulopathy (Chapter 149)
- Anabolic androgens
- Congenital cyanotic heart disease
- Viral infections:
  - HIV-associated nephropathy (HIVAN)
  - Parvovirus B 19[c]
  - Simian virus SV 40[c]
  - Cytomegalovirus (CMV)
  - Epstein–Barr virus (EBV)
  - Hepatitis C virus (HCV)
- Toxic agents (Chapter 82):
  - Heroin
  - Pamidronate-alendronate
  - Lithium
  - Interferon
  - Bevacizumab (anti VEGF monoclonal antibody)
  - Ciclosporin
- Ageing (Chapter 300)
- "Hypertensive nephrosclerosis" (see Chapters 100, 208)
- Hereditary conditions (see Chapter 45):
  - Gene defects in the slit diaphragm (Nephrin, podocin, CD2AP, actinin 4, TRCP6)
  - Mitochondrial cytopathies
  - WT1 mutation (Denys–Drash and Frasier syndromes)
  - Thin membrane disease with collagen mutations.

[a] These secondary forms require a supportive regimen. In some, such as HIVAN and HCV, antiviral treatment is required. When a toxic agent has been identified, its removal is mandatory.
[b] The size (diameter and volume) of glomeruli is often distinctly greater than normal. This 'glomerulomegaly' may induce a shear stretch on podocytes, see Chapters 136 and 139.
[c] Not definitely proven.

A common hypothesis is that FSGS is a secondary consequence of haemodynamic stretch on podocytes (Kriz et al., 2013) (see Chapter 136). It is argued that this could explain FSGS seen in obesity and nephron number reduction, such as, amongst others, remnant kidney, oligomeganephronia, reflux nephropathy, and various nephropathies with widespread glomerular obsolescence.

A particular issue is that of *viral aetiologies*. Following the identification of HIV-associated nephropathy, researchers claimed that such viruses as parvovirus B 19 (Moudgil et al., 2001), simian virus SV 40 (Li et al., 2002), hepatitis B virus (Sakai et al., 2011), and hepatitis C virus (Sperati, 2012) may cause the collapsing variant of FSGS. In fact, a critical review of the literature based on case reports and small case series does link infection with some common viruses and glomerular injury, including anecdotal cases of collapsing glomerulopathy associated with cytomegalovirus (CMV) and Epstein–Barr virus (EBV; Chandra and Kopp, 2013). However evidence for a pathogenic role is generally stronger for glomerulonephritis than specifically for collapsing glomerulopathy. The evidence linking collapsing glomerulopathy with CMV is relatively strong but not yet conclusive, whilst the evidence for a pathogenic role for EBV and parvovirus B19 is weaker.

The identification of *genetic forms* of FSGS has elicited an immense interest and a flurry of publications over the last two decades. Some forms are syndromic, that is, comprising extrarenal abnormalities and others non-syndromic with only kidney involvement. This major breakthrough is covered in Chapter 327. Interestingly, treatment responses are described for some genetic causes.

References

Bariéty, J., Bruneval, P., Hill, G., *et al.* (2001). Posttransplantation relapse of FSGS is characterized by glomerular epithelial cell transdifferentiation. *J Am Soc Nephrol*, 12, 261–74.

Bariéty, J., Nochy, D., Jacquot, C., *et al.* (1998a). Diversity and unity of focal and segmental glomerular sclerosis. *Adv Nephrol Necker Hosp*, 28, 1–42.

Bariéty, J., Nochy, D., Mandet, C., *et al.* (1998b). Podocytes undergo phenotypic changes and express macrophagic-associated markers in idiopathic collapsing glomerulopathy. *Kidney Int*, 53, 918–25.

Barisoni, L., Kriz, W., Mundel, P., *et al.* (1999). The dysregulated podocyte phenotype: a novel concept in the pathogenesis of collapsing idiopathic focal segmental glomerulosclerosis and HIV-associated nephropathy. *J Am Soc Nephrol*, 10, 51–61.

Barisoni, L., Mokrzycki, M., Sablay, L., *et al.* (2000). Podocyte cell cycle regulation and proliferation in collapsing glomerulopathies. *Kidney Int*, 58, 137–43.

Braden, G. L., Mulhern, J. G., O'Shea, M. H., *et al.* (2000). Changing incidence of glomerular diseases in adults. *Am J Kidney Dis*, 35, 878–83.

Cameron, J. S., Turner, D. R., Ogg, C. S., *et al.* (1974). The nephrotic syndrome in adults with 'minimal change' glomerular lesions. *QJM*, 43, 461–88.

Chandra, P. and Kopp, J. B. (2013). Viruses and collapsing glomerulopathy: a brief critical review. *Clin Kidney J*, 6, 1–5.

Churg, J., Habib, R., and White, R. H. (1970). Pathology of the nephrotic syndrome in children: a report for the International Study of Kidney Disease in Children. *Lancet*, 760, 1299–302.

Clarkson, M. R., O'Meara, Y. M., Murphy, B., *et al.* (1998). Collapsing glomerulopathy—recurrence in a renal allograft. *Nephrol Dial Transplant*, 13, 503–6.

Couser, W. G. and Stilmant, M. M. (1975). Mesangial lesions and focal glomerular sclerosis in the aging rat. *Lab Invest*, 33, 491–501.

Crook, E. D., Habeeb, D., Gowdy, O., *et al.* (2005). Effects of steroids in focal segmental glomerulosclerosis in a predominantly African-American population. *Am J Med Sci*, 330, 19–24.

D'Agati, V. D., Fogo, A. B., Bruijn, J. A., *et al.* (2004). Pathologic classification of focal segmental glomerulosclerosis: a working proposal. *Am J Kidney Dis*, 43, 368–82.

D'Agati, V. D., Kaskel, F. J., and Falk, R. J. (2011). Focal segmental glomerulosclerosis. *N Engl J Med*, 365, 2398–411.

Detwiler, R. K., Falk, R. J., Hogan, S. L., *et al.* (1994). Collapsing glomerulopathy: a clinically and pathologically distinct variant of focal segmental glomerulosclerosis. *Kidney Int*, 45, 1416–24.

Elger, M. and Kriz, W. (1998). Podocytes and the development of segmental glomerulosclerosis. *Nephrol Dial Transplant*, 13, 1368–73.

Filler, G., Young, E., Geier, P., *et al.* (2003). Is there really an increase in non-minimal change nephrotic syndrome in children? *Am J Kidney Dis*, 42, 1107–13.

Fogo, A., Glick, A. D., Horn, S. L., *et al.* (1995). Is focal segmental glomerulosclerosis really focal? Distribution of lesions in adults and children. *Kidney Int*, 47, 1690–6.

Fogo, A., Hawkins, E. P., Berry, P. L., *et al.* (1990). Glomerular hypertrophy in minimal change disease predicts subsequent progression to focal glomerular sclerosis. *Kidney Int*, 38, 115–23.

Fuiano, G., Comi, N., Magri, P., *et al.* (1996). Serial morphometric analysis of sclerotic lesions in primary "focal" segmental glomerulosclerosis. *J Am Soc Nephrol*, 7, 49–55.

Gallon, L., Leventhal, J., Skaro, A., *et al.* (2012). Resolution of recurrent focal segmental glomerulosclerosis after retransplantation. *N Engl J Med*, 366, 1648–9.

Genovese, G., Friedman, D. J., Ross, M. D., *et al.* (2010). Association of trypanolytic ApoL1 variants with kidney disease in African Americans. *Science*, 329, 841–5.

Habib, R. and Gubler, M. C. (1973). Focal sclerosing glomerulonephritis. *Perspect Nephrol Hypertens*, 1 Pt 1, 263–78.

Hayslett, J. P., Krassner, L. S., Bensch, K. G., *et al.* (1969). Progression of "lipoid nephrosis" to renal insufficiency. *N Engl J Med*, 281, 181–7.

Hyman, L. R. and Burkholder, P. M. (1973). Focal sclerosing glomerulonephropathy with segmental hyalinosis. A clinicopathologic analysis. *Lab Invest*, 28, 533–44.

Kaplan, C., Pasternack, B., Shah, H., *et al.* (1975). Age-related incidence of sclerotic glomeruli in human kidneys. *Am J Pathol*, 80, 227–34.

Kemper, M. J., Wolf, G., and Muller-Wiefel, D. E. (2001). Transmission of glomerular permeability factor from a mother to her child. *N Engl J Med*, 344, 386–7.

Kohaut, E. C., Singer, D. B., and Hill, L. L. (1976). The significance of focal glomerular sclerosis in children who have nephrotic syndrome. *Am J Clin Pathol*, 66, 545–50.

Kopp, J. B., Smith, M. W., Nelson, G. W., *et al.* (2008). MYH9 is a major-effect risk gene for focal segmental glomerulosclerosis. *Nat Genet*, 40, 1175–84.

Korbet, S. M. (2012). Treatment of primary FSGS in adults. *J Am Soc Nephrol*, 23, 1769–76.

Korbet, S. M., Schwartz, M. M., and Lewis, E. J. (1988). Recurrent nephrotic syndrome in renal allografts. *Am J Kidney Dis*, 11, 270–6.

Kriz, W., Elger, M., Nagata, M., *et al.* (1994). The role of podocytes in the development of glomerular sclerosis. *Kidney Int Suppl*, 45, S64–72.

Kriz, W., Shirato, I., Nagata, M., *et al.* (2013). The podocyte's response to stress: the enigma of foot process effacement. *Am J Physiol Renal Physiol*, 304, F333–47.

Lagrue, G., Niaudet, P., Guillot, F., *et al.* (1989). Pregnancy and glomerulonephritis. *Lancet*, 2, 1037.

Li, R. M., Branton, M. H., Tanawattanacharoen, S., *et al.* (2002). Molecular identification of SV40 infection in human subjects and possible association with kidney disease. *J Am Soc Nephrol*, 13, 2320–30.

Mathieson, P. W. (2010). Podocyte actin in health, disease and treatment. *Nephrol Dial Transplant*, 25, 1772–3.

Meehan, S. M., Pascual, M., Williams, W. W., *et al.* (1998). De novo collapsing glomerulopathy in renal allografts. *Transplantation*, 65, 1192–7.

Moudgil, A., Nast, C. C., Bagga, A., *et al.* (2001). Association of parvovirus B19 infection with idiopathic collapsing glomerulopathy. *Kidney Int*, 59, 2126–33.

Muda, A. O., Feriozzi, S., Cinotti, G. A., *et al.* (1994). Glomerular hypertrophy and chronic renal failure in focal segmental glomerulosclerosis. *Am J Kidney Dis*, 23, 237–41.

Nagata, M., Hattori, M., Hamano, Y., *et al.* (1998). Origin and phenotypic features of hyperplastic epithelial cells in collapsing glomerulopathy. *Am J Kidney Dis*, 32, 962–9.

Nash, M. A., Greifer, I., Olbing, H., *et al.* (1976). The significance of focal sclerotic lesions of glomeruli in children. *J Pediatr*, 88, 806–13.

Newman, W. J., Tisher, C. C., McCoy, R. C., *et al.* (1976). Focal glomerular sclerosis: contrasting clinical patterns in children and adults. *Medicine (Baltimore)*, 55, 67–87.

Niang, A., Niang, S. E., Ka El, H. F., *et al.* (2008). Collapsing glomerulopathy and haemophagocytic syndrome related to malaria: a case report. *Nephrol Dial Transplant*, 23, 3359–61.

Oda, T., Hotta, O., Taguma, Y., *et al.* (1998). Clinicopathological significance of intratubular giant macrophages in progressive glomerulonephritis. *Kidney Int*, 53, 1190–200.

Pakasa, N. M., Nseka, N. M., and Nyimi, L. M. (1997). Secondary collapsing glomerulopathy associated with Loa loa filariasis. *Am J Kidney Dis*, 30, 836–9.

Rumpelt, H. J. and Thoenes, W. (1974). Focal and segmental sclerosing glomerulopathy (-nephritis). *Virchows Arch A Pathol Anat Histol*, 362, 265–82.

Rydel, J. J., Korbet, S. M., Borok, R. Z., *et al.* (1995). Focal segmental glomerular sclerosis in adults: presentation, course, and response to treatment. *Am J Kidney Dis*, 25, 534–42.

Sakai, K., Morito, N., Usui, J., *et al.* (2011). Focal segmental glomerulosclerosis as a complication of hepatitis B virus infection. *Nephrol Dial Transplant*, 26, 371–3.

Schwartz, M. M., Evans, J., Bain, R., *et al.* (1999). Focal segmental glomerulosclerosis: prognostic implications of the cellular lesion. *J Am Soc Nephrol*, 10, 1900–7.

Schwartz, M. M., and Korbet, S. M. (1993). Primary focal segmental glomerulosclerosis: pathology, histological variants, and pathogenesis. *Am J Kidney Dis*, 22, 874–83.

Schwartz, M. M., Korbet, S. M., Rydell, J., *et al.* (1995). Primary focal segmental glomerular sclerosis in adults: prognostic value of histologic variants. *Am J Kidney Dis*, 25, 845–52.

Schwartz, M. M., and Lewis, E. J. (1985). Focal segmental glomerular sclerosis: the cellular lesion. *Kidney Int*, 28, 968–74.

Shankland, S. J., Eitner, F., Hudkins, K. L., *et al.* (2000). Differential expression of cyclin-dependent kinase inhibitors in human glomerular disease: role in podocyte proliferation and maturation. *Kidney Int*, 58, 674–83.

Sperati, J. (2012). Stabilization of hepatitis C associated collapsing focal segmental glomerulosclerosis with interferon a-2a and ribavirin. *Clin Nephrol.*

Stokes, M. B., Davis, C. L., and Alpers, C. E. (1999). Collapsing glomerulopathy in renal allografts: a morphological pattern with diverse clinicopathologic associations. *Am J Kidney Dis*, 33, 658–66.

Stokes, M. B., Markowitz, G. S., Lin, J., *et al*. (2004). Glomerular tip lesion: a distinct entity within the minimal change disease/focal segmental glomerulosclerosis spectrum. *Kidney Int*, 65, 1690–702.

Stokes, M. B., Valeri, A. M., Markowitz, G. S., *et al*. (2006). Cellular focal segmental glomerulosclerosis: Clinical and pathologic features. *Kidney Int*, 70, 1783–92.

Toth, C. M., Pascual, M., Williams, W. W., Jr., *et al*. (1998). Recurrent collapsing glomerulopathy. *Transplantation*, 65, 1009–10.

Velosa, J. A., Donadio, J. V., Jr., and Holley, K. E. (1975). Focal sclerosing glomerulonephropathy: a clinicopathologic study. *Mayo Clin Proc*, 50, 121–33.

Verani, R. R. and Hawkins, E. P. 1986. Recurrent focal segmental glomerulosclerosis. A pathological study of the early lesion. *Am J Nephrol*, 6, 263–70.

Weiss, M. A., Daquioag, E., Margolin, E. G., *et al*. (1986). Nephrotic syndrome, progressive irreversible renal failure, and glomerular "collapse": a new clinicopathologic entity? *Am J Kidney Dis*, 7, 20–8.

CHAPTER 58

Primary focal segmental glomerulosclerosis: treatment and outcome

Alain Meyrier and Patrick Niaudet

Introduction

The proportion of cases of primary focal segmental glomerulosclerosis (FSGS) responsive to treatment with corticosteroids is variable and depends on histological type, and duration and dose of steroid treatment, but overall complete remission rate is estimated at 20–25% in white and Asian patients, and lower in black patients. Partial response dependent on a high dose of steroids is possible. Despite anxieties about nephrotoxicity, there may be justification for adding calcineurin inhibitors to control nephrotic syndrome if it is severe. Data for additional agents are not very encouraging.

Plasma exchange appears to remove a circulating factor that causes proteinuria in FSGS, as illustrated by responses to this treatment when proteinuria recurs after kidney transplantation.

Treatment

The principles of management of FSGS are similar in children and adults. A genetic cause is more likely in young children, but may also present in adults, especially young adults.

General management

The general management of nephrotic syndrome is described in Chapter 52.

Aetiologic treatment

It can be considered as a paradox to envisage an aetiologic treatment of 'primary' nephrotic FSGS, as the aetiology of this condition is still unknown. This would be based on the assumption that FSGS is an immunological, autoimmune disease. We give further our reasons for believing that the favourable effect of some and not all immunosuppressive regimens are not a sufficient argument to endorse the concept of primary FSGS being an immunologic disease. This applies to corticosteroids, calcineurin inhibitors, antimetabolites, and rituximab (RTX) (see Table 45.1).

Corticosteroids

The mode of action of corticosteroids in idiopathic nephrotic syndrome was long considered as being that of immunosuppression and of an anti-inflammatory direct effect (Buttgereit et al., 2005). Recent research points to another interpretation based on a pharmacologic, specifically antiproteinuric effect of steroids on the podocyte. Xing et al. studied the direct effects of the glucocorticoid dexamethasone at concentrations designed to mimic *in vivo* therapeutic corticosteroid levels (Xing et al., 2006). A conditionally immortalized human podocyte cell line was transfected with a temperature-sensitive simian virus 40 (SV40) transgene. When the SV40 transgene was inactivated *in vitro*, these cells adopted the phenotype of differentiated podocytes. The study confirmed that expression of glucocorticoid receptors by podocytes was replicated by their podocyte cell line *in vitro*. Glucocorticoid receptors were present in both nuclear and cytoplasmic extracts. There was a suggestion that overall level of expression and nuclear localization of glucocorticoid receptors was upregulated by dexamethasone in a dose-dependent manner. Dexamethasone upregulated expression of nephrin and tubulin-a and downregulated vascular endothelial growth factor. Effects on the cell cycle comprised downregulation of cyclin kinase inhibitor p21 (that promotes podocyte proliferation) and augmentation of podocyte survival, without any effect on apoptosis. Cytokine production by podocytes, especially interleukin (IL)-6 and IL-8. IL-6 expression was suppressed by dexamethasone.

Notwithstanding these recent notions and from a practical standpoint, nephrotic, primary FSGS represents a constant indication of first-line corticosteroid treatment (Korbet, 2002; Chun et al., 2004; Braun et al., 2008; Meyrier, 2009b). However, the response of nephrotic FSGS to steroids depends on several factors.

The degree of fibrotic glomerular and tubulointerstitial lesions when treatment is undertaken matters, with usually the poorest results when serum creatinine concentrations are > 150–200 μmoles per litre. This indicates that treatment should be undertaken early in the course of FSGS. There is some wishful thinking in this assertion as the clinical onset of nephrotic syndrome, which marks the time when a kidney biopsy is performed is not as explosive in FSGS as it is in MCD, except in the glomerular tip lesion (GTL) variant (Stokes et al., 2004). It is conceivable that the kidney biopsy be carried out following a protracted period of clinically silent glomerular injury by the offending factor that causes the disease.

The histologic subtype according to the Columbia classification also matters as the rate of complete, partial remission, and failure is respectively approximately 59%, 14%, and 27% in the GTL

(Stokes et al., 2004), whereas 72–93% of collapsing glomerulopathies are treatment failures (Albaqumi et al., 2006; Stokes et al., 2006).

A series of 187 patients with FSGS was analysed by Stokes et al. according to the histopathologic type (Stokes et al., 2006). Seventy two patients were treated with steroids (the figures for the cell, the collapsing (CG), the GTL, and the NOS (not otherwise specified) variants were respectively 77.8%, 66.0%, 97.2%, and 63.0%). Other treatments comprised in a non-systematic fashion calcineurin inhibitors and/or alkylating agents in some but not all patients. The results show that on a whole the NOS variant reflects the overall rate of complete and partial remission and that the cell and the collapsing variant do not follow the same course, the latter enjoying remission in only 13% of cases whereas the former achieves complete or partial remission in nearly half of cases. The GTL in terms of response to treatment is quite comparable to that of MCD (Stokes et al., 2004). One-third of all patients and one-third of those with the NOS variant progressed to end-stage renal disease as compared with one-quarter for the cell subtype, two-thirds for CG, and only one-twentieth for the GTL.

Two drugs are commonly used: prednisone or prednisolone orally. The initial dosage in adults is in the order of 1 mg/kg/day without exceeding 80 mg/day in obese patients. The time to remission is slow and in adults the cumulative success rate of steroids requires about 4 months before pronouncing steroid resistance (Nakayama et al., 2002). Prednisone is typically given for 12 weeks, followed in cases of remission, even partial, by slow tapering over months to avoid a rebound effect. On a whole, patients with nephrotic FSGS do not enjoy complete (and more often partial) remission in more than approximately 20–25% of cases but with a wide spectrum of responses according to the mode of treatment and as illustrated above to the histologic subtype.

In white and Asian patients the overall rate of complete remission with corticosteroids alone is in the order of 20–25% (Braun et al., 2008). Conversely the response to therapy is extremely low in black patients (Schwartz et al., 1999; Crook et al., 2005).

Complete remission portends a favourable outcome (Korbet, 2002; Chun et al., 2004) and a partial remission is better in terms of renal function than no remission (Troyanov et al., 2005). However, a minority of patients with steroid-sensitive nephrotic FSGS achieve complete stable remission after tailing off steroids to a stop. Most are steroid dependent. When the threshold dose is high, > 10–15 mg/day, the patient is exposed to the well-known, long-term complications of steroids, including hip osteonecrosis.

The high incidence of patients with FSGS and steroid dependency on a high threshold dose or steroid resistance leads to discussing other treatment options that imply adding or substituting their own toxic effects to the complications of steroid therapy (Philibert and Cattran, 2008).

Alkylating agents

Two cytotoxic agents have been used in the treatment of steroid-resistant or -dependent idiopathic nephrotic syndrome: chlorambucil and cyclophosphamide.

The results of cytotoxic agents in idiopathic nephrotic syndrome largely depend on the previous response to steroids. Their best indication is corticosteroid dependency, where the rates of complete remission, partial remission, and failure are, respectively, 51%, 23%, and 26% of cases (Korbet, 1999; 2002; Chun et al., 2004). The main advantage of alkylating agents is that, in steroid-dependent and multirelapsing forms, the remission they yield is long-lasting. However, this is mostly true in MCD whereas multiple relapses following periods of complete remission are a very rare scenario in FSGS, although it has been published that, surprisingly, some cases of the worst form of FSGS, that is, collapsing glomerulopathy, achieved a spontaneous remission (Valeri et al., 1996). Steroid resistance, which is the usual case in FSGS is highly predictive of resistance to alkylating agents with the corresponding figures of 17%, 15%, and 69%. The results of cytotoxic drugs used in about 20% of all patients in the series of Stokes et al. were not different amongst the various histologic subtypes (Stokes et al., 2006).

Heering et al. conducted a prospective randomized study in two groups of patients with nephrotic FSGS (Heering et al., 2004). Thirty-four were treated with steroids and ciclosporin, whilst 23 received steroids and chlorambucil for 6 months. When FSGS was refractory to chlorambucil the patients were switched to ciclosporin. The results in terms of renal function and proteinuria were the same in the two groups. The authors concluded that adding treatment with chlorambucil to steroids was ineffective in FSGS.

In fact, cytotoxic agents are of no avail in a majority of steroid-resistant patients. Their short- and long-term toxicity has been established. They entail a risk of definitive sterility in the young male and hypofertility in females (Chapman, 1983). Cyclophosphamide is carcinogenic (Faurschou et al., 2008). Evidence-based analysis leads to considering that alkylating agents are dangerous and mostly inefficient in FSGS (Braun et al., 2008).

Immunophilin modulators

Calcineurin inhibitors

Calcineurin inhibitors operate on intracellular signal transduction pathways (Kaminuma, 2008; Rao, 2009). A T-cell-driven immune response develops in three phases. First, transcriptional activation of early genes such as the IL-2 receptor that elicits progression of T cells from the G0 to the G1 state. Second, T cells transduce the signal triggered by specific cytokines that permit entry into the cell cycle, resulting in clonal expansion and effector functions in the third phase of the immune response. Both ciclosporin and tacrolimus bind to the same family of immunophilins, cytosolic FK binding proteins (FKBP). Ciclosporin and FK-506 inhibit the first phase. Both inhibit the nuclear factor of activated T cells (NFAT) signalling in T cells.

Two calcineurin inhibitors have been tried in the treatment of idiopathic nephrotic syndrome: ciclosporin and tacrolimus (FK-506). The rationale for using them is the postulate that all subsets of idiopathic nephrotic syndrome are a T-cell driven immunologic disease. This hypothesis has some consistence in MCD but is not substantiated in FSGS where calcineurin inhibition cannot be considered as an aetiologic treatment (Meyrier, 2009b).

Ciclosporin

The mode of action of ciclosporin in reducing proteinuria is not necessarily immunological (Meyrier, 1992, 1999, 2009a, 2009b). Ciclosporin was shown to reduce proteinuria in glomerulopathies with no immunologic background, such as diabetic glomerulopathy and Alport syndrome. This effect was initially attributed to renal vasoconstriction and considered as more noxious than beneficial.

In fact ciclosporin is endowed with pharmacological properties that interfere with the glomerular permeability to albumin. Chen et al. in a model of Alport syndrome in the dog showed that

ciclosporin reduced albuminuria and preserved renal function, despite the fact that repeat electron microscopy showed that the glomerular basement menbrane lesions were progressing (Chen et al., 2003). Ambalavanan et al. treated nephrotic patients suffering from idiopathic membranous glomerulonephritis with ciclosporin (Ambalavanan et al., 1996); proteinuria diminished significantly and the glomerular filtration rate was unchanged. Repeat electron microscopy showed that the lesions had progressed. Other studies in membranous glomerulonephritis have confirmed a 'proteinuria-only' effect of calcineurin inhibitors (see Chapter 62).

Recent research has shed a new light on the non-specific effect of ciclosporin on proteinuria. It appears that the drug does not only exert an immunosuppressive action but also an antiproteinuric effect of its own. Faul et al. published an elegant study on the mode of action of ciclosporin on the podocyte (Faul et al., 2008). They examined the effect of ciclosporin from various angles. By confocal laser microscopy they showed that synaptopodin specifically interacts with 14-3-3, an intermediate filament protein. 14-3-3β, E64, and ciclosporin block the cathepsin L-mediated degradation of synaptopodin. Ciclosporin and E64 also ameliorate lipopolysaccharide-induced proteinuria following the cathepsin L mediated degradation of synaptopodin. These experiments collectively demonstrated that the antiproteinuric effect of ciclosporin does not result from the inhibition of NFAT signalling but from blocking the calcineurin-mediated dephosphorylation of the actin-organizing protein synaptopodin, which confers a stabilization of the actin cytoskeleton in podocytes. This antiproteinuric effect of ciclosporin might explain partial, but clinically beneficial remissions in FSGS. It is conceivable that tacrolimus diminishes proteinuria through a similar mechanism, but so far no study has been undertaken on this matter.

Since 1985, ciclosporin has been considered amongst the most useful immunosuppressive agents in the treatment of idiopathic nephrotic syndrome, including FSGS (Meyrier, 2009a). The efficacy of ciclosporin depends essentially on the previous response to steroids. In steroid-responsive cases, the percentages of complete remission, partial remission, and failure are, respectively, 73%, 7%, and 20%. In steroid-resistant cases of FSGS, that are by far the most frequent, the respective figures are 29%, 22%, and 49%. Evidence-based analysis of the results of three studies showed that the rate of complete plus partial remissions with 3.5–5 mg/kg of ciclosporin (Sandimmune®) in combination with low-dose (0.15 mg/kg/day) prednisone or prednisolone was significantly increased versus the glucocorticoid alone (Meyrier et al., 1994; Braun et al., 2008). Thus, ciclosporin is a steroid-sparing agent and steroids enhance the efficacy of ciclosporin. This synergic efficacy can be explained by the mode of action of both drugs described above (Xing et al., 2006; Faul et al., 2008).

Treatment of FSGS with ciclosporin has been the subject of numerous publications since 1986. In the first article on the effect of ciclosporin in adults with idiopathic nephrotic syndrome (three with MCD and three with FSGS), the authors observed that 'The results…suggest that minimal change lipoid nephrosis and focal segmental glomerulosclerosis are separate entities' (Meyrier et al., 1986). This is still true. The last review found in 2013 in a Medline search on ciclosporin treatment of FSGS in adults dates back to 2007 (Cattran et al., 2007). Since, all publications deal with idiopathic nephrotic syndrome in children and do not adduce substantial progress on the matter.

The acquired experience teaches that the drug, when used at low dosages in combination with steroids, has increased efficacy and lower toxicity (Braun et al., 2008; Meyrier, 2009a). A randomized study showed that despite its nephrotoxic potential, long-term ciclosporin treatment slowed the pace to end-stage renal disease in FSGS (Cattran et al., 1999). This unexpected finding might be interpreted as indicating a favourable effect of reduced proteinuria on the tubulointerstitium, more than reflecting an improvement of the glomerular lesions of FSGS (Meyrier et al., 1994).

The only controlled study to compare the efficacy (induction of remission) and safety of ciclosporin alone with those of supportive therapy in patients with steroid-resistant idiopathic nephrotic syndrome was carried out by Ponticelli et al. in 14 patients with FSGS (Ponticelli et al., 1993). There were three complete remissions, five partial remissions, and six failures. Yet steroid resistance in adults was pronounced after only 2 months of this treatment, which makes the interpretation of results difficult. However, the same group 6 years later published results indicating that FSGS requires a long course of ciclosporin treatment for obtaining and maintaining a remission (Ponticelli et al., 1999).

Guidelines regarding Sandimmune® dosage formulated nearly two decades ago (Meyrier et al., 1994; Niaudet, 1994) still apply and have been the subject of the consensus workshop cited earlier (Cattran et al., 2007). This update comprises caveats regarding the use of ciclosporin generics of the first galenic form of the drug in oily solution. The better bioavailability of the microemulsion (Neoral®) leads to recommending dosages distinctly < 5 mg/kg/day.

Ciclosporin dependency was observed from the very first trials of treatment. The probability of remaining in remission after abruptly stopping ciclosporin was 50% at 2 months, 30% at 4 months, 20% at 6 months, and nil at 9 months. This implied the worrisome prospect of indefinite treatment with a nephrotoxic drug. However, the notion of ciclosporin dependency was reconsidered when a series of 36 adults having undergone a repeat kidney biopsy was analysed after 1–5 years of ciclosporin treatment (Meyrier et al., 1994). Fourteen had been treated for 26 ± 14.5 months including four with FSGS. After > 1 year of remission, ciclosporin was progressively tapered to a stop. Remission was durable and maintained without steroids in 11 and with low-dose steroids in three. In five cases patients remained in remission with very low dosages, in the order of 3 mg/kg/day of the oily solution (Sandimmune®), and even in one with 1 mg/kg/day. Ciclosporin dependency to a low dosage most probably entails little renal toxicity over the years. This justifies long-term ciclosporin treatment to maintain remission (Ponticelli et al., 1999).

Tacrolimus (FK506)

Tacrolimus is a calcineurin inhibitor whose mode of action is similar to that of ciclosporin. However, FK506 also exerts a pharmacological action on the slit diaphragm molecules to diminish proteinuria in a hereditary autosomal dominant form of FSGS, that is, a mutation of the transient receptor potential cation channel 6 (TRPC6) (Mukerji et al., 2007). Preliminary experiments reveal that FK-506 can inhibit TRPC6 *in vitro* through blocking the TRPC6 channels (Winn, 2003, 2008; Winn et al., 2005; Mukerji et al., 2007).

Trials of treatment of FSGS with FK-506 in adults are few and comprise short series of patients (Westhoff and van der Giet, 2007).

Segarra et al. presented the results of combined therapy with tacrolimus and steroids in 25 patients with nephrotic FSGS in whom ciclosporin had not obtained remission. At 6 months, ten patients enjoyed complete remission, two partial remission, and five a significant reduction of proteinuria (Segarra et al., 2002). Time to remission was long (112 ± 24 days). Reversible nephrotoxicity was observed in 40%. A majority of patients were FK-506-dependent.

Duncan et al. studied prospectively six patients with nephrotic FSGS treated with tacrolimus alone, and a further five in remission on ciclosporin, converted to FK-506 in an attempt to arrest a decline in renal function (Duncan et al., 2004). They achieved partial remission after 6.5 ± 5.9 months. Proteinuria diminished by 75.2 ± 16.8%. Renal function declined modestly at 3 months and subsequently remained stable. Another group of five patients were converted from ciclosporin to FK-506. In two, FK506 maintained complete remission and the extant three patients had a further reduction in proteinuria. Overall, conversion from ciclosporin to FK-506 was followed by an improvement in renal function, but the follow up was short.

Sirolimus

Sirolimus is another immunosuppressive agent used in transplantation, but it may be acutely nephrotoxic in proteinuric glomerulopathies, including FSGS (Fervenza et al., 2004) and may induce *de novo* FSGS in transplanted patients (Letavernier et al., 2007). There is one report to the contrary (a paper based on 21 cases that presented rather favourable results of the drug (Tumlin et al., 2006)) but it cannot be recommended.

Antimetabolites

Azathioprine

Linshaw and Gruskin analysed the results of treatment with azathioprine and concluded that there was a lack of efficacy of this antimetabolite in children (Linshaw and Gruskin, 1974). Conversely, Cade et al. treated with azathioprine 13 adults in whom idiopathic nephrotic syndrome had appeared in childhood in two and after age 14 in 11 (Cade et al., 1986). Five had FSGS. Six had been steroid resistant from the outset. Seven others were multirelapsers, of whom four evolved to steroid resistance. At 3 months, all patients showed clinical improvement. At 18 months the six patients with selective proteinuria were in remission. At 24 months, 12 out of 13 of the patients still followed up were in complete remission.

Since 1986, azathioprine has occasionally been cited in papers dealing with treatment of idiopathic nephrotic syndrome, but there are no hard data on its place in the treatment of FSGS.

Mycophenolate mofetil

Since preliminary publications dating back to 1998 (Briggs et al., 1998), mycophenolate mofetil (MMF) had been tried in a few cases of FSGS, amongst other causes of nephrotic syndrome (Choi et al., 2002; Bagga et al., 2003; Barletta et al., 2003; Cattran et al., 2004; Gellermann and Querfeld, 2004; Segarra et al., 2007). Of 11 adults with FSGS, two went into remission with stable renal function. In the others, proteinuria diminished whilst renal function declined. In the three paediatric series, a total of four children were treated with MMF. The drug allowed corticosteroid sparing and seemed to be beneficial in terms of renal function.

Cattran et al. performed an open 6-month trial of MMF in 18 patients resistant to a course of corticosteroid therapy (Cattran et al., 2004). Seventy-five per cent had also failed to respond to a cytotoxic agent and/or a calcineurin inhibitor. A substantial improvement in proteinuria was seen in 8/18 of the patients by 6 months. This was sustained for up to 1 year post treatment in 4/8 of this group. No patient had a complete remission. No deterioration in renal function was observed over the treatment period, but three patients progressed to chronic kidney failure during follow-up. Adverse effects were mild. Relapses were common, suggesting that more prolonged or combination therapy may be required.

Segarra et al. published on a series of 22 patients (Segarra et al., 2007). All had received a previous 6-month course of prednisone and a 6-month course of ciclosporin. Five had also been treated with cyclophosphamide and four with FK-506. Over a 12-month follow-up period of MMF, two went into complete remission, 10 into a partial remission, and 10 were failures. When obtained the time to remission was very long, in the order of 150 days. The authors concluded that 'MMF causes a moderate decrease in proteinuria in 50% of the patients who do not have other treatment options. The response to therapy is largely influenced by a preserved renal function and requires sustained MMF treatment'.

Rituximab

The majority of publications on RTX in steroid-dependent and steroid-resistant idiopathic nephrotic syndrome deal with the disease in children and are analysed in Chapter 56. RTX has been tried in very few adult patients with FSGS. From this sparse experience it appears that RTX in this setting is poorly efficient in FSGS that resisted other treatments, including in cases that relapse following transplantation (Yabu et al., 2008; Fernandez-Fresnedo et al., 2009; Kisner et al., 2012).

Other treatment options

The hypothesis that FSGS is the consequence of a circulating factor that induces proteinuria, followed by podocyte lesions has led to attempts to remove it by plasmapheresis or by adsorption on staphylococcal protein A-coated columns.

Plasmapheresis was considered of doubtful avail in primary FSGS (Feld et al., 1998) although a few studies observed a favourable result of plasma and LDL apheresis (Yokoyama et al., 2007). In fact plasmapheresis is widely used in the special case of nephrotic syndrome recurring after transplantation. Relapse of nephrotic syndrome and glomerular lesions are observed in approximately 30% of patients undergoing renal transplantation for end-stage FSGS (Ponticelli, 2010). Recurrence often leads to loss of the transplant, with an increased incidence when the primary disease was a collapsing glomerulopathy (Swaminathan et al., 2006). Repeated sessions of plasmapheresis (Artero et al., 1994) are carried out alone or with increased immunosuppression (Canaud et al., 2009). The efficacy of pre-emptive or curative plasmapheresis per se in these recurrent forms is not clearly established as large, randomized studies are lacking (Gohh et al., 2005). In fact pre-emptive plasmapheresis can be repeated over days and weeks in case of transplantation with a living donor and thus achieve substantial removal of the glomerular permeability factor, whereas in case of deceased donor transplantation

there is no sufficient time for performing more than three plasma exchanges before grafting.

Plasma protein adsorption on columns coated with staphylococcal protein A have led to a dead end. Dantal et al. had published enticing results of this costly technique (Dantal et al., 1994), but the excitement was damped when another group showed that immunoadsorption diminished proteinuria in various glomerulopathies with no specificity regarding FSGS (Esnault et al., 1999).

Antifibrotic agents have been tried in FSGS as in other glomerulopathies.

Endothelin has a fibrosing effect on the injured glomerulus (Barton, 2008) and animal experiments indicate that endothelin receptor antagonists (ERAs) exert a preventive effect on the progression of glomerulosclerosis in the rat. So far these drugs have only been the subject of anecdotal reports in human FSGS. In fact clinical development of ERAs has been hampered by problems with dosing, with the make-up of study cohorts, and adverse events (Barton and Kohan, 2011).

Pirfenidone, an orally available antifibrotic drug (Cho et al., 2007) and the monoclonal antibody adalimumab, an antitumour necrosis factor inhibitor associated with rosiglitazone—an antidiabetic agent—have been the subject of preliminary trials (Peyser et al., 2010).

Treatment of steroid-resistant FSGS in children

The prognosis of steroid-resistant FSGS is poor, with a high proportion of children progressing to end-stage renal failure. This explains that intensive treatment regimens have been tried. The results of immunosuppressive treatments should take into account the fact that children with genetic forms of idiopathic nephrotic syndrome most often fail to respond to any therapy. However, many published trials include patients who had not been tested for mutations in the genes involved in steroid-resistant idiopathic nephrotic syndrome. Moreover, most studies are non-randomized and include a small number of patients.

Calcineurin inhibitors

A combination of calcineurin inhibitor with low-dose steroid therapy for at least 6 months is presently the best known option as a first-line therapy.

Ciclosporin

The rate of complete remission is significantly higher when ciclosporin is given in combination with steroids (Niaudet and Habib, 1994). Three randomized trials involving 49 children showed that complete remissions and partial remissions were observed in 31% and 38% of patients which was significantly higher than in the control arms (Garin et al., 1988; Ponticelli et al., 1993; Lieberman and Tejani, 1996).

Ingulli et al. reported that prolonged ciclosporin treatment in children with steroid-resistant FSGS reduces proteinuria and blunts the progression to end-stage renal failure (Ingulli et al., 1995). The dose of ciclosporin (4–20 mg/kg/day) was titrated to the serum cholesterol level to achieve a remission. In this study, only 5 of the 21 treated patients (24%) progressed to end-stage renal failure compared to 42 of 54 patients from an historical group who had not received this treatment. Ehrich et al. reported a retrospective study including 25 children with steroid-resistant FSGS who received prolonged and intensified treatment with combined ciclosporin and steroids including methylprednisolone pulses. This treatment resulted in sustained remission in 84% of children with non-genetic forms of steroid resistant idiopathic nephrotic syndrome (Ehrich et al., 2007).

Tacrolimus

There is evidence from case series that tacrolimus is effective in children with steroid-resistant FSGS (Loeffler et al., 2004; Bhimma et al., 2006; Gulati et al., 2008). Tacrolimus was found to be as effective as ciclosporin in a randomized trial involving 41 children (Choudhry et al., 2009). Both groups received alternate-day steroids and enalapril. The rate of remission at 6 months was 85.7% with tacrolimus and 80% with ciclosporin. Interestingly, the proportion of patients with relapses was significantly higher in the ciclosporin group. Conversely, Wang et al. found that tacrolimus was associated with higher efficacy and lower renal toxicity in comparison to ciclosporin, with no difference in the rate of relapse (Wang et al., 2012).

Pulse methylprednisolone

Methylprednisolone pulse therapy has been proposed by Mendoza et al. It consists of methylprednisolone (30 mg/kg intravenously), administered every other day for 2 weeks, weekly for 8 weeks, every other week for 8 weeks, monthly for 9 months, and then every other month for 6 months in association with oral prednisone and, if necessary, cyclophosphamide or chlorambucil (Mendoza et al., 1990). At an average of > 6 years of follow-up, 21 of 32 children were in complete remission and the 5-year incidence of end-stage renal disease was approximately 5% versus 40% in historical controls (Tune et al., 1995). Two publications reported similar results (Yorgin et al., 2001; Pena et al., 2007). Although these results are better than those seen in any other study, other reports described less favourable results (Waldo et al., 1992; Hari et al., 2001).

Alkylating agents

Although alkylating agents have little therapeutic effect in steroid-resistant FSGS, for unknown reasons they are still widely used either alone or in combination with corticosteroids. The International Study of Kidney Disease in Children reported on 60 children with steroid-resistant FSGS who were randomly allocated to receive either prednisone 40 mg/m^2 on alternate days for 12 months (control group) or cyclophosphamide, 2.5 mg/kg body weight for 3 months plus prednisone 40 mg/m^2 on alternate days for 12 months (Tarshish et al., 1996). Complete remissions were observed in 28% of children in the control group and in 25% of children who received cyclophosphamide. The authors concluded that there was no beneficial effect of cyclophosphamide in these patients. Rennert et al. treated 10 children with steroid-resistant FSGS with cyclophosphamide pulses. Only two of the five initial non-responders went into remission whereas all five late non-responders achieved complete remission (Rennert et al., 1999). In a prospective study of 24 patients, Bajpai et al. also found that therapy with intravenous cyclophosphamide had limited efficacy in patients with initial corticosteroid resistance whilst sustained remission was likely to occur in patients with late resistance and those with absence of significant tubulointerstitial changes on renal histology (Bajpai et al., 2003).

Mycophenolate mofetil

There is no convincing data for the beneficial effect of MMF in children with steroid-resistant FSGS. Menzibal et al. treated five

patients and only one achieved complete remission (Mendizabal et al., 2005). MMF in association with methylprednisolone pulses and angiotensin converting-enzyme inhibitors was reported to significantly reduce proteinuria (Montane et al., 2003). Another study involving 52 patients found a rate of complete remission of 23% and partial remission of 35.5% following therapy with MMF (de Mello et al., 2010). A prospective trial of the NIH compared ciclosporin to a combination of oral pulse dexamethasone and MMF (Gipson et al., 2011). Partial or complete remission was achieved in 22 of the 66 patients in the mycophenolate/dexamethasone group and 33 of the 72 ciclosporin-treated patients at 12 months. The authors concluded that the small sample size might have prevented detection of a moderate treatment effect.

Rituximab

At present, there is no evidence that RTX is effective in patients with steroid-resistant nephrotic syndrome, although retrospective case series report that treatment with RTX was effective in some patients (Peters et al., 2008; Gulati et al., 2010; Prytula et al., 2010; Sinha and Bagga, 2013). Gulati et al. reported that RTX had induced a complete remission in 27% of 33 steroid-resistant patients and a partial remission in 21% of them. The rate of remission was higher in patients with minimal changes on renal biopsy and in patients who were late non-responders (Gulati et al., 2010). Magnasco et al. reported the results of an open-label randomized trial including 31 children aged 2 to 16 years. All received calcineurin inhibitors and prednisolone and 16 of them received in addition two RTX infusions. Proteinuria remained unchanged in these patients and none entered partial or complete remission (Magnasco et al., 2012).

Post-transplantation recurrence of FSGS

(See also Chapter 289.)

Post-transplantation recurrence of nephrotic FSGS is frequent, and affects about 30–50% of patients (Ponticelli, 2010). Recurrence, which often occurs within the first hours after transplantation, is characterized by profuse proteinuria with foot process fusion followed by histologic lesions of FSGS. Recurrence leads to loss of the graft in at least half of cases. The risk is particularly high in patients whose primary glomerulopathy was of the cellular and the collapsing variants. Other risk factors are a rapid progression to renal failure (<3 years), a non-genetic form of FSGS, and recurrence on a previous graft. It is interesting to note that removing a grafted kidney affected by recurrence and grafting it in a patient whose primary glomerulopathy was not FSGS can be followed by an abrogation of proteinuria and a resolution of the podocytopathy (see section 'Reversibility of the cellular FSGS lesion' in Chapter 57).

It is difficult to propose recommendations regarding the optimal preventive and curative treatment. Plasma exchanges or immunoabsorption with high doses of calcineurin inhibitors lead to complete or partial remission in more than half of patients (Canaud et al., 2009). RTX has been found to be effective in patients who do not respond to plasma exchanges or are dependent on this therapy. In fact, the question will remain open as long as the factor, or factors, responsible for one of the most difficult to treat of all glomerulopathies has not been identified and counteracted.

References

Albaqumi, M., Soos, T. J., Barisoni, L., *et al.* (2006). Collapsing glomerulopathy. *J Am Soc Nephrol*, 17, 2854–63.

Ambalavanan, S., Fauvel, J. P., Sibley, R. K., *et al.* (1996). Mechanism of the antiproteinuric effect of cyclosporine in membranous nephropathy. *J Am Soc Nephrol*, 7, 290–8.

Artero, M. L., Sharma, R., Savin, V. J., *et al.* (1994). Plasmapheresis reduces proteinuria and serum capacity to injure glomeruli in patients with recurrent focal glomerulosclerosis. *Am J Kidney Dis*, 23, 574–81.

Bagga, A., Hari, P., Moudgil, A., *et al.* (2003). Mycophenolate mofetil and prednisolone therapy in children with steroid-dependent nephrotic syndrome. *Am J Kidney Dis*, 42, 1114–20.

Bajpai, A., Bagga, A., Hari, P., *et al.* (2003). Intravenous cyclophosphamide in steroid-resistant nephrotic syndrome. *Pediatr Nephrol*, 18, 351–6.

Barletta, G. M., Smoyer, W. E., Bunchman, T. E., *et al.* (2003). Use of mycophenolate mofetil in steroid-dependent and -resistant nephrotic syndrome. *Pediatr Nephrol*, 18, 833–7.

Barton, M. (2008). Reversal of proteinuric renal disease and the emerging role of endothelin. *Nat Clin Pract Nephrol*, 4, 490–501.

Barton, M. and Kohan, D. E. (2011). Endothelin antagonists in clinical trials: lessons learned. *Contrib Nephrol*, 172, 255–60.

Bhimma, R., Adhikari, M., Asharam, K., *et al.* (2006). Management of steroid-resistant focal segmental glomerulosclerosis in children using tacrolimus. *Am J Nephrol*, 26, 544–51.

Braun, N., Schmutzler, F., Lange, C., *et al.* (2008). Immunosuppressive treatment for focal segmental glomerulosclerosis in adults. *Cochrane Database Syst Rev*, CD003233.

Briggs, W. A., Choi, M. J., and Scheel, P. J., Jr. (1998). Successful mycophenolate mofetil treatment of glomerular disease. *Am J Kidney Dis*, 31, 213–7.

Buttgereit, F., Burmester, G. R., and Lipworth, B. J. (2005). Optimised glucocorticoid therapy: the sharpening of an old spear. *Lancet*, 365, 801–3.

Cade, R., Mars, D., Privette, M., *et al.* (1986). Effect of long-term azathioprine administration in adults with minimal-change glomerulonephritis and nephrotic syndrome resistant to corticosteroids. *Arch Intern Med*, 146, 737–41.

Canaud, G., Zuber, J., Sberro, R., *et al.* (2009). Intensive and prolonged treatment of focal and segmental glomerulosclerosis recurrence in adult kidney transplant recipients: a pilot study. *Am J Transplant*, 9, 1081–6.

Cattran, D. C., Alexopoulos, E., Heering, P., *et al.* (2007). Cyclosporin in idiopathic glomerular disease associated with the nephrotic syndrome : workshop recommendations. *Kidney Int*, 72, 1429–47.

Cattran, D. C., Appel, G. B., Hebert, L. A., *et al.* (1999). A randomized trial of cyclosporine in patients with steroid-resistant focal segmental glomerulosclerosis. North America Nephrotic Syndrome Study Group. *Kidney Int*, 56, 2220–6.

Cattran, D. C., Wang, M. M., Appel, G., *et al.* (2004). Mycophenolate mofetil in the treatment of focal segmental glomerulosclerosis. *Clin Nephrol*, 62, 405–11.

Chapman, R. M. (1983). Gonadal injury resulting from chemotherapy. *Am J Ind Med*, 4, 149–61.

Chen, D., Jefferson, B., Harvey, S. J., *et al.* (2003). Cyclosporine a slows the progressive renal disease of Alport syndrome (X-linked hereditary nephritis): results from a canine model. *J Am Soc Nephrol*, 14, 690–8.

Cho, M. E., Smith, D. C., Branton, M. H., *et al.* (2007). Pirfenidone slows renal function decline in patients with focal segmental glomerulosclerosis. *Clin J Am Soc Nephrol*, 2, 906–13.

Choi, M. J., Eustace, J. A., Gimenez, L. F., *et al.* (2002). Mycophenolate mofetil treatment for primary glomerular diseases. *Kidney Int*, 61, 1098–114.

Choudhry, S., Bagga, A., Hari, P., *et al.* (2009). Efficacy and safety of tacrolimus versus cyclosporine in children with steroid-resistant nephrotic syndrome: a randomized controlled trial. *Am J Kidney Dis*, 53, 760–9.

Chun, M. J., Korbet, S. M., Schwartz, M. M., *et al.* (2004). Focal segmental glomerulosclerosis in nephrotic adults: presentation, prognosis, and response to therapy of the histologic variants. *J Am Soc Nephrol*, 15, 2169–77.

Crook, E. D., Habeeb, D., Gowdy, O., *et al.* (2005). Effects of steroids in focal segmental glomerulosclerosis in a predominantly African-American population. *Am J Med Sci*, 330, 19–24.

Dantal, J., Bigot, E., Bogers, W., *et al.* (1994). Effect of plasma protein adsorption on protein excretion in kidney-transplant recipients with recurrent nephrotic syndrome. *N Engl J Med*, 330, 7–14.

De Mello, V. R., Rodrigues, M. T., Mastrocinque, T. H., *et al.* (2010). Mycophenolate mofetil in children with steroid/cyclophosphamide-resistant nephrotic syndrome. *Pediatr Nephrol*, 25, 453–60.

Duncan, N., Dhaygude, A., Owen, J., *et al.* (2004). Treatment of focal and segmental glomerulosclerosis in adults with tacrolimus monotherapy. *Nephrol Dial Transplant*, 19, 3062–7.

Ehrich, J. H., Geerlings, C., Zivicnjak, M., *et al.* (2007). Steroid-resistant idiopathic childhood nephrosis: overdiagnosed and undertreated. *Nephrol Dial Transplant*, 22, 2183–93.

Esnault, V. L., Besnier, D., Testa, A., *et al.* (1999). Effect of protein A immunoadsorption in nephrotic syndrome of various etiologies. *J Am Soc Nephrol*, 10, 2014–7.

Faul, C., Donnelly, M., Merscher-Gomez, S., *et al.* (2008). The actin cytoskeleton of kidney podocytes is a direct target of the antiproteinuric effect of cyclosporine A. *Nat Med*, 14, 931–8.

Faurschou, M., Sorensen, I. J., Mellemkjaer, L., *et al.* (2008). Malignancies in Wegener's granulomatosis: incidence and relation to cyclophosphamide therapy in a cohort of 293 patients. *J Rheumatol*, 35, 100–5.

Feld, S. M., Figueroa, P., Savin, V., *et al.* (1998). Plasmapheresis in the treatment of steroid-resistant focal segmental glomerulosclerosis in native kidneys. *Am J Kidney Dis*, 32, 230–7.

Fernandez-Fresnedo, G., Segarra, A., Gonzalez, E., *et al.* (2009). Rituximab treatment of adult patients with steroid-resistant focal segmental glomerulosclerosis. *Clin J Am Soc Nephrol*, 4, 1317–23.

Fervenza, F. C., Fitzpatrick, P. M., Mertz, J., *et al.* (2004). Acute rapamycin nephrotoxicity in native kidneys of patients with chronic glomerulopathies. *Nephrol Dial Transplant*, 19, 1288–92.

Garin, E. H., Orak, J. K., Hiott, K. L., *et al.* (1988). Cyclosporine therapy for steroid-resistant nephrotic syndrome. A controlled study. *Am J Dis Child*, 142, 985–8.

Gellermann, J. and Querfeld, U. (2004). Frequently relapsing nephrotic syndrome: treatment with mycophenolate mofetil. *Pediatr Nephrol*, 19, 101–4.

Gipson, D. S., Trachtman, H., Kaskel, F. J., *et al.* (2011). Clinical trial of focal segmental glomerulosclerosis in children and young adults. *Kidney Int*, 80, 868–78.

Gohh, R. Y., Yango, A. F., Morrissey, P. E., *et al.* (2005). Preemptive plasmapheresis and recurrence of FSGS in high-risk renal transplant recipients. *Am J Transplant*, 5, 2907–12.

Gulati, A., Sinha, A., Jordan, S. C., *et al.* (2010). Efficacy and safety of treatment with rituximab for difficult steroid-resistant and -dependent nephrotic syndrome: multicentric report. *Clin J Am Soc Nephrol*, 5, 2207–12.

Gulati, S., Prasad, N., Sharma, R. K., *et al.* (2008). Tacrolimus: a new therapy for steroid-resistant nephrotic syndrome in children. *Nephrol Dial Transplant*, 23, 910–13.

Hari, P., Bagga, A., Jindal, N., *et al.* (2001). Treatment of focal glomerulosclerosis with pulse steroids and oral cyclophosphamide. *Pediatr Nephrol*, 16, 901–5.

Heering, P., Braun, N., Mullejans, R., *et al.* (2004). Cyclosporine A and chlorambucil in the treatment of idiopathic focal segmental glomerulosclerosis. *Am J Kidney Dis*, 43, 10–18.

Ingulli, E., Singh, A., Baqi, N., *et al.* (1995). Aggressive, long-term cyclosporine therapy for steroid-resistant focal segmental glomerulosclerosis. *J Am Soc Nephrol*, 5, 1820–5.

Kaminuma, O. (2008). Selective inhibitors of nuclear factor of activated T cells: potential therapeutic drugs for the treatment of immunological and inflammatory diseases. *Inflamm Allergy Drug Targets*, 7, 35–40.

Kisner, T., Burst, V., Teschner, S., *et al.* (2012). Rituximab treatment for adults with refractory nephrotic syndrome: a single-center experience and review of the literature. *Nephron Clin Pract*, 120, c79–85.

Korbet, S. M. (1999). Clinical picture and outcome of primary focal segmental glomerulosclerosis. *Nephrol Dial Transplant*, 14 Suppl 3, 68–73.

Korbet, S. M. (2002). Treatment of primary focal segmental glomerulosclerosis. *Kidney Int*, 62, 2301–10.

Letavernier, E., Bruneval, P., Mandet, C., *et al.* (2007). High sirolimus levels may induce focal segmental glomerulosclerosis de novo. *Clin J Am Soc Nephrol*, 2, 326–33.

Lieberman, K. V. and Tejani, A. (1996). A randomized double-blind placebo-controlled trial of cyclosporine in steroid-resistant idiopathic focal segmental glomerulosclerosis in children. *J Am Soc Nephrol*, 7, 56–63.

Linshaw, M. A. and Gruskin, A. B. (1974). Management of the nephrotic syndrome. *Clin Pediatr (Phila)*, 13, 45–51.

Loeffler, K., Gowrishankar, M., and Yiu, V. (2004). Tacrolimus therapy in pediatric patients with treatment-resistant nephrotic syndrome. *Pediatr Nephrol*, 19, 281–7.

Magnasco, A., Ravani, P., Edefonti, A., *et al.* (2012). Rituximab in children with resistant idiopathic nephrotic syndrome. *J Am Soc Nephrol*, 23, 1117–24.

Mendizabal, S., Zamora, I., Berbel, O., *et al.* (2005). Mycophenolate mofetil in steroid/cyclosporine-dependent/resistant nephrotic syndrome. *Pediatr Nephrol*, 20, 914–9.

Mendoza, S. A., Reznik, V. M., Griswold, W. R., *et al.* (1990). Treatment of steroid-resistant focal segmental glomerulosclerosis with pulse methylprednisolone and alkylating agents. *Pediatr Nephrol*, 4, 303–7.

Meyrier, A. (1992). Antiproteinuric and immunological effects of cyclosporin A in the treatment of glomerular diseases. *Nephrol Dial Transplant*, 7 Suppl 1, 80–4.

Meyrier, A. (1999). Treatment of primary focal segmental glomerulosclerosis. *Nephrol Dial Transplant*, 14 Suppl 3, 74–8.

Meyrier, A. (2009a). An update on the treatment options for focal segmental glomerulosclerosis. *Expert Opin Pharmacother*, 10, 615–28.

Meyrier, A. Y. (2009b). Treatment of focal segmental glomerulosclerosis with immunophilin modulation: when did we stop thinking about pathogenesis? *Kidney Int*, 76, 487–91.

Meyrier, A., Noel, L. H., Auriche, P., *et al.* (1994). Long-term renal tolerance of cyclosporin A treatment in adult idiopathic nephrotic syndrome. Collaborative Group of the Societe de Nephrologie. *Kidney Int*, 45, 1446–56.

Meyrier, A., Simon, P., Perret, G., *et al.* (1986). Remission of idiopathic nephrotic syndrome after treatment with cyclosporin A. *Br Med J (Clin Res Ed)*, 292, 789–92.

Mukerji, N., Damodaran, T. V., and Winn, M. P. (2007). TRPC6 and FSGS: the latest TRP channelopathy. *Biochim Biophys Acta*, 1772, 859–68.

Nakayama, M., Katafuchi, R., Yanase, T., *et al.* (2002). Steroid responsiveness and frequency of relapse in adult-onset minimal change nephrotic syndrome. *Am J Kidney Dis*, 39, 503–12.

Niaudet, P. (1994). Treatment of childhood steroid-resistant idiopathic nephrosis with a combination of cyclosporine and prednisone. French Society of Pediatric Nephrology. *J Pediatr*, 125, 981–6.

Niaudet, P., and Habib, R. (1994). Cyclosporine in the treatment of idiopathic nephrosis. *J Am Soc Nephrol*, 5, 1049–56.

Pena, A., Bravo, J., Melgosa, M., *et al.* (2007). Steroid-resistant nephrotic syndrome: long-term evolution after sequential therapy. *Pediatr Nephrol*, 22, 1875–80.

Peters, H. P., van de kar, N. C., and Wetzels, J. F. (2008). Rituximab in minimal change nephropathy and focal segmental glomerulosclerosis: report of four cases and review of the literature. *Neth J Med*, 66, 408–15.

Peyser, A., Machardy, N., Tarapore, F., *et al.* (2010). Follow-up of phase I trial of adalimumab and rosiglitazone in FSGS: III. Report of the FONT study group. *BMC Nephrol*, 11, 2.

Philibert, D. and Cattran, D. (2008). Remission of proteinuria in primary glomerulonephritis: we know the goal but do we know the price? *Nat Clin Pract Nephrol*, 4, 550–9.

Ponticelli, C. (2010). Recurrence of focal segmental glomerular sclerosis (FSGS) after renal transplantation. *Nephrol Dial Transplant*, 25, 25–31.

Ponticelli, C., Rizzoni, G., Edefonti, A., *et al.* (1993). A randomized trial of cyclosporine in steroid-resistant idiopathic nephrotic syndrome. *Kidney Int*, 43, 1377–84.

Ponticelli, C., Villa, M., Banfi, G., *et al.* (1999). Can prolonged treatment improve the prognosis in adults with focal segmental glomerulosclerosis? *Am J Kidney Dis*, 34, 618–25.

Prytula, A., Iijima, K., Kamei, K., *et al.* (2010). Rituximab in refractory nephrotic syndrome. *Pediatr Nephrol*, 25, 461–8.

Rao, A. (2009). Signaling to gene expression: calcium, calcineurin and NFAT. *Nat Immunol*, 10, 3–5.

Rennert, W. P., Kala, U. K., Jacobs, D., *et al.* (1999). Pulse cyclophosphamide for steroid-resistant focal segmental glomerulosclerosis. *Pediatr Nephrol*, 13, 113–16.

Schwartz, M. M., Evans, J., Bain, R., *et al.* (1999). Focal segmental glomerulosclerosis: prognostic implications of the cellular lesion. *J Am Soc Nephrol*, 10, 1900–7.

Segarra, A., Amoedo, M. L., Martinez Garcia, J. M., *et al.* (2007). Efficacy and safety of 'rescue therapy' with mycophenolate mofetil in resistant primary glomerulonephritis--a multicenter study. *Nephrol Dial Transplant*, 22, 1351–60.

Segarra, A., Vila, J., Pou, L., *et al.* (2002). Combined therapy of tacrolimus and corticosteroids in cyclosporin-resistant or -dependent idiopathic focal glomerulosclerosis: a preliminary uncontrolled study with prospective follow-up. *Nephrol Dial Transplant*, 17, 655–62.

Sinha, A. and Bagga, A. (2013). Rituximab therapy in nephrotic syndrome: implications for patients' management. *Nat Rev Nephrol*, 9, 154–69.

Stokes, M. B., Markowitz, G. S., Lin, J., *et al.* (2004). Glomerular tip lesion: a distinct entity within the minimal change disease/focal segmental glomerulosclerosis spectrum. *Kidney Int*, 65, 1690–702.

Stokes, M. B., Valeri, A. M., Markowitz, G. S., *et al.* (2006). Cellular focal segmental glomerulosclerosis: Clinical and pathologic features. *Kidney Int*, 70, 1783–92.

Swaminathan, S., Lager, D. J., Qian, X., *et al.* (2006). Collapsing and non-collapsing focal segmental glomerulosclerosis in kidney transplants. *Nephrol Dial Transplant*, 21, 2607–14.

Tarshish, P., Tobin, J. N., Bernstein, J., *et al.* (1996). Cyclophosphamide does not benefit patients with focal segmental glomerulosclerosis. A report of the International Study of Kidney Disease in Children. *Pediatr Nephrol*, 10, 590–3.

Troyanov, S., Wall, C. A., Miller, J. A., *et al.* (2005). Focal and segmental glomerulosclerosis: definition and relevance of a partial remission. *J Am Soc Nephrol*, 16, 1061–8.

Tumlin, J. A., Miller, D., Near, M., *et al.* (2006). A prospective, open-label trial of sirolimus in the treatment of focal segmental glomerulosclerosis. *Clin J Am Soc Nephrol*, 1, 109–16.

Tune, B. M., Kirpekar, R., Sibley, R. K., *et al.* (1995). Intravenous methylprednisolone and oral alkylating agent therapy of prednisone-resistant pediatric focal segmental glomerulosclerosis: a long-term follow-up. *Clin Nephrol*, 43, 84–8.

Valeri, A., Barisoni, L., Appel, G. B., *et al.* (1996). Idiopathic collapsing focal segmental glomerulosclerosis: a clinicopathologic study. *Kidney Int*, 50, 1734–46.

Waldo, F. B., Benfield, M. R., and Kohaut, E. C. (1992). Methylprednisolone treatment of patients with steroid-resistant nephrotic syndrome. *Pediatr Nephrol*, 6, 503–5.

Wang, W., Xia, Y., Mao, J., *et al.* (2012). Treatment of tacrolimus or cyclosporine A in children with idiopathic nephrotic syndrome. *Pediatr Nephrol*, 27, 2073–9.

Westhoff, T. H. and van der Giet, M. (2007). Tacrolimus in the treatment of idiopathic nephrotic syndrome. *Expert Opin Investig Drugs*, 16, 1099–110.

Winn, M. P. (2003). Approach to the evaluation of heritable diseases and update on familial focal segmental glomerulosclerosis. *Nephrol Dial Transplant*, 18 Suppl 6, vi14–20.

Winn, M. P. (2008). 2007 Young Investigator Award: TRP'ing into a new era for glomerular disease. *J Am Soc Nephrol*, 19, 1071–5.

Winn, M. P., Conlon, P. J., Lynn, K. L., *et al.* (2005). A mutation in the TRPC6 cation channel causes familial focal segmental glomerulosclerosis. *Science*, 308, 1801–4.

Xing, C. Y., Saleem, M. A., Coward, R. J., *et al.* (2006). Direct effects of dexamethasone on human podocytes. *Kidney Int*, 70, 1038–45.

Yabu, J. M., Ho, B., Scandling, J. D., *et al.* (2008). Rituximab failed to improve nephrotic syndrome in renal transplant patients with recurrent focal segmental glomerulosclerosis. *Am J Transplant*, 8, 222–7.

Yokoyama, H., Wada, T., Zhang, W., *et al.* (2007). Advances in apheresis therapy for glomerular diseases. *Clin Exp Nephrol*, 11, 122–7.

Yorgin, P. D., Krasher, J., and Al-Uzri, A. Y. (2001). Pulse methylprednisolone treatment of idiopathic steroid-resistant nephrotic syndrome. *Pediatr Nephrol*, 16, 245–50.

CHAPTER 59

Pathogenesis of proteinuria in minimal change disease and focal segmental glomerulosclerosis

Patrick Niaudet and Alain Meyrier

Introduction

It is now well established that the podocyte, and in particular the slit diaphragm structure, is critical to the barrier to serum albumin entering glomerular filtrate in large quantities. In minimal change disease (MCD) there is proteinuria without podocyte changes other than reversible foot process flattening and fusion whereas in focal segmental glomerulosclerosis (FSGS) there is not only podocyte dysfunction but also podocyte loss.

The location of the defect in minimal change disease

The permeability of the glomerular basement membrane (GBM) is determined not only by the size but also to some extent by the charge of the proteins. In conventional teaching the anionic charges of the GBM repulse the negatively charged albumin molecules, whose isoelectric point is 4.6. The mechanism of proteinuria in the absence of histological alterations on light microscopy has suggested to earlier authors an electrochemical disorder of the GBM. However, several lines of argument conflict with this, including the observations in knockout mice that deleting proteoglycan types did not cause proteinuria (see Chapter 320). Other genetic defects point to the primacy of the podocyte in controlling the egress of protein into glomerular filtrate. Several models that have been proposed for the mechanisms of glomerular filtration have recently been summarized by Moeller and Tenten (2013).

Circulating permeability factors

Several arguments support the role of a circulating permeability factor in the pathogenesis of MCD and FSGS. These arguments stem from the recurrence of proteinuria after renal transplantation in patients with steroid-resistant nephrotic syndrome (FSGS), the remission of proteinuria in FSGS after plasma exchanges (Hoyer et al., 1972; Dantal et al., 1994; Canaud et al., 2009), and the disappearance of proteinuria when a kidney from a patient with MCD has been transplanted in a patient without nephrotic syndrome (Ali et al., 1994).

Lagrue et al. first described the vascular permeability factor (VPF), a lymphokine found in the supernatant of concanavalin A-activated lymphocytes from patients with MCD which enhances vascular permeability when injected intradermally in the guinea pig (Lagrue et al., 1975). The group of Bakker reported that haemopexin induces nephrin-dependent reorganization of the actin cytoskeleton in cultured podocytes and causes proteinuria when injected in rats (Bakker et al., 2005a; Lennon et al., 2008). The authors suggested that haemopexin may exist in an altered isoform, showing enhanced protease activity in patients during a relapse as compared with patients in remission or patients with other forms of primary glomerulopathy (Bakker et al., 2005b). Savin et al., using isolated rat glomeruli incubated *in vitro* with the sera of patients with recurrence of proteinuria after renal transplantation, described a glomerular permeability factor (Savin et al., 1996). Using this assay, the same group identified the cardiotrophin-like cytokine 1 (CLC-1), a protein of the interleukin (IL)-6 family (McCarthy et al., 2010). Its concentration is 100 times higher in the sera of patients with recurrent proteinuria after transplantation compared to normal subjects. The injection of CLC-1 in rats induces proteinuria. CLC-1 decreases nephrin expression in cultured podocytes. However, the role of CLC-1 being the 'FSGS factor' has not been convincingly demonstrated.

The Buffalo/Mna strain of rats spontaneously develops proteinuria with FSGS at 2 months of age. The nephrotic syndrome recurs when these rats receive a kidney from a healthy LEW.1W rat (Le Berre et al., 2002). Conversely, proteinuria and renal lesions regress when kidneys from a Buffalo/Mna rat are transplanted into normal LEW.1W rats.

However, this putative factor has not yet been identified, and it is currently unclear whether idiopathic nephrotic syndrome may be caused by several different factors, and whether this or these factors are identical in MCD and FSGS (Tesar and Zima, 2008).

Immunological abnormalities

T lymphocytes

In 1974, Shalhoub postulated that MCD might be secondary to a disorder of the immune system (Shalhoub, 1974). He hypothesized that the production of a lymphokine may increase the permeability

of the glomerular filtration barrier to proteins. The arguments supporting this hypothesis were the response of the disease to corticosteroids and to alkylating agents, the remission occurring in association with measles, which depresses cell-mediated immunity, the susceptibility of patients to pneumococcal infections, and the occurrence of minimal change nephrotic syndrome in patients with Hodgkin disease.

Alterations in the production of several lymphokines have been reported in patients with idiopathic nephrotic syndrome (Bakker and van Luijk, 1989). Increased IL-2 levels have been found in lymphocyte culture supernatants from patients with idiopathic nephrotic syndrome and IL-2 can induce proteinuria and a reduction of the anionic sites of the GBM when injected into the rat kidney (Heslan et al., 1991). *IL-13* gene expression in both CD4+ and CD8+ T cells is upregulated in children with steroid-sensitive idiopathic nephrotic syndrome during relapse (Yap et al., 1999). IL-13 is one of the cytokines secreted by T-helper 2 (Th2) cells, and it has been shown that T-cell activation early evolves towards Th2 phenotype in MCD (Sahali et al., 2002). Receptors for IL-13 have been found in podocytes, with direct effects of IL-13 on podocytes and their signalling pathways (Van Den Berg et al., 2000). The IL-13 promoter contains two nuclear factor kappa B (NF-κB) responsive elements and high and sustained plasma levels of NF-κB, a member of the chromatin remodelling complex, have been detected during relapse (Sahali et al., 2001; Audard et al., 2012), suggesting a disorder of transcriptional regulation in minimal change nephrotic syndrome.

The impairment of T-regulatory (Treg) cell function in patients during relapse leads to an enhanced cytokine release by T effector cells (Araya et al., 2009). In the Buffalo/Mna rat model of nephrotic syndrome, the transfer of CD4+CD25+ T cells (Treg) significantly reduces proteinuria (Le Berre et al., 2009).

CD-80 (B7-1), a transmembrane protein on antigen-presenting cells, which acts as a costimulatory signal in T-lymphocyte activation, is also expressed on podocytes in several experimental models of nephrotic syndrome. Upregulation of CD-80 in podocytes of mice leads to nephrotic-range proteinuria whereas mice lacking CD-80 are protected from lipopolysaccharide-induced nephrotic syndrome, suggesting a link between podocyte B7-1 expression and proteinuria (Reiser et al., 2004). Urinary CD-80 levels are increased in children with MCD (Garin et al., 2009).

A remarkable experiment by Koyama et al. consisted in creating a T-cell hybridoma derived from the T cells of a patient with minimal change nephrotic syndrome and isolating a glomerular permeability factor (GPF) (Koyama et al., 1991). When this GPF was injected intravenously into rats, it induced a significant proteinuria and in normal human lymphocyte culture the GPF enhanced concanavalin-A (Con-A)-induced lymphocyte blastogenesis by > 10-fold. Electron microscopy with polyethyleneimine (PEI) staining, indicated that the GPF induced the changes in the arrangement of PEI particles and partial fusion of glomerular epithelial cells. The molecular weight of GPF was estimated to be between 60,000 and 160,000 Da. The molecular weight of the GPF and its tumour necrosis factor-like activity led to speculation that the factor was a lymphokine.

B lymphocytes

Anomalies of B lymphocytes have been reported. Patients with MCD have depressed serum IgG levels. This is more pronounced during relapses, but persists during remission (Giangiacomo et al., 1975). Conversely, serum IgM is elevated. Altered serum levels of IgG and IgM may be secondary to abnormal T-cell regulation of immunoglobulin synthesis (Yokoyama et al., 1987). Several reports indicate that most patients with steroid-dependent MCD respond to rituximab, a chimeric monoclonal antibody that depletes the CD20 lymphocyte population, suggesting a role for B cells in addition to T cells in the pathogenesis of MCD (Sinha and Bagga, 2013). Rituximab could act by the induction of regulatory T lymphocytes, as this has been observed in patients with lupus nephritis (Sfikakis et al., 2007; Vallerskog et al., 2007). Fornoni et al. showed that rituximab also acts directly on podocytes by stabilizing the podocyte cytoskeleton and preventing apoptosis through an interaction with the sphingomyelin phosphodiesterase acid-like 3b protein (SMPDL-3b) which is expressed in podocytes (Fornoni et al., 2011).

Glomerular permeability factors in focal segmental glomerulonephritis

As discussed elsewhere in this chapter there is increasing evidence that, at least in adults, MCD and idiopathic FSGS do not share a common pathophysiology. The quest for the elusive factor or factors leading to the *structural* podocyte disease that characterizes FSGS is more recent than that of T-cell secreted substances that induce the *functional* podocytopathy of MCD (Maas et al., 2014).

Relapse of nephrotic FSGS following transplantation led to studying the effect of nephrotic serum in rats. In a seminal paper, Zimmerman studied the serum from a patient who had had recurrence of nephrotic syndrome and FSGS after two cadaveric renal allografts (Zimmerman, 1984). Serum was infused into the aorta of rats. During infusion there were significant increases in mean urinary protein and rat albumin excretion, which persisted after infusion. When sera from 10 patients with nephrotic syndrome secondary to other glomerulopathies were infused no changes in urinary protein or albumin excretion were noted. Likewise no changes in urine protein or albumin excretion were produced with infusion of serum from a patient with FSGS without recurrence after transplantation. Increases in urine protein and albumin excretion noted during and after infusion of recurrent FSGS serum were independent of changes in glomerular filtration rate, urine volume, or fractional excretion of sodium. Zimmerman suggested there was a factor or factors present in the serum of this patient capable of producing enhanced urinary protein excretion in the rat. He rightly concluded that this factor which is heat stable at 56°C could possibly play a role in the pathogenesis of recurrent nephrotic syndrome.

The same experiment was carried out by Sharma et al. about two decades later (Sharma et al., 2002). Whilst the same group had previously elaborated an *in vitro* test based on variations of isolated glomeruli volume (GVV) following a hypotonic stress in the presence of nephrotic plasma (Savin et al., 1996). This bioassay was applied to identifying the GPF in the plasma of patients with FSGS who relapsed following transplantation and to predicting such relapse before grafting. The test was subsequently used to further identify the 'FSGS factor' and find molecules that counteract its proteinuric effect (Sharma et al., 1999, 2000).

Unfortunately other investigators who used this bioassay in various forms of glomerulonephritis observed such an overlap with

FSGS that the specificity and the predictive value of the test were not confirmed (Godfrin et al., 1997).

A role of soluble urokinase receptor (suPAR) in the pathogenesis of FSGS have been proposed (Wei et al., 2011). In mice models, suPAR activates podocyte β(3) integrin causing foot process effacement, proteinuria, and FSGS-like glomerulopathy. However, Cathelin et al. showed that the administration of recombinant suPAR to C57BL/6J wild-type mice does not result in albuminuria nor podocyte architecture alterations (Cathelin et al., 2014). With regard to FSGS, Wei et al. indicated that suPAR is elevated in two-thirds of subjects with primary FSGS, but not in people with other glomerular diseases. They further found that a higher concentration of suPAR before transplantation underlies an increased risk for recurrence of FSGS after transplantation. These findings might suggest that the renal disease only develops when suPAR sufficiently activates podocyte β(3) integrin and that the disease could possibly be abrogated by lowering serum suPAR concentrations through plasmapheresis, or by interfering with the suPAR-β(3) integrin interaction through antibodies and small molecules targeting either uPAR or β(3) integrin (Jefferson and Alpers, 2013). Wei et al. analysed circulating suPAR levels in two cohorts of children and adults with biopsy-proven primary FSGS (Wei et al., 2012). There were 70 patients from the North America-based FSGS clinical trial (CT) and 94 patients from PodoNet, the Europe-based consortium studying steroid-resistant nephrotic syndrome. Circulating suPAR levels were elevated in 84.3% and 55.3% of patients with FSGS patients in the CT and PodoNet cohorts, respectively, compared with 6% of controls. An interesting observation demonstrated that suPAR can be transmitted from mother to child. A mother with FSGS and high suPAR levels (4635 pg/mL, about twice the level in controls) gave birth to a proteinuric child with suPAR levels of 5225 pg/mL. At 12 months of age, proteinuria had become undetectable in the child (Kemper et al., 2013). This, however, does not demonstrate that suPAR was the cause of proteinuria, as another unknown factor could conceivably have been transmitted through the placenta along with suPAR.

suPAR is not specific to FSGS. It is released from cells during inflammatory diseases and elevated levels predict outcomes in a host of viral, microbial, and parasitic infections and in malignancies. suPAR concentrations are also high in an intensive care setting, with a prognostic value. Moreover in these inflammatory conditions, despite extremely high suPAR serum levels there is no significant proteinuria.

A definitive role of suPAR in the pathogenesis of FSGS is still debated. (Bock et al., 2013; Maas et al., 2013a; Sever et al., 2013). Three studies including a total of 1151 patients, 212 of whom had primary FSGS, concluded that levels of suPAR do not discriminate between primary FSGS and other causes of glomerular diseases (Meijers et al., 2014; Sinha et al., 2014; Wada et al., 2014). For Maas and co-authors the measurement of suPAR using currently available assays has no value at the present time in decision-making in routine clinical practice (Maas et al., 2013). Altogether, these data argue against suPAR being per se the FSGS factor (Deegens and Wetzels, 2014).

References

Ali, A. A., Wilson, E., Moorhead, J. F., *et al.* (1994). Minimal-change glomerular nephritis. Normal kidneys in an abnormal environment? *Transplantation*, 58, 849–52.

Araya, C., Diaz, L., Wasserfall, C., *et al.* (2009). T regulatory cell function in idiopathic minimal lesion nephrotic syndrome. *Pediatr Nephrol*, 24, 1691–8.

Audard, V., Pawlak, A., Candelier, M., *et al.* (2012). Upregulation of nuclear factor-related kappa B suggests a disorder of transcriptional regulation in minimal change nephrotic syndrome. *PLoS One*, 7, e30523.

Bakker, W. W., Borghuis, T., Harmsen, M. C., *et al.* (2005a). Protease activity of plasma hemopexin. *Kidney Int*, 68, 603–10.

Bakker, W. W., Van Dael, C. M., Pierik, L. J., *et al.* (2005b). Altered activity of plasma hemopexin in patients with minimal change disease in relapse. *Pediatr Nephrol*, 20, 1410–5.

Bakker, W. W. and Van Luijk, W. H. (1989). Do circulating factors play a role in the pathogenesis of minimal change nephrotic syndrome? *Pediatr Nephrol*, 3, 341–9.

Bock, M. E., Price, H. E., Gallon, L., *et al.* (2013). Serum soluble urokinase-type plasminogen activator receptor levels and idiopathic FSGS in children: a single-center report. *Clin J Am Soc Nephrol*, 8, 1304–11.

Canaud, G., Zuber, J., Sberro, R., *et al.* (2009). Intensive and prolonged treatment of focal and segmental glomerulosclerosis recurrence in adult kidney transplant recipients: a pilot study. *Am J Transplant*, 9, 1081–6.

Cathelin, D., Placier, S., Ploug, M., *et al.* (2014). Administration of recombinant soluble urokinase receptor per se is not sufficient to induce podocyte alterations and proteinuria in mice. *J Am Soc Nephrol*.

Dantal, J., Bigot, E., Bogers, W., *et al.* (1994). Effect of plasma protein adsorption on protein excretion in kidney-transplant recipients with recurrent nephrotic syndrome. *N Engl J Med*, 330, 7–14.

Deegens, J. K. and Wetzels, J. F. (2014). Glomerular disease: the search goes on: suPAR is not the elusive FSGS factor. *Nat Rev Nephrol*, 10, 431–2.

Fornoni, A., Sageshima, J., Wei, C., *et al.* (2011). Rituximab targets podocytes in recurrent focal segmental glomerulosclerosis. *Sci Transl Med*, 3, 85ra46.

Garin, E. H., Diaz, L. N., Mu, W., *et al.* (2009). Urinary CD80 excretion increases in idiopathic minimal-change disease. *J Am Soc Nephrol*, 20, 260–6.

Giangiacomo, J., Cleary, T. G., Cole, B. R., *et al.* (1975). Serum immunoglobulins in the nephrotic syndrome. A possible cause of minimal-change nephrotic syndrome. *N Engl J Med*, 293, 8–12.

Godfrin, Y., Dantal, J., Perretto, S., *et al.* (1997). Study of the in vitro effect on glomerular albumin permselectivity of serum before and after renal transplantation in focal segmental glomerulosclerosis. *Transplantation*, 64, 1711–15.

Heslan, J. M., Branellec, A. I., Pilatte, Y., *et al.* (1991). Differentiation between vascular permeability factor and IL-2 in lymphocyte supernatants from patients with minimal-change nephrotic syndrome. *Clin Exp Immunol*, 86, 157–62.

Hoyer, J. R., Vernier, R. L., Najarian, J. S., *et al.* (1972). Recurrence of idiopathic nephrotic syndrome after renal transplantation. *Lancet*, 2, 343–8.

Jefferson, J. A. and Alpers, C. E. (2013). Glomerular disease: 'suPAR'-exciting times for FSGS. *Nat Rev Nephrol*, 9, 127–8.

Kemper, M. J., Wei, C., and Reiser, J. (2013). Transmission of glomerular permeability factor soluble urokinase plasminogen activator receptor (suPAR) from a mother to child. *Am J Kidney Dis*, 61, 352.

Koyama, A., Fujisaki, M., Kobayashi, M., *et al.* (1991). A glomerular permeability factor produced by human T cell hybridomas. *Kidney Int*, 40, 453–60.

Lagrue, G., Branellec, A., Blanc, C., *et al.* (1975). A vascular permeability factor in lymphocyte culture supernants from patients with nephrotic syndrome. II. Pharmacological and physicochemical properties. *Biomedicine*, 23, 73–5.

Le Berre, L., Bruneau, S., Naulet, J., *et al.* (2009). Induction of T regulatory cells attenuates idiopathic nephrotic syndrome. *J Am Soc Nephrol*, 20, 57–67.

Le Berre, L., Godfrin, Y., Gunther, E., *et al.* (2002). Extrarenal effects on the pathogenesis and relapse of idiopathic nephrotic syndrome in Buffalo/Mna rats. *J Clin Invest*, 109, 491–8.

Lennon, R., Singh, A., Welsh, G. I., *et al.* (2008). Hemopexin induces nephrin-dependent reorganization of the actin cytoskeleton in podocytes. *J Am Soc Nephrol*, 19, 2140–9.

Maas, R. J., Deegens, J. K., and Wetzels, J. F. (2013). Serum suPAR in patients with FSGS: trash or treasure? *Pediatr Nephrol*, 28(7), 1041–8.

Maas, R. J., Deegens, J. K., and Wetzels, J. F. (2014). Permeability factors in idiopathic nephrotic syndrome: historical perspectives and lessons for the future. *Nephrol Dial Transplant*, 29, 2207–16.

McCarthy, E. T., Sharma, M., and Savin, V. J. (2010). Circulating permeability factors in idiopathic nephrotic syndrome and focal segmental glomerulosclerosis. *Clin J Am Soc Nephrol*, 5, 2115–21.

Meijers, B., Maas, R. J., Sprangers, B., *et al.* (2014). The soluble urokinase receptor is not a clinical marker for focal segmental glomerulosclerosis. *Kidney Int*, 85, 636–40.

Moeller, M. J., and Tenten, V. (2013). Renal albumin filtration: alternative models to the standard physical barriers. *Nat Rev Nephrol*, 9, 266–77.

Reiser, J., Von Gersdorff, G., Loos, M., *et al.* (2004). Induction of B7–1 in podocytes is associated with nephrotic syndrome. *J Clin Invest*, 113, 1390–7.

Savin, V. J., Sharma, R., Sharma, M., *et al.* (1996). Circulating factor associated with increased glomerular permeability to albumin in recurrent focal segmental glomerulosclerosis. *N Engl J Med*, 334, 878–83.

Sahali, D., Pawlak, A., Le Gouvello, S., *et al.* (2001). Transcriptional and post-transcriptional alterations of IkappaBalpha in active minimal-change nephrotic syndrome. *J Am Soc Nephrol*, 12, 1648–58.

Sahali, D., Pawlak, A., Valanciute, A., *et al.* (2002). A novel approach to investigation of the pathogenesis of active minimal-change nephrotic syndrome using subtracted cDNA library screening. *J Am Soc Nephrol*, 13, 1238–47.

Sever, S., Trachtman, H., Wei, C., *et al.* (2013). Is there clinical value in measuring suPAR levels in FSGS? *Clin J Am Soc Nephrol*, 8, 1273–5.

Sfikakis, P. P., Souliotis, V. L., Fragiadaki, K. G., *et al.* (2007). Increased expression of the FoxP3 functional marker of regulatory T cells following B cell depletion with rituximab in patients with lupus nephritis. *Clin Immunol*, 123, 66–73.

Shalhoub, R. J. (1974). Pathogenesis of lipoid nephrosis: a disorder of T-cell function. *Lancet*, 2, 556–60.

Sharma, M., Sharma, R., McCarthy, E. T., *et al.* (1999). "The FSGS factor": enrichment and in vivo effect of activity from focal segmental glomerulosclerosis plasma. *J Am Soc Nephrol*, 10, 552–61.

Sharma, R., Sharma, M., McCarthy, E. T., *et al.* (2000). Components of normal serum block the focal segmental glomerulosclerosis factor activity in vitro. *Kidney Int*, 58, 1973–9.

Sharma, M., Sharma, R., Reddy, S. R., *et al.* (2002). Proteinuria after injection of human focal segmental glomerulosclerosis factor. *Transplantation*, 73, 366–72.

Sinha, A., and Bagga, A. (2013). Rituximab therapy in nephrotic syndrome: implications for patients' management. *Nat Rev Nephrol*, 9, 154–69.

Sinha, A., Bajpai, J., Saini, S., *et al.* (2014). Serum-soluble urokinase receptor levels do not distinguish focal segmental glomerulosclerosis from other causes of nephrotic syndrome in children. *Kidney Int*, 85, 649–58.

Tesar, V., and Zima, T. (2008). Recent progress in the pathogenesis of nephrotic proteinuria. *Crit Rev Clin Lab Sci*, 45, 139–220.

Vallerskog, T., Gunnarsson, I., Widhe, M., *et al.* (2007). Treatment with rituximab affects both the cellular and the humoral arm of the immune system in patients with SLE. *Clin Immunol*, 122, 62–74.

Wada, T., Nangaku, M., Maruyama, S., *et al.* (2014). A multicenter cross-sectional study of circulating soluble urokinase receptor in Japanese patients with glomerular disease. *Kidney Int*, 85, 641–8.

Wei, C., El Hindi, S., Li, J., *et al.* (2011). Circulating urokinase receptor as a cause of focal segmental glomerulosclerosis. *Nat Med*, 17, 952–60.

Wei, C., Trachtman, H., Li, J., *et al.* (2012). Circulating suPAR in two cohorts of primary FSGS. *J Am Soc Nephrol*, 23, 2051–9.

Yap, H. K., Cheung, W., Murugasu, B., *et al.* (1999). Th1 and Th2 cytokine mRNA profiles in childhood nephrotic syndrome: evidence for increased IL-13 mRNA expression in relapse. *J Am Soc Nephrol*, 10, 529–37.

Yokoyama, H., Kida, H., Abe, T., *et al.* (1987). Impaired immunoglobulin G production in minimal change nephrotic syndrome in adults. *Clin Exp Immunol*, 70, 110–15.

Zimmerman, S. W. (1984). Increased urinary protein excretion in the rat produced by serum from a patient with recurrent focal glomerular sclerosis after renal transplantation. *Clin Nephrol*, 22, 32–8.

CHAPTER 60

Membranous glomerulonephritis: overview

Daniel C. Cattran and Heather N. Reich

Membranous glomerulonephritis (MGN) is the most common cause of adult-onset nephrotic syndrome, and a common glomerular cause of end-stage renal failure (see Chapter 61). It is caused by antibodies to podocyte surface molecules, usually autoantibodies. In most patients with primary membranous nephropathy the target is the phospholipase A2 receptor (see Chapter 64). It is hoped that robust assays for this antibody will help to guide therapy but it has not been possible to test this adequately yet. Primary MGN accounts for about 70% of cases with regional variations.

MGN is more common in men (~2:1) and its peak incidence is in middle adult life (see Chapter 61). Secondary membranous nephropathy (see Chapter 63) occurs in lupus and some other immune or autoimmune disorders, in hepatitis B infection, after exposure to some drugs or toxins, and in some cancers, and other conditions shown in Box 60.1.

Box 60.1 Conditions and medications associated with secondary MGN

Immunologic

- SLE and mixed connective tissue disease
- Rheumatoid arthritis
- Antiphospholipid antibody syndrome
- Ankylosing spondylitis
- Sjögren syndrome
- Sarcoidosis
- Autoimmune hepatitis
- Autoimmune thyroiditis (Hashimoto)
- Graft-versus-host disease following bone marrow transplantation.

Neoplasm

- Solid tumours—lung, breast, gastrointestinal, renal, prostate, etc.
- Haematological—lymphoma, usually non-Hodgkin.

Infection

- Viral—hepatitis, usually hepatitis B, also C, HIV
- Bacterial—syphilis, streptococcus,
- Mycobacterial—leprosy
- Parasitic—malaria, schistosomiasis, filariasis.

Medication

- D-Penicillamine
- Gold
- Captopril
- Probenecid
- Bucillamine
- Non-steroidal anti-inflammatories.

Other

- Mercury
- Sickle-cell disease
- Diabetes.

Pathologically it is characterized by deposition of immunoglobulin G in a granular pattern beneath the podocyte, between it and the glomerular basement membrane (GBM) (see Chapter 61). On light microscopy, the GBM appears thickened, with growth of normal GBM material around deposits giving rise to 'spikes' on silver stains (Fig. 60.1). Later there is increased mesangial matrix and glomerulosclerosis.

Supportive therapy for nephrotic syndrome, with particular attention to use of angiotensin-converting enzyme inhibitors and blood

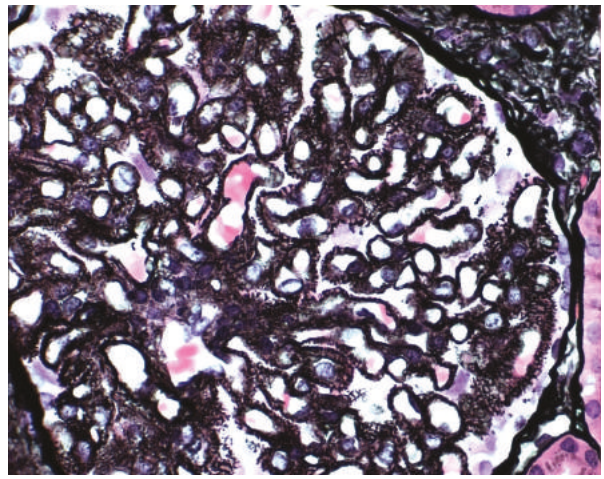

Fig. 60.1 Membranous glomerulonephritis is characterized by the presence of subepithelial immune complex deposits, best visualized with electron microscopy. Image courtesy of Dr Andrew Herzenberg and Dr Rohan John.

pressure control, is indicated for all patients (see Chapter 62). It remits spontaneously in some patients, but persistent heavy proteinuria, age, male sex, and reduced glomerular filtration rate predict poorer outcomes. The only treatments of proven value in primary MGN involve alkylating agents (cyclophosphamide or chlorambucil) used over a period of 6 months. Published studies have always combined this with high-dose corticosteroid therapy. These treatments are toxic, so are reserved by most clinicians for patients who are deteriorating despite conservative measures after a period of observation and optimization of supportive management. Other potential treatments, notably anti-B-cell antibodies, are unproven at present and need to be tested in randomized controlled trials.

CHAPTER 61

Membranous glomerulonephritis: clinical features and diagnosis

Daniel C. Cattran and Heather N. Reich

Epidemiology

Membranous glomerulonephritis (MGN) is the most common cause of nephrotic syndrome observed in adult populations, accounting for approximately 25% of biopsies done for the investigation of this clinical syndrome (Haas et al., 1995, 1997; Schena, 1997), and 50% of primary glomerulonephritis in adults (Braden et al., 2000). In large biopsy series, its overall frequency is 10% of all histologic diagnoses (Davison et al., 1984; Zucchelli and Pasquali, 1998). Due to its relatively high incidence as a cause of glomerular disease, it is the second or third most common primary glomerulonephritis to lead to end-stage renal disease in the industrialized nations (Maisonneuve et al., 2000; United States Renal Data System, 2009). The majority of cases of MGN are idiopathic in nature (idiopathic or primary MGN); however, up to one-third of cases are related to systemic diseases (secondary MGN) (Row et al., 1975; Ehrenreich et al., 1976; Gluck et al., 1973; Abe et al., 1986; Cahen et al., 1989; Adu and Cameron, 1989; Glassock, 1992).

Differences in incidence of MGN have been observed over time and across different ethnic and geographic populations. Although for the past 50 years, MGN has been the most common cause of nephrotic syndrome in industrialized countries, focal segmental glomerulosclerosis (FSGS) has been emerging in some studies as a the new number one aetiology. Some of this trend is related to the inclusion of larger numbers of subjects of African origin, in whom FSGS has a higher incidence than MGN (D'Agati, 1994; Korbet et al., 1996), but an increasing proportion of FSGS has also been observed in non-African ethnic groups (Haas et al., 1997). One large recent biopsy series in an American metropolitan setting has documented a halving of the prevalence of MGN in biopsies done between 1990 and 1994 compared with the period from 1975 to 1979 from 38.3% to 14.5%, with a proportionate increase in the relative frequency of FSGS (Braden et al., 2000). In elderly populations, however, MGN remains the most common cause of primary glomerulonephritis overall (Abrass, 1985; Donadio, 1990; Passerini et al., 1993). Even in this group, most cases are primary. Some cases associated with cancer may also be primary disease coincidentally associating with cancer (see Chapter 63).

The aetiology of MGN also differs depending upon the geographic location of the study population, largely due the local prevalence of associated infectious diseases including hepatitis and malaria.

MGN consistently occurs more frequently in males, who account for up to 70% of patients with MGN in several large series (Mallick et al., 1983; Davison et al., 1984; Honkanen, 1986; Kida et al., 1986; MacTier et al., 1986). The peak age of onset of MGN is the fourth or fifth decade of life (Rosen, 1971; Gluck et al., 1973; Noel et al., 1979; Ehrenreich et al., 1976; Davison et al., 1984; Honkanen, 1986; Honkanen et al., 1992). While familial MGN is rare, it is reported and it has been documented to occur in cases of identical twins (Guella et al., 1997).

Clinical features

The characteristics of patients at the time of biopsy vary from study to study, given differing thresholds and criteria for biopsy at various centres. Therefore, although the development of MGN may in fact be quite insidious, the presence of 'full-blown' nephrotic syndrome is often the event prompting referral and biopsy. Table 61.1 illustrates the range in clinical features historically present in patients at the time of diagnosis or biopsy in several of the larger studies of patients with predominantly idiopathic MGN.

The majority of patients with MGN, ranging from half to over 90%, have nephrotic syndrome at the time of biopsy. In a recent series from the Toronto Glomerulonephritis registry 27% of the total cohort of 395 with idiopathic MGN had lower levels of proteinuria at the time of biopsy. A substantial proportion of these subjects (40%) maintained sub-nephrotic levels of proteinuria throughout their follow-up (Hladunewich et al., 2009).

Perhaps surprisingly, microscopic haematuria (usually low level) is reported in roughly half the patients surveyed. Macroscopic haematuria is also rarely reported (Ramzy et al., 1981; MacTier et al., 1986).

Hypertension is present in a significant proportion of patients (30–50%) at presentation, often in the absence of renal insufficiency. The finding of systolic hypertension at presentation is noted particularly frequently in older patients although its relationship to the disease as opposed to age per se is not clear (O'Callaghan et al., 2002).

The degree of impairment of renal function varies widely (Table 61.1). In general about 20% have abnormal renal function

Table 61.1 Clinical features present at diagnosis or time of biopsy, expressed as per cent (%) of patients

Study	Nephrotic syndrome	Non-nephrotic proteinuria	Haematuria	Hypertension	Renal insufficiency
Gluck et al., 1973	92	N/A	42	N/A	18
Noel et al., 1979	76	24	55	6[a]	6
Ramzy et al., 1981	74	26	54	~ 50	46
Davison et al., 1984	81	19	19	N/A	33
Honkanen, 1986	76	N/A	28	27	8
Kida et al., 1986	54	30	N/A	N/A	29
MacTier et al., 1986	93	7	71	45	N/A
Murphy et al., 1988	54	N/A	33	40	18
Donadio et al., 1988	83	N/A	N/A	30	N/A[b]
Hay et al., 1992	92	8	N/A	33[c]	16
Schieppati et al., 1993	~ 63	37	N/A	55	N/A[b]

[a] 7/116 subjects had hypertension in absence of renal insufficiency. A further 7 had moderate renal insufficiency.

[b] Mean creatinine 1.2 ± 0.5 mg/dL in Donadio et al. study, clearance 95.5 ± 36 mL/min/1.73 m^2 in the Schieppati study.

[c] An additional 22% had 'controlled' blood pressure on medications.

N/A = not available.

at diagnosis, usually mild to moderate in severity. In some cases this may be due to changes in intra- and extracellular fluid volume rather than parenchymal disease. The finding of acute renal failure is rare in MGN, and should direct investigation towards other diagnoses or related conditions, such as bilateral renal vein thrombosis, excessive diuresis, the use of nephrotoxic medications with tubular injury, or superimposed crescentic disease.

Serology

Immunoassays for antibodies to M-type PLA2R are becoming available, and are likely to become an established part of clinical assessment (see Chapter 63).

Pointers to secondary MGN

There are several features that should prompt the clinician to suspect a secondary cause of MGN. Extremes of age in presentation (i.e. > 60 or < 20 years), presence of weight loss, rash, arthritis, or risk factors for hepatitis, raise the likelihood that the MGN is secondary in nature. Due to the wide range of causes of secondary MGN, the clinical features present at diagnosis are more variable. History should carefully probe exposure to drugs and toxins. Skin-lightening creams have been repeatedly associated with MGN (see Chapter 82) and should be directly asked about.

Laboratory testing may reveal positive autoimmune serology (i.e. antinuclear and anti-DNA antibodies, and/or low complement level), positive hepatitis serology, or cryoglobulin tests, in contrast with primary MGN, in which these investigations are negative or normal.

Pathology

The hallmark finding in MGN is the presence of subepithelial immune complex deposits, best seen on electron microscopy (EM) (see Fig. 61.1). These are found in cases of both primary and secondary forms of the disease. The principal findings evident in MGN by conventional diagnostic techniques will be described in this section. Although many of the features are common to both primary and secondary forms of MGN, there are some characteristics which are highly suggestive of a secondary cause.

Light microscopy

Early in the disease, glomeruli may appear normal, and changes may only be evident on EM or immunofluorescence (IF). One

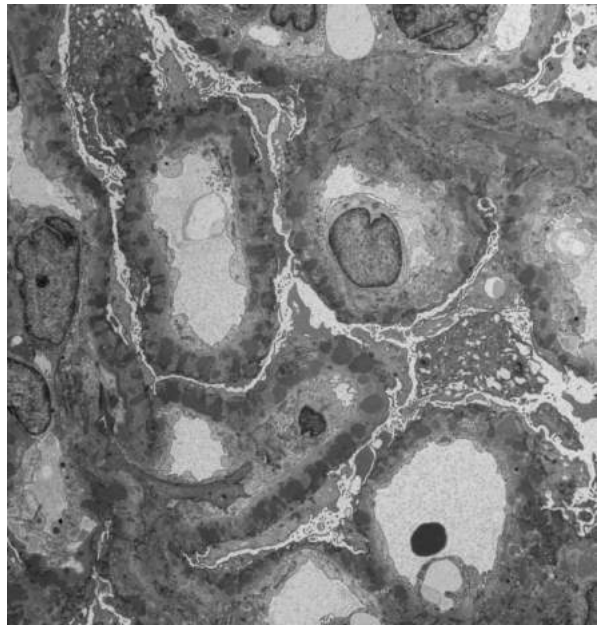

Fig. 61.1 Silver stain revealing 'spikes', representing growth of the glomerular basement membrane matrix between and around subepithelial immune deposits. Image courtesy of Dr Andrew Herzenberg and Dr Rohan John.

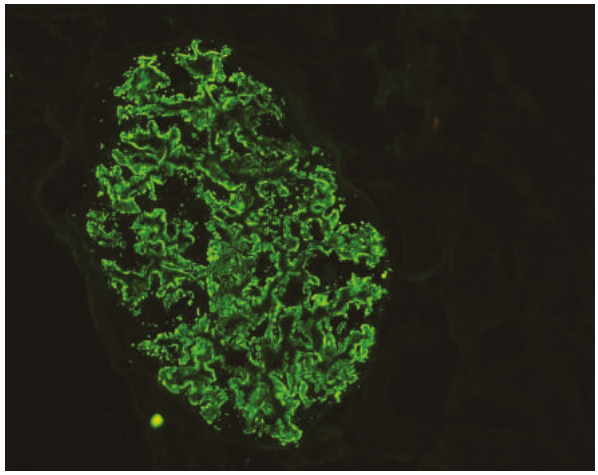

Fig. 61.2 IgG immunofluorescence in membranous glomerulonephritis.
Image courtesy of Dr Andrew Herzenberg and Dr Rohan John.

of the earliest changes seen by light microscopy (LM) is a 'moth eaten' appearance of the basement membrane when observed 'en face' using silver stains (Kern et al., 1999). Silver stain is also useful to observe the linear 'spike' projections protruding from the outer (epithelial) surface of the GBM (Fig. 61.2). With disease progression, and larger numbers of immune deposits, capillary walls and the GBM may appear globally thickened. The mesangium usually exhibits normal cellularity in idiopathic MGN. With advanced disease, segmental and global glomerulosclerosis may be observed.

The tubulointerstitial compartment, and vessels are usually unremarkable in early disease. With disease chronicity, however, tubulointerstitial atrophy and fibrosis are frequently observed. Vascular injury with arterial and arteriolar sclerosis may be evident.

Immunofluorescence

The characteristic finding on IF microscopy is granular capillary wall staining for immunoglobulin and complement. IgG is most commonly present. The predominant IgG subclass represented in idiopathic MGN biopsies is IgG4 (Doi et al., 1984). Complement C3 is commonly found by IF within deposits in MGN, despite the lack of predilection for IgG4 to fix complement. In patients where a broader range of immunoglobulins, or of IgG subtypes are found, secondary MGN is more likely (see 'Pathologic findings in secondary MGN').

A more diverse spectrum of IgG subtypes may be observed in MGN secondary to lupus (Haas, 1994) and IgG1 and IgG2 may be found more commonly in MGN diagnosed in the context of a malignancy (Ohtani et al., 2004). The finding of intense C1q staining, and the presence of other immunoglobulins such as IgM and IgA are suggestive of lupus-associated membranous nephropathy (Jennette et al., 1983).

Electron microscopy

EM is essential for diagnosing MGN, and is especially useful in detecting changes that may suggest SLE as a primary cause. Several 'staging' systems have been used to describe the pathologic changes observed (Ehrenreich and Churg, 1968; Bariety et al., 1970; Gartner et al., 1977). A modified version of the Ehrenreich and Churg scale, which includes the addition of a stage V lesion, is the most widely used system to describe the histopathologic variations seen on biopsy by EM:

- *Stage I—subepithelial deposits*: at this stage of disease, LM findings are frequently normal. The GBM is usually normal in thickness, with minimal evidence of spike formation. A few small, flat, electron-dense deposits may be seen on the epithelial surface of the GBM.
- *Stage II—spike formation*: spikes protruding from epithelial surface of GBM become clearly visible. The spikes extend between the electron-dense deposits, and are present in virtually every capillary loop. With progression, the spike tips may have a widened or clubbed appearance. The number and size of deposits are increased compared with stage I.
- *Stage III –incorporation of deposits*: electron-dense deposits become surrounded by and incorporated into the GBM. This results in an irregular thickening of the GBM, and capillary wall. The capillary wall may appear to have a split appearance due to interruption of the GBM by this immune-complex material.
- *Stage IV—disappearing deposits*: the deposits incorporated within the GBM lose their electron density. As a result, the GBM has a very irregular, thickened appearance. Areas of the GBM occupied by deposit material which has lost electron density will have a vacuolated or lucent appearance.
- *Stage V—repair stage*: during this 'healing' phase, the deposits have become completely rarified, and the GBM appearance is returning to normal. The GBM appears partially thickened, and may still contain areas of lucency. Some consider stage V to include an 'end-stage' appearance, with glomerular obsolescence and sclerosis (Donadio et al., 1988).

In addition to the pathologic changes described by the staging system, diffuse effacement of visceral epithelial cell foot processes is also frequently observed on EM in MGN. This finding may be associated with heavy proteinuria.

A review of 350 biopsies of patients with primary MGN at the University of North Carolina, United States, reveals that most patients present with stage I or II biopsy findings (total 70%) (Falk et al., 2000). Some disagreement exists regarding whether a biopsy should be staged according to the predominant lesion observed in the majority of glomeruli, versus the most advance lesion evident in the specimen. Tornroth et al. (1987) first demonstrated that in biopsies of patients with a protracted clinical course, lesions representing all stages of disease are often present in one specimen. They suggested that based upon serial biopsies of patients with MGN, the presence of subepithelial deposits correlate with an 'active' clinical stage, whereas replacement by lucent intramembranous lesions occurred during clinical remission. Cessation of formation of immune complexes may result in the presence of intramembranous healing changes, such as in a patient who has one 'flare' of proteinuria, then achieves a remission. However, ongoing formation of immune complexes, as may occur in the patient with a more protracted and progressive course, may result in the continued formation of new subepithelial deposits that will be seen in addition to more 'chronic' intramembranous lesions. In addition, deposits which are large and extend from the subepithelial space deep into the membrane are also suggestive of continuous deposition of immune complex material.

Pathologic findings in secondary MGN

Emerging data suggest that the presence or absence of anti-PLA2R antibodies may be informative to distinguish idiopathic versus secondary MGN; this is discussed further in Chapter 64. However some pathological features are suggestive of a secondary aetiology.

A more diverse spectrum of IgG subtypes is characteristic of MGN secondary to lupus (Haas, 1994), and IgG1 and IgG2 may be found more commonly in MGN diagnosed in the context of a malignancy (Ohtani et al., 2004). The finding of intense C1q staining, and the presence of other immunoglobulins such as IgM and IgA are suggestive of lupus-associated membranous nephropathy (Jennette et al., 1983).

The location of immune deposits differed in 28 patients with lupus from Jennette et al.'s series of 170 patients with a pathologic diagnosis of MGN (Jennette et al., 1983). The final diagnosis of systemic lupus erythematosus (SLE) was based on serologic and clinical criteria. Mesangial, subendothelial, and tubular basement membrane electron dense deposits in addition to the subepithelial deposits were highly suggestive of SLE-related MGN.

In primary MGN, immune complex formation occurs in situ, along the basal surface of the podocyte. Other mechanisms such as immune-complex formation in the circulation, or deposition of foreign antigen, may operate in secondary causes of MGN, so that deposits may be found throughout the glomerulus, as opposed to only in the subepithelial zone. Although not routinely sought, hepatitis antigens may be found in in the glomeruli of MGN due to hepatitis B; in one study, 100% of biopsies were positive for glomerular core antigen, and 88% for the e-antigen, with only a minority of biopsies demonstrating the presence of surface antigen deposition (Lin, 1990).

Mesangial hypercellularity is uncommon in idiopathic MGN, and may be suggestive of a systemic disease. Other suggestive findings include tubuloreticular inclusions (associated with viral or SLE-associated MGN), and intense C1q staining (suggest SLE).

The presence of crescents in MGN biopsies is a rare finding, and even in context of minimal subepithelial deposits and proliferation this lesion should elicit consideration of underlying severe lupus nephritis (Basford et al., 2011). Multiple reports have described cases of anti-GBM antibody glomerulonephritis, occurring in the presence of or diagnosed shortly after the development of biopsy-proven membranous nephropathy (see Chapter 72) (Klassen et al., 1974, reviewed in Basford et al., 2011). In rarer instances, a necrotizing crescentic glomerulonephritis may also be observed and this may be accompanied by the presence of antineutrophil cytoplasmic antibodies (Nasr et al., 2009).

References

Abe, S., Amagasaki, Y., Konishi, K., *et al.* (1986). Idiopathic membranous glomerulonephritis: aspects of geographical differences. *J Clin Pathol*, 39, 1193–8.

Abrass, C. K. (1985). Glomerulonephritis in the elderly. *Am J Nephrol*, 5, 409–18.

Adu, D. and Cameron, J. S. (1989). Aetiology of membranous nephropathy. *Nephrol Dial Transplant*, 4, 757–8.

Bariety, J., Druet, P., Lagrue, G., *et al.* (1970). 'Extra-membranous' glomerulopathies (E.M.G.). Morphological study with optic microscopy, electron microscopy and immunofluorescence. *Pathol Biol (Paris)*, 18, 5–32.

Basford, A. W., Lewis, J., Dwyer, J. P., *et al.* (2011). Membranous nephropathy with crescents. *J Am Soc Nephrol*, 22(10), 1804–8.

Braden, G. L., Mulhern, J. G., O'Shea, M. H., *et al.* (2000). Changing incidence of glomerular diseases in adults. *Am J Kidney Dis*, 35, 878–83.

Cahen, R., Francois, B., Trolliet, P., *et al.* (1989). Aetiology of membranous glomerulonephritis: a prospective study of 82 adult patients. *Nephrol Dial Transplant*, 4, 172–80.

D'Agati, V. (1994). The many masks of focal segmental glomerulosclerosis. *Kidney Int*, 46, 1223–41.

Davison, A. M., Cameron, J. S., Kerr, D. N., *et al.* (1984). The natural history of renal function in untreated idiopathic membranous glomerulonephritis in adults. *Clin Nephrol*, 22, 61–7.

Doi, T., Mayumi, M., Kanatsu, K., *et al.* (1984). Distribution of IgG subclasses in membranous nephropathy. *Clin Exp Immunol*, 58, 57–62.

Donadio, J. V., Jr. (1990). Treatment of glomerulonephritis in the elderly. *Am J Kidney Dis*, 16, 307–11.

Donadio, J. V., Jr., Torres, V. E., Velosa, J. A., *et al.* (1988). Idiopathic membranous nephropathy: the natural history of untreated patients. *Kidney Int*, 33, 708–15.

Ehrenreich, T. and Churg, J. (1968). Pathology of membranous nephropathy. In S.C. Scommers (ed.) *Pathology Annual*, pp.145–86. New York : Appleton-Century-Crofts.

Ehrenreich, T., Porush, J. G., Churg, J., *et al.* (1976). Treatment of idiopathic membranous nephropathy. *N Engl J Med*, 295, 741–6.

Falk, R., Jennette, J. C., and Nachman, P. H. (2000). Primary glomerular disease. In B. M. Brenner (ed.) *Brenner and Rector's The Kidney*, pp. 1263–92. Philadelphia, PA: W. B. Saunders.

Gartner, H. V., Watanabe, T., Ott, V., *et al.* (1977). Correlations between morphologic and clinical features in idiopathic perimembranous glomerulonephritis. A study on 403 renal biopsies of 367 patients. *Curr Top Pathol*, 65, 1–29.

Glassock, R. J. (1992). Secondary membranous glomerulonephritis. *Nephrol Dial Transplant*, 7 Suppl 1, 64–71.

Gluck, M. C., Gallo, G., Lowenstein, J., *et al.* (1973). Membranous glomerulonephritis. Evolution of clinical and pathologic features. *Ann Intern Med*, 78, 1–12.

Guella, A., Akhtar, M., Ronco, P. (1997). Idiopathic membranous nephropathy in identical twins. *Am J Kidney Dis*, 29, 115–8.

Haas, M. (1994). IgG subclass deposits in glomeruli of lupus and nonlupus membranous nephropathies. *Am J Kidney Dis*, 23, 358–64.

Hay, N. M., Bailey, R. R., Lynn, K. L., *et al.* (1992). Membranous nephropathy: a 19 year prospective study in 51 patients. *N Z Med J*, 105, 489–91.

Hladunewich, M. A., Troyanov, S., Calafati, J., *et al.* (2009). The natural history of the non-nephrotic membranous nephropathy patient. *Clin J Am Soc Nephrol*, 4, 1417–22.

Honkanen, E. (1986). Survival in idiopathic membranous glomerulonephritis. *Clin Nephrol*, 25, 122–8.

Honkanen, E., Tornroth, T., and Grönhagen-Riska, C. (1992). Natural history, clinical course and morphological evolution of membranous nephropathy. *Nephrol Dial Transplant*, 7 (Suppl 1), 35–41.

Jennette, J. C., Iskandar, S. S., and Dalldorf, F. G. (1983). Pathologic differentiation between lupus and nonlupus membranous glomerulopathy. *Kidney Int*, 24, 377–85.

Kern, W. F., Silva, F. G., Laszik, Z. G., *et al.* (1999). *Atlas of Renal Pathology*. Philadelphia, PA: W. B. Saunders.

Kida, H., Asamoto, T., Yokoyama, H., *et al.* (1986). Long-term prognosis of membranous nephropathy. *Clin Nephrol*, 25, 64–9.

Klassen, J., Elwood, C., Grossberg, A. L., et al. (1974). Evolution of membranous nephropathy into anti-glomerular-basement-membrane glomerulonephritis. *N Engl J Med*, 290(24), 1340–4.

Korbet, S. M., Genchi, R. M., Borok, R. Z., *et al.* (1996). The racial prevalence of glomerular lesions in nephrotic adults. *Am J Kidney Dis*, 27, 647–51.

Lin, C. Y. (1990). Hepatitis B virus-associated membraneous nephropathy: clinical features, immunological profiles and outcome. *Nephron*, 55, 37–44.

MacTier, R., Boulton Jones, J. M., Payton, C. D., *et al.* (1986). The natural history of membranous nephropathy in the West of Scotland. *QJM*, 60, 793–802.

Maisonneuve, P., Agodoa, L., Gellert, R., *et al.* (2000). Distribution of primary renal diseases leading to end-stage renal failure in the United States, Europe, and Australia/New Zealand: results from an international comparative study. *Am J Kidney Dis*, 35, 157–65.

Mallick, N. P., Short, C.D., Manos, J. (1983). Clinical membranous nephropathy. *Nephron*, 34, 209–19.

Murphy, B. F., Fairley, K.F., and Kincaid-Smith, P.S. (1988). Idiopathic membranous glomerulonephritis: long-term follow-up in 139 cases. *Clin Nephrol*, 30, 175–81.

Nasr, S. H., Said, S. M., Valeri, A. M., *et al.* (2009). Membranous glomerulonephritis with ANCA-associated necrotizing and crescentic glomerulonephritis. *Clin J Am Soc Nephrol*, 4, 299–308.

Noel, L. H., Zanetti, M., Droz, D., *et al.* (1979). Long-term prognosis of idiopathic membranous glomerulonephritis. Study of 116 untreated patients. *Am J Med*, 66, 82–90.

Ohtani, H., Wakui, H., Komatsuda, A., *et al.* (2004). Distribution of glomerular IgG subclass deposits in malignancy-associated membranous nephropathy. *Nephrol Dial Transplant*, 19, 574–9.

Passerini, P., Como, G., Viganò, E., *et al.* (1993). Idiopathic membranous nephropathy in the elderly. *Nephrol Dial Transplant*, 8, 1321–5.

Ramzy, M. H., Cameron, J. S., Turner, D. R., *et al.* (1981). The long-term outcome of idiopathic membranous nephropathy. *Clin Nephrol*, 16, 13–9.

Rosen, S. (1971). Membranous glomerulonephritis: current status. *Human Pathology*, 2, 209–31.

Row, P. G., Cameron, J. S., Turner, D. R., *et al.* (1975). Membranous nephropathy. Long-term follow-up and association with neoplasia. *QJM*, 44, 207–39.

Schena, F. P. (1997). Survey of the Italian Registry of Renal Biopsies. Frequency of the renal diseases for 7 consecutive years. The Italian Group of Renal Immunopathology. *Nephrol Dial Transplant*, 12, 418–26.

Schieppati, A., Mosconi, L., Perna, A., *et al.* (1993). Prognosis of untreated patients with idiopathic membranous nephropathy. *N Engl J Med*, 329, 85–9.

Tornroth, T., Honkanen, E., Pettersson, E. (1987). The evolution of membranous glomerulonephritis reconsidered: new insights from a study on relapsing disease. *Clin Nephrol*, 28, 107–17.

United States Renal Data System (2009). *USRDS 2009 Annual Data Report: Atlas of Chronic Kidney Disease and End-Stage Renal Disease in the United States*. Bethesda, MD: National Institutes of Health, National Institute of Diabetes and Digestive and Kidney Diseases.

Zucchelli, P., and Pasquali, S. (1998). Membranous nephropathy. In A. M. Davison, J. S. Cameron, J. -P. Grunfeld, *et al.* (ed.) *Oxford Textbook of Clinical Nephrology* (2nd ed.), pp. 571–90. Oxford: Oxford University Press.

CHAPTER 62

Membranous glomerulonephritis: treatment and outcome

Daniel C. Cattran and Heather N. Reich

Natural history

There have been few studies of untreated subjects with primary MGN, and multiple inconsistencies in the literature contribute to the difficulty establishing the natural history reliably. The outcomes of secondary MGN are considered in Chapter 63.

Marx and Marx's (Marx and Marx, 1997) review emphasizes the lack of uniformity with respect to diagnostic criteria, selection of endpoints, baseline severity, statistical techniques, and assessment of treatment effects. Noting these shortcomings, Table 62.1 shows studies examining the natural history of membranous glomerulonephritis (MGN) selected on the basis of relatively large sample size, and including a significant percentage of patients who received only supportive therapy. In addition, these studies provided data regarding the clinical status at diagnosis, and clear statistics regarding patient outcomes.

The disease course of MGN is considered to be indolent when left untreated, as is evident in most of the studies quoted in this table. The 10-year survival rates are favourable, averaging 82%. The study by Murphy et al. (1988) also provides information regarding survival if the patients had nephrotic syndrome at presentation; the 5-year and 8-year survival were 85% and 82% respectively, which did not differ from the overall patient survival. This relatively good prognosis is likely an underestimate of today's outcome, given the introduction within the last decade of more potent antihypertensive medications, angiotensin-converting enzyme inhibitors (ACEIs), and lowered ideal levels for target blood pressure. However, this assumption is speculative, as there are still no data indicating that these advances have yielded an improvement in the natural history of this disease.

Despite intentional selection of studies in Table 62.1 based upon some degree of uniformity in data and sample size, the variability in outcome evident in the table illustrate some of the limitations of the available literature. For example, the year of diagnosis and entry into study has important implications; the accuracy of the diagnosis may even be questioned, given the lack of electron microscopy prior to around 1975. In addition, some discrepancies in outcome may reflect differences in the quality of 'conservative therapy' that have evolved over time. The study by Noel et al. (1979), for instance, included patients diagnosed between 1958 and 1975, and is potentially subject to inconsistencies in diagnosis, which may explain differences in outcome compared with the other studies.

Another element to be considered when interpreting the data is the presence or absence of nephrotic syndrome at diagnosis. In the Donadio et al. (1988) study, 83% had nephrotic syndrome at diagnosis, compared to only 60% in the study by Kida et al. (1986). This may explain part of the differences in survival observed, given that the presence of the nephrotic syndrome impacts negatively on prognosis. One large study by Zucchelli et al. (1987) including 82 subjects with at least 10 years of follow-up reported far more ominous prognosis, with 20% reaching end-stage renal disease (ESRD), 17% chronic renal insufficiency, and 20% of patients suffering from non-renal mortality. These surprising results may be due to the methodological differences described, and the study was therefore not included in the table. For instance, all patients were diagnosed prior to 1976, and 100% had the nephrotic syndrome.

A final element to be considered is the control group used for comparison (if available). The study by Donadio et al. (1989) compares survival to actuarial life tables in healthy individuals, as opposed to comparing survival of patients who did or did not receive treatment (Zucchelli et al., 1987). Most recently, a large retrospective review of 328 subjects with the nephrotic syndrome, confirmed spontaneous remission in approximately one-third of subjects (Polanco et al., 2010). Although spontaneous remission rates were lower in patients with higher grades of proteinuria, approximately 20–25% of subjects with proteinuria of 8–12 g/day or more achieved spontaneous remission. The time to complete spontaneous remission, however, ranged from 25 to 41 months. Patients who did not achieve a spontaneous remission had a high rate of non-renal morbidity and mortality in addition to poor renal outcomes.

Multiple reviews combining studies of pooled treated and untreated patients have examined overall survival rates. A review of 11 reports of the natural history of idiopathic MGN revealed an overall renal survival of 65–90% by 10 years (Cattran et al., 1992). Similarly, a large pooled analysis of 32 studies examined the course of 1189 patients, estimated renal survival at 86% at 5 years, 65% at 10 years, and 60% at 15 years (Hogan et al., 1995).

Non-renal-related morbidity and mortality in patients with idiopathic MGN is difficult to determine from the literature. There are, however, quite alarming mortality rates in several of the earlier studies of the natural history of the disease. Reviews of these earlier papers have indicated that a high proportion of deaths in

Table 62.1 Selected studies of the natural history of idiopathic MGN. Survival rates reflect overall patient survival, that is, patient alive with adequate independent renal function, with number of patients at risk in parentheses when available

Study (year, country)	Number of subjects	Mean follow-up in months	Outcomes
Noel et al. (1979, France)	116	54	CR 23.5%, PR 14.5%, NR 43%, deterioration 19% (9.5% ESRD) 88% 5 YS (N = 60), 76% 10 YS (N = 12)
Davison et al. (1984, Great Britain)	64	N/A—range 2–15 years	47% no change, 42% doubling creatinine, or level > 400 µmol/L, 8% slow deterioration, 3% excluded
Kida et al. (1986, Japan)	104 (59 received no treatment)	138	CR 40%, PR 30%, 10–15% persistent disease 94.% 5 YS (N = 72), 90% 10 YS (N = 56), 80% 15 YS (N = 34)[a]
MacTier et al. (1986, Scotland)	37	64	30% CR, 16% persistent proteinuria, 19% proteinuria and CRF, 22% ESRD, 13% non-renal mortality
Donadio et al. (1988, USA)	140 (89 received no treatment)	127.4	64% stable, 20% ESRD, 16% CRF 85% 5 YS, 71% 10 YS[a]
Murphy et al. (1988, Australia)	139 (79 received no treatment)	52	50% alive, normal function, 13% CRF, 6% ESRD, 11% non-renal death, 20% lost to follow-up 88% 5 YS (N = 68), 81% 10 YS (N = 38)[a]
Schieppati et al. (1993, Italy)	100	52	68% normal function, 18% CRF, 14% ESRD (including 6% mortality) 5YS (renal) 88% (N = 37), 8 YS 73%

[a] Survival rates calculated using both treated and untreated patients. Statistical analysis indicated no difference in survival between treated and non-treated groups.

CR = complete remission; CRF = chronic renal failure; ESRD = end-stage renal disease; NR = no response; PR = partial remission; YS = year survival.

this population—up to 60%—are non-renal related (Donadio et al., 1988; Laluck and Cattran, 1999). One early study of 32 subjects, for instance, indicated 41% mortality in the follow-up period (Franklin et al., 1973). A high mortality rate was confirmed in larger more recent series as well, where 6–20% of patients died by the end of the follow-up period (Kida et al., 1986; Zucchelli et al., 1987; Murphy et al., 1988; Wehrmann et al., 1989). These deaths tend to occur at a young age (mean 51 years), and are most often due to cardiovascular disease or malignancy (Honkanen, 1986). The reasons for this high rate of premature non-renal deaths may be comorbid conditions, the effects of the disease itself (i.e. complications of the nephrotic syndrome) or of the treatment. The precise cause of death in many of the aforementioned studies is unclear. An as yet unidentified element of glomerulonephritis may increase the risk of mortality. A compelling review of 2380 subjects who underwent biopsy for glomerulonephritis revealed that by 10 years following diagnosis, 32% of the subjects had died (Heaf et al., 1999). More recently, it was demonstrated that the achievement of a remission is important with respect to minimizing morbidity and mortality; in patients who do not achieve a spontaneous remission of nephrotic syndrome have a substantially higher rate of mortality compared to those who do achieve this endpoint (10.7% vs 1.9%, P = 0.002) (Polanco et al., 2010). This is an area in the natural history of MGN about which little information exists, and where further investigation is certainly warranted.

Prognostic factors

In univariate analysis, gender, advanced age, hypertension, renal insufficiency at presentation, and urinary protein excretion all appear to be predictive. These can be combined into a predictive equation.

Sex

Males have a worse prognosis. This was first observed in a study of prednisone for treatment of MGN (Collaborative Study of the Adult Idiopathic Nephrotic Syndrome, 1979), and later confirmed in a case series (Hopper et al., 1981). Two studies in 1984 confirmed by univariate analysis that male gender is associated with an unfavourable prognosis (Davison et al., 1984; Tu et al., 1984). The gender ratio at presentation versus at end stage of disease also illustrates this effect. In several populations, it has been observed that the gender ratio is almost equal at presentation of MGN (Abe et al., 1986; Harrison 1986; Simon et al., 1994), whereas at end-stage renal disease it is consistently 2–3:1, male:female (United States Renal Data System, 1999; Maisonneuve et al., 2000). The role of gender in the progression of MGN has been evaluated in a large meta-analysis, which included 21 studies containing 1894 patients followed for an average of 84 months (Neugarten et al., 2000). The pooled analysis confirmed that male gender is highly significantly associated with a more rapid rate of progression in MGN (standardized effect size estimate 0.30, 95% confidence interval (CI) 0.16–0.36, P < 0.00001). Our own data suggest that females demonstrate a lower rate of renal function decline and lower risk of kidney failure compared to males (0.63, 95% CI 0.40–1.00, P = 0.05), and that this may be related, in part, to lower degrees of proteinuria and blood pressure both at presentation and follow-up (Cattran et al., 2008).

Age

Older age is generally associated with a higher risk of development of chronic renal insufficiency. We performed a retrospective study of 74 patients > 60 years of age at time of diagnosis of idiopathic MGN and compared them to the younger subjects in the same registry, who presented over a 19-year period (Zent et al., 1997). Older subjects had significantly higher median creatinine levels (1.3 mg/dL vs 1.0 mg/dL, P < 0.001), and lower calculated creatinine

clearance (55 mL/min vs 95 mL/min, P < 0.0001) than those in the younger-onset group. The incidence of chronic renal insufficiency defined as a clearance of < 50 mL/min was significantly higher in the elderly after a mean observation time of nearly 4 years (59% vs 25%, P < 0.0001), although the incidence of ESRD was not different. The rate of deterioration in renal function, however, was not different between younger and older patients. The observed difference in rates of chronic renal failure may therefore be due in large part to loss of reserve of functional nephron mass with age, such that there is less remaining to compensate for the additive detrimental effects of a glomerular disease. A more recent large retrospective study including 44 patients presenting over 60 years of age confirmed that older patients more often have worse renal impairment than a younger cohort, and also found a worse prognosis for renal survival (O'Callaghan et al., 2002). Smaller individual studies have found a somewhat more variable association with age and renal prognosis (Row et al., 1975; Davison et al., 1984; Tu et al., 1984; Zucchelli et al., 1987; Schieppati et al., 1993; Honkanen et al., 1994).

Proteinuria

Nephrotic syndrome and nephrotic-range proteinuria were shown early on to be associated with a worse prognosis, when analysed in univariate analysis (Row et al., 1975; Noel et al., 1979; Mallick et al., 1983; Davison et al., 1984; Tu et al., 1984; Gerstoft et al., 1986; Donadio et al., 1988; Murphy et al., 1988; Wehrmann et al., 1989). Multivariable analysis, however, does not consistently reveal that the degree of proteinuria at the time of diagnosis predicts outcome (Tu et al., 1984; Schieppati et al., 1993; Marx and Marx, 1999), and this may suggest that if reduction of proteinuria over time is achieved prognosis will be favourable (Ponticelli et al., 1992a; Laluck and Cattran, 1999). In one of the largest series to date, it is not surprising that complete remission of proteinuria from the nephrotic range is associated with an excellent prognosis (Troyanov et al., 2004). However patients achieving a partial remission of proteinuria, defined as reduction of proteinuria of 50% from peak value to sub-nephrotic levels (< 3.5 g/day) also benefit from a marked reduction in risk of kidney failure (hazard ratio 0.08, 95% CI 0.03–0.19, P < 0.001) compared to those with no remission (Troyanov et al., 2004). Given the variable course of the disease, a dynamic view of proteinuria over time is likely more indicative of prognosis (see below). Hypertension and renal insufficiency at the time of biopsy have shown similar results to initial proteinuria with respect to prediction of adverse outcome (Troyanov et al., 2004, as well as the rate of renal function decline 2006). There is general agreement that impaired function at the time of biopsy and hypertension do not portend a favourable prognosis; however, studies indicate conflicting results when incorporating these factors in multivariable models.

The prognosis of patients who have non-nephrotic range proteinuria has recently been described in a review of 395 patients with idiopathic biopsy-proven MGN. One hundred and eight (27% of the total) patients presented with sub-nephrotic proteinuria and almost 40% (42 of 108) remained sub-nephrotic throughout the average followed up of 68 months. Their long-term rate of renal function declined as measured by slope of creatinine clearance (slope) was −0.93 mL/min/year. In contrast, those who subsequently developed nephrotic range proteinuria had a progression rate almost four times faster (−3.52 mL/min/year). The majority who developed nephrotic syndrome did so within the first year of follow-up. The only distinguishing baseline feature between the two groups was a higher level of urine protein in the group that subsequently developed nephrotic syndrome (1.98 (0.3–3.4) versus 2.43 (0.5–3.4) g/day). Baseline creatinine clearance as well as baseline and follow-up mean arterial pressure were not significantly different nor were the mean number of blood pressure medications between those that remained some nephrotic versus those that converted to nephrotic range proteinuria (Hladunewich et al., 2009).

Pathological findings

The relationship between pathologic findings and both cross-sectional and long-term clinical parameters has been an area of conflicting results. This likely relates in part to relatively small study sample sizes, a mixture of idiopathic and secondary disease in studies, as well as a lack of consensus regarding standardized pathologic staging for the disease. With respect to the stage of intramembranous deposits, more 'advanced' stages of pathology have been found to correlate with an adverse prognosis in some studies according to univariate analysis. For instance, several analyses suggest an association between stage III and IV deposits and an adverse long term prognosis, compared with stages I and II deposits (Gluck et al., 1973; Franklin et al., 1973; Noel et al., 1979; Zucchelli et al., 1986, 1987; Tornroth et al., 1987; Hay et al., 1992), but not in all studies (Abe et al., 1986). Furthermore, biopsies with heterogenous deposits at various phases of evolution have been associated with a worse outcome (Yoshimoto et al., 2004). However, when a large sample of 389 cases was reviewed, neither stage nor synchronicity of EM deposits was found to be associated with clinical variables evaluated at the time of biopsy or with progressive renal insufficiency (Troyanov et al., 2006). The extent of C3 deposition (measured on a semi-quantitative scale) did correlate with the degree of proteinuria at the time of biopsy, as well as the rate of renal function decline. However, there was no difference in renal survival or likelihood of remission according to the extent of deposition (Troyanov et al., 2006). Mesangial immune complexes have been associated with a favourable prognosis in idiopathic MGN, although this finding may more commonly suggest a secondary aetiology (Davenport et al., 1994). Focal and segmental glomerulosclerosis lesions are more consistently associated with a trend towards worse renal survival (Wakai and Magil, 1992; Toth and Takebayashi, 1994; Dumoulin et al., 2003, Troyanov et al., 2006).

The findings of interstitial fibrosis and tubular atrophy are more consistently associated with a poor prognosis (Noel et al., 1979; Ramzy et al., 1981) even in multivariable analysis (Wehrmann et al., 1989; Ponticelli et al., 1989; Marx and Marx, 1999). While lesions of tubulointerstitial fibrosis correlate with a worse renal survival, these lesions do not affect the rate of renal function decline; this may reflect the fact that patients with interstitial fibrosis at the time of biopsy tend to have more impaired clearance at the time of kidney biopsy (Zent et al., 1997; Troyanov et al., 2006).

Other markers

Several other factors have been correlated with outcome, but are not yet part of the usual diagnostic assessment. Urinary excretion of immunoglobulin (Ig)-G and alpha-1-microglobulin has been correlated with outcome more closely than total proteinuria (Bazzi, 2001). Further, urinary excretion of beta-2-microglobulin, potentially reflecting proximal tubular cell injury, is independently predictive of progression and adds to data obtained from routine

clinical evaluation (Branten et al., 2005; Hofstra et al., 2008). In addition, there has been significant investigation into the role of genetic factors that are associated with the course of MGN.

Predicting prognosis

Given the potential toxicities of the medications involved in treating MGN, as well as the significant potential for a patient to undergo a spontaneous remission, it is desirable to predict the clinical course of an individual patient before making therapeutic decisions. Ideally, the data required to offer prognostic information should be obtainable as soon as possible period after diagnosis. The difficulty associating the above factors with long-term outcome is their poor specificity, qualitative nature, and the fact that they reflect only cross sectional data at diagnosis, which has varied in each study. Another approach is to use an observation period to gather further information on progression (and to allow time for spontaneous remission). It is highly likely that in the near future autoantibody titres (e.g. to PLA2R, see Chapter 64) will be able to usefully supplement this information.

Box 62.1 Prediction model for risk of progression in idiopathic MGN (Pei et al., 1992; Cattran, 2001)

Logistic regression model:

$X = 1.26 + (0.3 \times PP) - (0.3 \times \text{slopeCcr}) - (0.05 \times Ccr_i)$

PP: the level of persistent proteinuria in g/24 hours. This is measured as the lowest level observed over a period of 6 months.

SlopeCcr: the slope of the creatinine clearance over the period used to observe persistent proteinuria (e.g. 6 months). Measured in mL/min/month.

Ccr_i: the initial creatinine clearance documented at the beginning of the observation period, in mL/min.

Then, use the calculated X to obtain a probability of progression (R) by substituting as follows:

$R = e^x/(1+ e^X)$

Sample calculation of risk of progression

Patient A:

Would like to know patient A's risk of progression. One requires 6 months of follow-up for the calculation.

PP = 5 g/24 hours

Month	Proteinuria (g/24 hours)	Creatinine clearance (mL/min)
0	10	95
3	5	75
6	7	75

SlopeCcr = Ccr final – Ccr initial = 75 mL/min – 95 mL/min = –3.33

Time 6 months

$X = 1.26 + (0.3 \times PP) - (0.3 \times \text{slope Ccr}) - (0.05 \times \text{Ccri})$

$= 1.26 + (0.3 \times 5) - (0.3 \times -3.33) - (0.05 \times 95)$

$= -0.991$

$e^X = 0.37$

$R = 0.37 / (1 + 0.37) = 0.27$ or a 27% chance of progressing.

Box 62.1 presents one prediction model. A less mathematical variant is presented in Box 62.2 and Fig. 62.1.

The prediction model in Box 62.1 incorporates the clinical parameters of proteinuria and creatinine clearance estimates over fixed periods of time. The approach demonstrates an impressive overall accuracy of predicting progression to chronic renal failure. When the sensitivity, specificity, and positive and negative predictive values are considered, the accuracy of this formula, meaning its ability to predict whether or not a patient will progress, can be determined. This accuracy changes according to proteinuria values over a 6-month observation period; when values were persistently > 4 g/day, for instance, the algorithm can accurately predict whether a patient will or will not progress with a rate of 71%. At ≥ 6 g/day accuracy is 79% and at ≥ 8 g/day accuracy is 84% (Cattran et al., 1997). If the patient's renal function was impaired or deteriorated over the 6 months, sensitivity and specificity were even higher. In addition, the regression formula has been validated in two populations—101 patients from Italy, and 78 patients from Finland, and found to have consistent accuracy in identifying the risk of progression compared to the original Canadian (Cattran et al., 1997).

There are several advantages to this algorithm. Firstly, all of the factors are easily obtainable standard laboratory measurements. In addition, the dynamic nature of the algorithm allows it to be recalculated and reapplied over the course of a patient's disease. It is important to note that the individual risk factors of age, gender, biopsy findings, and presence of hypertension were not found to be independent of the factors in the model, and although relevant to each individual case, they do not add to the predictive value of the algorithm.

Treatment of primary membranous glomerulonephritis

The general management of nephrotic syndrome is described in Chapter 52. Here we examine specific issues and specific therapies for MGN. Specific measures for secondary MGN are mentioned in Chapter 63.

Non-immunologic therapy

Dietary manipulation alone has not been shown to induce a complete remission of the nephrotic syndrome in idiopathic MGN. However, some data suggests that protein restriction results in a reduction in proteinuria and possibly progression of disease, demonstrated specifically in patients with MGN, and as well in patients with other causes of the nephrotic syndrome (Cupisti et al., 1990; D'Amico 1992; Pedrini et al., 1996; Kopple et al., 1997). Dietary management is discussed in Chapters 47 and 101.

The additional benefit of ACE inhibition has been demonstrated specifically in idiopathic MGN (Thomas et al., 1991; Rostoker et al., 1995).

It is assumed that there is benefit from HMG-CoA reductase inhibitors in all patients with long-term heavy proteinuria, including MGN.

Thrombotic complications pose a significant risk in patients with nephrotic syndrome, and it may be greater when the syndrome is due to MGN (See Chapter 52) (Trew et al., 1978; Wagoner et al., 1983; Llach, 1985; Bellomo and Atkins, 1993; Bellomo et al., 1993; Rabelink et al., 1994). Even when adjusted for the degree of proteinuria, the disease-specific risk of clinically evident thrombotic events is highest in MGN compared to other forms of idiopathic

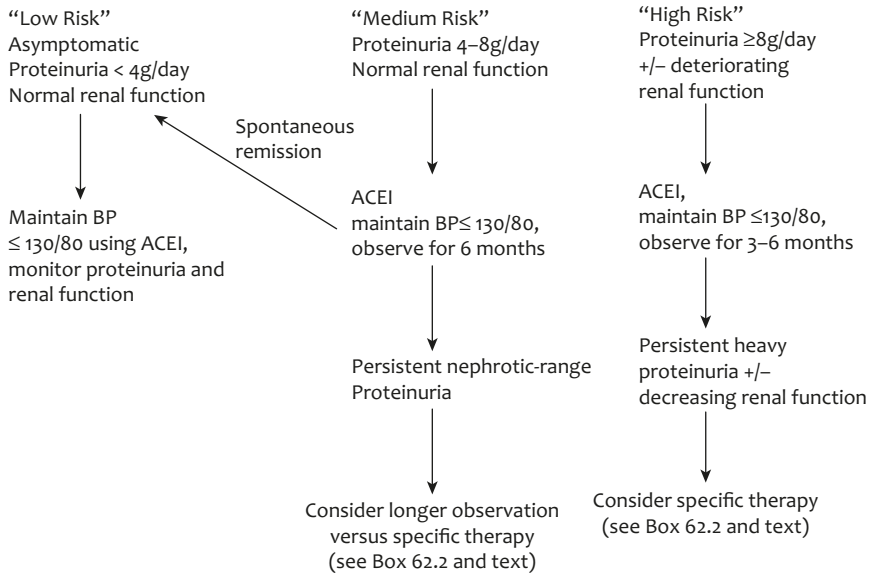

Fig. 62.1 Treatment algorithm. Patients may change from one category to another during the course of follow-up. BP = blood pressure; ACEI = angiotensin-converting-enzyme inhibiting drug.

glomerulonephritis (Barbour et al., 2011). Some have observed that the risk is heavily weighted towards the first few months around the time of diagnosis (Kumar et al., 2012). Given the risks associated with anticoagulation in this population, prophylactic anticoagulation even in the high-risk group has not been adopted by all clinicians (see Chapter 52).

Specific immunotherapy

The majority of trials of immunosuppressive agents in idiopathic MGN do not meet rigorous standards; they are often non-controlled, have small patient numbers, and short observation periods (Geddes and Cattran, 2000). The regimens examined in controlled trials include corticosteroids alone or in combination with either chlorambucil, cyclophosphamide, or ciclosporin, and the latter three drugs used as single agents. These medications all have significant adverse effects, and therefore the decision to subject a patient to these risks, must be weighed against the potential benefits. They are reviewed with our recommendations. Evidence for other potential therapies is also discussed.

Recommendations for specific treatment

Given the variable clinical outcomes of patients, it is useful to first establish categories of progression, as determined by factors in the previously discussed algorithm. We recommend an initial observation period of 6 months, during which time non-immunologic interventions should be maximized. In order to determine the categories of progression, specific therapeutic trials were retrospectively divided according to their subjects' initial laboratory characteristics. This allows the separation of the effectiveness of any one therapy by the category of risk of progression of the patients in that trial. After making a therapeutic decision regarding a patient, one can then compare a patient with the most similar risk profile, and treatment strategy can be determined for each individual patient, and a better estimate of risk:benefit ratio can be determined. While in general a 6-month wait period is advocated to fully evaluate risk, the presence of life-threatening complications of the nephrotic syndrome (i.e. refractory oedema) and declining renal function not explained by other complications (e.g. renal vein thrombosis or acute tubular necrosis) may prompt consideration for earlier initiation of immunotherapy. A summary of the discussed treatment algorithm is presented in Box 62.2 and Fig. 62.1.

Possible regimens

Corticosteroid monotherapy: ineffective

Corticosteroids alone have been shown to be ineffective in inducing remission. Some studies have shown some improvement in proteinuria, but corticosteroids have not been found to prevent progression when used as monotherapy in controlled studies (Collaborative Study of the Adult Idiopathic Nephrotic Syndrome, 1979; Kobayashi et al., 1982; Cattran et al., 1989). Entry criteria in these trials would have placed the participants into the 'medium-risk' category. Although the total follow-up periods were < 4years, and the protocol for administration differed, it is generally held that corticosteroids alone do not have a role in treatment of idiopathic MGN (Lewis, 1993; Muirhead, 1999). A possible exception is patients of Asian ancestry (specifically Japanese) who in several retrospective studies have shown better response to steroid monotherapy. This race-specific difference in response to steroid monotherapy has not been validated in randomized controlled prospective studies and there are insufficient data to warrant use as a single agent in Asian populations (Shiiki et al., 2004).

Corticosteroids (high risk): ineffective

This subgroup of patients is relatively small, and very few trials have exclusively studied subjects in this risk category. Corticosteroid treatment alone was examined in the subgroup of 55 patients with renal insufficiency from the relatively large randomized trial of Canadian subjects (Cattran et al., 1989). All in this subgroup had an initial creatinine clearance of < 72 mL/min. Those treated with prednisone (dose of 45 mg/m^2 on alternate days for 6 months) did

Box 62.2 Stratification of risk of progression in MGN

Low risk of progression

Asymptomatic proteinuria, peak < 4g/day, with normal serum creatinine at presentation and stable creatinine clearance over 6 months of observation.

Blood pressure management and antiproteinuric strategies with agents such as ACEIs are very important in this group. However, given the generally favourable outcome, immunosuppressive therapy is not recommended.

The prognosis of these patients is generally good. Our study of three cohorts of patients from Canada (N = 184), Finland (N = 78), and Italy (N = 101) showed that 17–28% of patients present in this category. Of these, only 6%, 0%, and 24% developed sustained renal insufficiency (clearance < 60 mL/min/1.73 m^2) after a mean follow-up of 70, 104, and 59 months respectively. The average overall risk was only 5%. However, the numbers of patients in this group included in trials are relatively small and observation time limited. A small percentage will progress, so monitoring of renal function, proteinuria, and blood pressure is necessary to assess if their category has changed.

Medium risk of progression

Proteinuria consistently 4–8 g/day over 6 months, but normal creatinine and creatinine clearance at presentation and during observation.

In addition to general therapy, only the combination of alkylating agents with high-dose corticosteroids for a period of 6 months is of unequivocal benefit. However, toxicity is significant, and the natural history of this group is varied. Longer monitoring and further consideration may be justified.

Steroids alone, and probably MMF, have no lasting effect on the disease, though steroids may have a transient effect on proteinuria. Calcineurin inhibitors have not been proven to alter long-term outcome, but do reduce proteinuria, which is useful in managing intractable nephrotic syndrome and may translate into long-term improvement in renal survival.

High risk of progression

Persistent proteinuria ≥ 8g/day over the 6 months of observation, and/or deteriorating renal function.

In addition to general therapy, the best evidence is for alkylating agents delivered with high-dose corticosteroids for a period of 6 months. IV cyclophosphamide is probably safer and less toxic than chlorambucil, but toxicity of these regimens is significant.

The rate of reaching renal endpoints remains high in treated patients. The role of steroids in these regimens is unproven, but it is known that they are ineffective when administered alone. Ciclosporin was no better than supportive management in a randomized study in this group with high-grade proteinuria in association with deteriorating renal function (Howman et al., 2013) although this was in contrast to earlier smaller studies (Cattran at al., 1995).

We regard the role of anti-B-cell antibodies such as rituximab as still unproven but deserving of further investigation.

not demonstrate an improvement in rate of deterioration of renal function. In the one randomized study of corticosteroids alone in high risk subjects (mean proteinuria was 10.6 g/day) (Cameron et al., 1990), prednisolone at 100–150 mg on alternate days given for 8 weeks prior to taper did not confer benefit with respect to rate of deterioration of function or proteinuria. Somewhat in contrast, a small study of 15 patients and declining function suggested that 5 days of 1 g intravenous (IV) methylprednisolone followed by a tapering course of prednisolone was associated with an initial stabilization in renal function in nine of the subjects (Short et al., 1987). At last follow-up, however, two patients had died, and five had reached ESRD, suggesting that any positive effects of the treatment were transient.

Alkylating agents with corticosteroids: beneficial but toxic

There is evidence of benefit, however, when corticosteroids are in combination with a cytotoxic agent used in this risk group. A significant increase in both remission of proteinuria and renal survival was demonstrated, with follow-up out to 10 years in a trial comparing a regimen of prednisone and chlorambucil to symptomatic treatment (Ponticelli et al., 1984, 1992b, 1995). The regimen consisted of 1 g of IV methylprednisolone daily for the first 3 days of months 1, 3, and 5, followed by 27 days of oral methylprednisolone 0.5 mg/kg/day for the remainder of the month. In alternating months (months 2, 4, and 6), chlorambucil 0.2 mg/kg/day was used instead of the corticosteroid. At 10 years, the probability of survival without dialysis was 92% in the treatment group, and 60% in the group receiving symptomatic therapy (P = 0.004). The probability of achieving a complete or partial remission was 83% in treated group, and only 38% in controls (P < 0.001), and at last follow-up, 62% of treated patients were in either a complete or partial remission, compared with 33% of controls (P < 0.05).

Chlorambucil is not easily available in some countries, has more gastrointestinal side effects, and increased toxicity in renal impairment (Howman et al., 2013). A further trial from the same group (Ponticelli et al., 1998) compared the original regimen employing chlorambucil, with a similar regimen substituting cyclophosphamide 2.5 mg/kg/day. Benefits similar to those found in the first trial were demonstrated. There was, however, a substantial rate of relapse of nephrotic syndrome. At 2 years, up to 30% of patients in both groups had relapsed to nephrotic range proteinuria. Overall, the regimens were reasonably well tolerated, with approximately 10% of patients discontinuing treatment due to adverse effects. A more recent open-label study using the same regimen in patients of Asian descent yielded similar results to the initial trials of Ponticelli et al. In addition, quality of life as measured by a visual analogue scale was significantly better in the immunotherapy treatment group versus the conservative treatment group throughout the follow-up period. The complication rate was not different in the two groups. Significant cross-over to the treatment protocol occurred following the initial period which may have contributed to the minimal long-term difference observed in renal function between the two groups (Jha et al., 2007).

One small, open-label randomized study (N = 26) examined the efficacy of cyclophosphamide for 12 months plus moderate-dose steroids in MGN patients considered to be at high risk of progression. The study compared an early-start group (urinary abnormalities at

baseline) versus a group started only after serum creatinine had risen by > 25–50%. All patients were felt to be at high risk of progression based on urinary IgG and urine beta-2-microglobulin levels that were previously correlated with a high risk of progressive renal insufficiency. They found a more rapid remission in proteinuria in early-start patients, but no differences between the two groups in overall remission rates, serum creatinine levels, average proteinuria, relapse rates, or adverse events after 6 years (Hofstra et al., 2010).

Five studies have examined high-risk patients treated with alkylating agents and corticosteroids. A substantial improvement in renal function in more than half of patients, and a decline in proteinuria was noted in one study of eight patients (Mathieson et al., 1988). Similarly, half of the 21 subjects in a subsequent study were noted to have a stabilization or improvement in renal function (Warwick et al., 1994), but subjects with an initial creatinine between 180 and 480 μmol/L continued to show deterioration in function. When the outcome of these subjects was compared to historical controls, however, there did appear to be a trend to improved renal survival (Stirling et al., 1998). The success noted by these small trials must, however, be balanced by the high incidence of serious complications; in the aforementioned study by Stirling et al., for instance, half of patients had significant side effects related to therapy (infectious and myelosuppressive), necessitating discontinuation of medications in 6 of 19 patients. This particular study population, particularly those with significantly impaired renal function, may be the group most vulnerable to drug toxicity. Most recently, one study of 39 subjects compared conservative therapy in patients treated between 1975 and 1989, to a group treated between 1990 and 2000 with a regimen of oral chlorambucil (0.15 mg/kg/day for 14 weeks) with oral prednisone for 6 months (Torres et al., 2002). Those receiving the chlorambucil had a 90% probability of renal survival at 4 years of follow-up, compared with only 55% probability in subjects receiving only conservative therapy ($P < 0.001$). This benefit persisted at 7 years.

A UK multicentre study randomized 108 patients with declining renal function (≥ 20% decline within 2 years of trial entry and average creatinine clearance 50 mL/min) to alternating months of chlorambucil/ high-dose steroids for 6 months, ciclosporin for 12 months, or supportive management. Risk of a 20% decline in GFR over 3 years was significantly reduced in the alkylating agent group but still high (58 vs 84%). The outcome for the ciclosporin group was not similar to that of supportive care alone. Adverse events were common in all groups but significantly more common in the chlorambucil/ steroids group. Less than 50% of subjects were evaluable at 12 months (Howman et al., 2013). It is possible that IV cyclophosphamide rather than oral chlorambucil would have caused fewer side effects. It is also worth questioning the need for corticosteroids in these regimens.

Monthly pulse cyclophosphamide for 6 months plus prednisone compared to prednisone alone produced no additional benefit in a randomized trial including 36 who would be considered 'high risk' by virtue of renal insufficiency (Falk et al., 1992). Two non-randomized case–control studies in similar populations involving long-term oral cyclophosphamide with or without prednisone did indicate a benefit to the therapy (Bruns et al., 1991; Jindal et al., 1992). However, aside from the limitations of the non-randomized design, long-term cyclophosphamide exposure carries the significant risks of infertility, infection, and malignancy, limiting the applicability of these trials. Direct comparison of cyclophosphamide and chlorambucil was undertaken in two trials that included patients with progressive deterioration in renal function. The first compared a traditional Italian regimen with chlorambucil to a modified routine with IV cyclophosphamide pulses at months 2, 4, 6, and three daily 1 g IV methylprednisolone pulses at months 1, 3, and 5 (Reichert et al., 1994). The authors concluded that cyclophosphamide administered in this manner was not beneficial after 6–36 months of follow-up. The same group then examined 27 patients receiving one of two treatment strategies. The first (N = 15) received a regimen of 3 days of IV methylprednisolone followed by 0.5 mg/kg/day of oral prednisone given in months 1, 3, and 5 alternating with oral chlorambucil 0.15 mg/kg/day in months 2, 4, and 6. The comparison group (N = 17) received cyclophosphamide 1.5–2.0 mg/kg/day for 1 year, and steroids in comparable doses to the first group (Branten et al., 1998). Those treated with cyclophosphamide showed a greater benefit with a greater fall in serum creatinine (61 vs 121 μmol/L fall, $P < 0.01$), lower incidence of ESRD (4 vs 1 patients, $P < 0.05$), and more frequent remission of proteinuria, and fewer short-term side effects, in a total of 27 subjects.

Calcineurin inhibitors: weak evidence of benefit

Given the high doses of steroid required in these studies, as well as the potential for both gonadal toxicity and malignancy with use of cyclophosphamide, efforts have focused on identification of alternate approaches to treatment of MGN that may spare some of the treatment-associated toxicity. In retrospect, many of these studies have reached misleading conclusions because of the propensity of calcineurin inhibitors to reduce proteinuria in a dose-related manner without necessarily impacting on the progression of the underlying disease (see Chapters 45, 58).

One study of subjects in the medium risk category examined the effectiveness of ciclosporin in combination with low-dose prednisone (Cattran et al., 2001). Fifty-one subjects were enrolled in this multicentre, placebo-controlled, single-blind randomized trial. All had failed to achieve remission after at least 8 weeks of therapy with prednisone 1 mg/kg/day. Study subjects receiving active treatment (N = 28) were given ciclosporin in a liquid formulation starting at 3.5 mg/kg/day divided in two doses, and adjusted to achieve whole blood 12-hour trough levels of 125–225 micrograms/L (monoclonal assay). Control subjects (N = 23) received a placebo liquid, and all subjects were given prednisone at a dose of 0.15 mg/kg/day to a maximum of 15 mg/day. Subjects received 26 weeks of therapy, after which the ciclosporin/placebo was stopped, and steroid dose was tapered. By 26 weeks, 75% of treated subjects had reached a partial or complete remission, compared with only 22% of controls ($P = 0.001$). The relapse rate was significant in both the treatment and control groups. The fraction of patients remaining in remission, however, remained significantly different at the 1year mark —39% of ciclosporin-treated subjects remained in remission, versus 13% in the placebo group ($P = 0.007$). This improvement in remission rate was not at the expense of a change in renal function, since there was no significant change noted in creatinine clearance in either group. A significant number of subjects did not respond to therapy. Further investigations are necessary to determine if a longer course of treatment, higher dose, or re-treatment of relapses may increase the rate and perhaps the duration of response. The high relapse rate may suggest that ciclosporin controls rather than

cures this disease especially in those patients that only achieve partial remission status.

Only one RCT in MGN patients has compared tacrolimus (N = 39, given for 6–9 months) to oral cyclophosphamide (N = 34 given for 4 months) (Chen et al., 2010). Both groups in this study of patients of Asian descent also received prednisone tapered off over 8 months. The results indicated no difference between treatment groups in terms of partial or complete remission of proteinuria (79% vs 69%), or adverse events at 12 months of follow-up. Relapses occurred in approximately 15% of each group. These data would suggest that the use of tacrolimus is an effective alternative to an oral alkylating-agent regimen with similar short-term outcomes. However, the long-term efficacy of a tacrolimus-based regimen for MGN remains to be determined.

One RCT explored the use of tacrolimus monotherapy in MGN, in patients with normal kidney function and mean proteinuria of approximately 8 g per 24 hours (moderate to high risk, N = 25). The patients received tacrolimus 0.05 mg/kg/day for 12 months followed by a 6-month taper, and were compared to conservatively treated controls (N = 23) (Praga et al., 2007). After 18 months, the probability of remission was 94% in the tacrolimus group but only 35%, in the control group. Six patients in the control group and only one in the tacrolimus group reached the secondary endpoint of a 50% increase in serum creatinine. Almost half of the patients relapsed after tacrolimus was withdrawn, similar to patients treated with ciclosporin. Tacrolimus monotherapy is therefore appealing for patients who are intolerant of corticosteroids; however, as in the case of ciclosporin, concerns regarding relapse rate after a 12-month course also merit further investigation into appropriate duration of therapy.

In our RCT of high-risk patients with renal insufficiency (mean creatinine was 195 μmol/L), 1 year of ciclosporin was administered at a dose of 3.8 mg/kg, and compared with placebo (Cattran et al., 1995). Ciclosporin-treated patients demonstrated significantly reduced proteinuria, and a slowed rate of progression of renal failure ($P = 0.02$, and $P < 0.02$ respectively). These positive results were sustained in more than half of the patients as late as 2 years after treatment. The number of patients in the study, however, was small, and there was a trend towards transient increases in creatinine noted in the treatment group. A similar benefit was noted in an uncontrolled study of 15 individuals with steroid-resistant progressive disease, however the relapse rate was high (Rostoker et al., 1993). A retrospective review from a large collaborative group included 41 patients considered high risk due to the severity of proteinuria (> 10 g/day), and resistance to other immunosuppressive drugs (Fritsche et al., 1999). Thirty-four per cent achieved a complete remission after a mean treatment time of 225 days, at a mean dose of 3.3 mg/kg, thus confirming the drug's efficacy, but also the need for prolonged therapy before assuming resistance to the medication.

Mycophenolate mofetil: no evidence of benefit

One uncontrolled study including 17 patients with MGN amongst a group of 46 subjects with primary glomerulonephritis, included a treatment protocol with a minimum of 3 months with mycophenolate mofetil (MMF) (Choi 2002). Patients with MGN were somewhat heterogeneous with regard to risk profile, and received variable doses of prednisone in addition to the MMF. There was a significant decrease in proteinuria, and trend towards improved renal function.

The findings of this preliminary study were supported by a small pilot RCT in 21 drug-naïve adults at medium risk of progression. Patients received either MMF 2 g/day with prednisone 0.5 mg/kg/day for 2 of the 6 months of immunotherapy, or alternating cycles of steroids and cyclophosphamide. There was no significant difference in the proportion of patients achieving remission: 64% with MMF, 80% with Ponticelli routine (Senthil Nayagam et al., 2008). The frequency of relapses and incidence of infections were similar in both groups. Similar results were observed in a small study comparing the same two regimens in patients of Chinese ancestry (Chan et al., 2007). In the latter the relapse rate was 23% at 2 years.

Uncontrolled studies of MMF in patients with MGN at high risk of progression have demonstrated conflicting results. MMF was evaluated in 16 patients with nephrotic syndrome due to MGN who would be categorized as either medium to high risk by virtue of their renal parameters alone (Miller et al., 2000). Nearly all had steroid-resistant disease, and half had failed cytotoxic and ciclosporin therapy. Moderate success was noted after a mean of 8 months of treatment, with six patients achieving a halving of their proteinuria. No difference was noted with respect to renal function; side effects were infrequent. In contrast, more recent studies using MMF as initial therapy in MGN have not consistently demonstrated efficacy in inducing remissions or delaying the onset of progressive CKD (Branten et al., 2007). Thirty-two patients with MGN and impairment of kidney function (creatinine > 132 μmol/L) were treated with oral MMF 1 g twice daily for 12 months, in combination with corticosteroids, and compared to 32 patients—historical controls—treated for the same duration with oral cyclophosphamide in combination with corticosteroids (cyclophosphamide; 1.5 mg/kg/day). Cumulative incidences of complete and partial remission of proteinuria at 12 months were 66% with MMF versus 72% with cyclophosphamide ($P = 0.3$). Adverse effects occurred at a similar rate in the two groups, but relapses were much more common with MMF beginning even before completion of the 12 months of treatment and approaching an 80% relapse rate within 10 months of stopping therapy.

Data from another randomized controlled study in this population do not support an advantage of MMF over standard care; 36 patients with MGN and nephrotic syndrome received either conservative therapy (renin–angiotensin system blockade, statins, low-salt and low-protein diet, and diuretics) plus MMF (2 g/day, without concomitant steroids) (N = 19) or conservative therapy alone (N = 17) for 12 months (Dussol et al., 2008). The probability of a complete or partial remission did not differ between the two groups after 12 months.

In summary, in patients at high risk of progressive renal disease, the studies evaluating a role for MMF are of limited number, include small sample sizes, and demonstrate inconsistent results with limited data beyond short-term endpoints. A regimen of MMF plus steroids may have comparable efficacy to the standard regimen of cyclical alkylating agents and steroids but is associated with more relapses, substantially reducing enthusiasm to adopt this approach to therapy of MGN. In this population, monotherapy with MMF appears to be ineffective.

Anti-B-cell antibodies: possibly effective, RCTs needed

A promising agent described is rituximab, a monoclonal antibody directed against the surface antigen CD20 of B cells. In addition to the lack of possible direct nephrotoxicity, this medication has the potential advantage of ease of administration with a limited

number of infusions and no need for frequent drug monitoring. While this drug likely has broad effects on immune system regulation and inflammation, its primary mechanism of action is causing apoptosis of CD20+ B cells. Interference with B-cell function should inhibit production of antibodies to the putative human auto-antigen implicated in the pathogenesis of MGN. The lack of relationship between levels of CD20+ cells and response to therapy supports the concept that the immunomodulatory effects of this drug extend beyond its ability to deplete this B-cell line.

A role for rituximab was first established in small observational studies. Seven patients received the IV medication in an uncontrolled trial (Remuzzi et al., 2002). The mean creatinine clearance was 68.7 mL/min/1.73 m^2, and mean proteinuria was 8.6 g/day (all had > 3.5g/day). All were on full doses of ACEIs, and had not reached remission after an observation of 1 year following biopsy. By 20 weeks following drug administration (the last follow-up), urine protein had decreased to a mean of 3.7 g/day, two patients achieved a full remission, and three a partial remission. Adverse effects were mild, and infusion-related. A second observational study from the same investigators suggested that rituximab is likely to be most effective in patients with minimal degrees of tubulointerstitial injury (Ruggenenti et al., 2006).

To reduce the cumulative dose of rituximab, the investigators subsequently performed a matched-cohort controlled study using circulating B-cell counts to guide dosing. At 1 year, the proportion of patients who achieved disease remission with lymphocyte-guided dosing was identical to that of 24 historical patients who were given a standard rituximab protocol (four weekly doses of 375 mg/m^2). Lymphocyte-guided therapy resulted in less cumulative exposure to rituximab with substantial cost-saving benefits (Cravedi et al., 2007).

Two pilot studies have been completed in North American patients. The first (Fervenza et al., 2008) was a prospective observational study in 15 patients with MGN and proteinuria > 4 g per 24 hours—despite ACEI/ARB use for > 3 months and systolic blood pressure < 130 mmHg— treated with 1 g rituximab at days 0 and 15. At 6 months, patients who remained with proteinuria >3 g per 24 hours, and with some recovery of their CD19/20+ B-cell count received a second identical course of rituximab. The mean baseline proteinuria of participants was 13.0 ± 5.7 g per 24 hours. The mean decline in proteinuria from baseline to 12 months was 6.2 ± 5.1 g/day and was statistically significant (P = 0.002). Rituximab was well tolerated, and was effective in reducing proteinuria in most of the patients. The complete and partial remission rate was almost 60%, higher than would have been expected based on known spontaneous remission rates.

The second study carried out by the same group was a prospective observational study in 20 patients with MGN and baseline persistent proteinuria > 5.0 g/day (Fervenza et al., 2010). It was designed to test whether the standard four-dose regimen would be more efficacious than the 1 g, two-dose regimen given in the first study. All patients received rituximab (375 mg/m^2 weekly for four doses), with retreatment at 6 months regardless of proteinuria response. Baseline proteinuria was 11.9 g/day and decreased to 4.2 g/day and 2.0 g/day at 12 and 24 months, respectively, while creatinine clearance increased from 72.4 to 88.4 mL/min per 1.73 m^2 at 24 months. Among 18 patients who completed 24 months of follow-up, four achieved complete remission, 12 achieved partial remission (total complete plus partial remission of 80%). One patient relapsed during follow-up. More than half of the patients in this pilot trial had not responded to prior therapy. No short-term toxicity of rituximab was observed. This study also reinforced the observation, originally made in patients receiving alkylating agents, that proteinuria declines gradually in patients with MGN and many months may be required for proteinuria to reach its nadir.

Comparison with alkylating agents in long term randomized studies is needed.

Adrenocorticotrophic hormone: experimental

Adrenocorticotrophic hormone (ACTH) was originally administered for the treatment of dyslipidaemia. The observation that patients receiving this drug for dyslipidaemia associated with the nephrotic syndrome also resulted in reduction in proteinuria first prompted consideration of potential anti-proteinuric drug activity particularly in patients with MGN. One observational study and one small randomized controlled trial provide preliminary support for the use of long-acting ACTH as initial therapy in MGN. Depot synthetic ACTH (Synacthen®) administered for 1 year in an observational study decreased proteinuria in patients with MGN (Berg and Nilsson-Ehle, 1994; Berg et al., 1999; Berg and Arnadottir, 2004).

More recently, a small, open-label, pilot RCT compared IV methylprednisolone and oral corticosteroids plus a cytotoxic agent (N = 16) versus synthetic ACTH (N = 16) as initial therapy in MGN, and found them to be of similar efficacy, at least over a short-term follow-up (Ponticelli et al., 2006). Side effects associated with the use of synthetic ACTH included dizziness, glucose intolerance, diarrhoea, and the development of bronze-coloured skin, all of which resolved after the end of therapy.

Larger RCTs are required before synthetic ACTH can be considered as recommended therapy for MGN. Preliminary reports of uncontrolled studies showing a similar effect of native, intact (porcine) ACTH in a gel formulation have been published but no RCTs have been conducted with this formulation of ACTH (it should be noted that in the United States intact native ACTH in gel formulation is approved by the Food and Drug Administration for treatment of the nephrotic syndrome including MGN in the absence of renal failure) (Bomback et al., 2011; Hladunewich et al., 2014).

Strategies to reduce the side effects of immunosuppressive therapy

Bone loss due to corticosteroid treatment, is related to both dose and duration of therapy, and is greatest in the first 3–6 months of treatment. Prolonged low-dose exposure, however, has also been associated with significant loss of bone density (Canalis and Giustina, 2001). The effects of steroids on the intestinal absorption of calcium and promotion of calciuria suggest that calcium and vitamin D should be incorporated into the treatment regimen. There are, however, significant data indicating the protective effects of antiresorptive medications on bone loss and fractures induced by glucocorticoids. Several agents, including etidronate and alendronate, have been shown in multicentred, well-designed trials to improve these outcomes (Adachi et al., 1997; Saag et al., 1998).

Avascular necrosis of the femoral head is another potentially serious skeletal complication of prednisone. Patients must be informed of this potential side effect, as it is not preventable, and is not necessarily dose related.

Ongoing monitoring for excessive myelosuppression should be employed in order to reduce the potential for infections, but individuals receiving alkylating agents and high-dose corticosteroids are at substantial risk of infectious complications. The use of trimethoprim-sulfamethoxazole significantly reduces the incidence of *Pneumocystis* pneumonia in this population (Ognibene et al., 1995).

Gonadal toxicity due to alkylating agents is of significant concern but is linked to cumulative dose. IV cyclophosphamide given for 3 months is unlikely to reach levels of high concern, but may be enough to bring on early menopause in some patients. As re-treatment may be necessary, cryopreservation of sperm or oocytes should be considered prior to initiation of therapy, where it is available.

Acrolein is the toxic metabolite produced from cyclophosphamide which induces urothelial damage. Hydration and MESNA can be used if administering the intravenous formulation of cyclophosphamide. Patients should be warned of the long-term risk of bladder and also of other malignancies, including skin cancers and haematological malignancy (Radis et al., 1995).

References

Abe, S., Amagasaki, Y., Konishi, K., *et al.* (1986). Idiopathic membranous glomerulonephritis: aspects of geographical differences. *J Clin Pathol*, 39, 1193–8.

Adachi, J. D., Bensen, W. G., Brown, J., *et al.* (1997). Intermittent etidronate therapy to prevent corticosteroid-induced osteoporosis. *N Engl J Med*, 337, 382–7.

Barbour, S. J., Greenwald, A., Djurdjev, O., *et al.* (2011). Disease-specific risk of venous thromboembolic events is increased in idiopathic glomerulonephritis. *Kidney Int*, 81, 190–5.

Bazzi, C. (2001). Urinary excretion of IgG and alpha(1)-microglobulin predicts clinical course better than extent of proteinuria in membranous nephropathy. *Am J Kidney Dis*, 38, 240–8.

Bellomo, R. and Atkins, R.C. (1993). Membranous nephropathy and thromboembolism: is prophylactic anticoagulation warranted? *Nephron*, 63, 249–54.

Bellomo, R., Wood, C., Wagner, I., *et al.* (1993). Idiopathic membranous nephropathy in an Australian population: the incidence of thromboembolism and its impact on the natural history. *Nephron*, 63, 240–1.

Berg, A. L. and Arnadottir, M. (2004). ACTH-induced improvement in the nephrotic syndrome in patients with a variety of diagnoses. *Nephrol Dial.Transplant*, 19, 1305–7.

Berg, A. L. and Nilsson-Ehle, P. (1994). Direct effects of corticotropin on plasma lipoprotein metabolism in man—studies in vivo and in vitro. *Metabolism*, 43, 90–7.

Berg, A. L., Nilsson-Ehle, P., and Arnadottir, M. (1999). Beneficial effects of ACTH on the serum lipoprotein profile and glomerular function in patients with membranous nephropathy. *Kidney Int*, 56, 1534–43.

Bomback, A. S., Tumlin, J. A., Baranski, J., *et al.* (2011). Treatment of nephrotic syndrome with adrenocorticotropic hormone (ACTH) gel. *Drug Des Devel Ther*, 5, 147–53.

Branten, A. J., Reichert, L. J., Koene, R. A., *et al.* (1998). Oral cyclophosphamide versus chlorambucil in the treatment of patients with membranous nephropathy and renal insufficiency. *QJM*, 91, 359–66.

Branten, A. J., du Buf-Vereijken, P. W., Klasen, I. S., *et al.* (2005). Urinary excretion of beta2-microglobulin and IgG predict prognosis in idiopathic membranous nephropathy: a validation study. *Am Soc Nephrol*, 16, 169–74.

Branten, A. J., du Buf-Vereijken, P. W., Vervloet, M., *et al.* (2007). Mycophenolate mofetil in idiopathic membranous nephropathy: a clinical trial with comparison to a historic control group treated with cyclophosphamide. *Am J Kidney Dis.*, 50, 248–56.

Bruns, F. J., Adler, S., Fraley, D. S., *et al.* (1991). Sustained remission of membranous glomerulonephritis after cyclophosphamide and prednisone. *Ann Intern Med*, 114, 725–30.

Cameron, J. S., Healy, M. J., and Adu, D. (1990). The Medical Research Council trial of short-term high-dose alternate day prednisolone in idiopathic membranous nephropathy with nephrotic syndrome in adults. The MRC Glomerulonephritis Working Party. *QJM*, 74, 133–56.

Canalis, E. and Giustina, A. (2001). Glucocorticoid-induced osteoporosis: summary of a workshop. *J Clin Endocrinol Metab*, 86, 5681–5.

Cattran, D. C. (2001). Idiopathic membranous glomerulonephritis. *Kidney Int*, 59, 1983–94.

Cattran, D. C., Appel, G. B., Hebert, L. A., *et al.* (2001). Cyclosporine in patients with steroid-resistant membranous nephropathy: a randomized trial. *Kidney Int*, 59, 1484–90.

Cattran, D. C., Delmore, T., Roscoe, J., *et al.* (1989). A randomized controlled trial of prednisone in patients with idiopathic membranous nephropathy. *N Engl J Med*, 320, 210–15.

Cattran, D. C., Greenwood, C., Ritchie, S., *et al.* (1995). A controlled trial of cyclosporine in patients with progressive membranous nephropathy. Canadian Glomerulonephritis Study Group. *Kidney Int*, 47, 1130–5.

Cattran, D. C., Pei, Y., and Greenwood, C. (1992). Predicting progression in membranous glomerulonephritis. *Nephrol Dial Transplant*, 7 Suppl 1, 48–52.

Cattran, D. C., Pei, Y., Greenwood, C. M., *et al.* (1997). Validation of a predictive model of idiopathic membranous nephropathy: its clinical and research implications. *Kidney Int*, 51, 901–7.

Cattran, D. C., Reich, H. N., Beanlands, H. J., *et al.* (2008). The impact of sex in primary glomerulonephritis. *Nephrol Dial Transplant*, 23, 2247–53.

Chan, T. M., Lin, A. W., Tang, S. C., *et al.* (2007). Prospective controlled study on mycophenolate mofetil and prednisolone in the treatment of membranous nephropathy with nephrotic syndrome. *Nephrology*, 12, 576–81.

Chen, M., Li, H., Li, X. Y., *et al.* (2010). Tacrolimus combined with corticosteroids in treatment of nephrotic idiopathic membranous nephropathy: a multicenter randomized controlled trial. *Am J Med Sci*, 339, 233–8.

Choi, M. J., Eustace, J. A., Gimenez, L. F., *et al.* (2002). Mycophenolate mofetil treatment for primary glomerular diseases. *Kidney Int*, 61, 1098–114.

Collaborative Study of the Adult Idiopathic Nephrotic Syndrome (1979). A controlled study of short-term prednisone treatment in adults with membranous nephropathy. *N Engl J Med*, 301, 1301–6.

Cravedi, P., Ruggenenti, P., Sghirlanzoni, M. C., *et al.* (2007). Titrating rituximab to circulating B cells to optimize lymphocytolytic therapy in idiopathic membranous nephropathy. *Clin J Am Soc Nephrol*, 2, 932–7.

Cupisti, A., Morelli, E., Ciardella, F., *et al.* (1990). Dietary proteins affect proteinuria in primary membranous glomerulonephritis with nephrotic syndrome and normal renal function. *Contrib Nephrol*, 83, 166–9.

D'Amico, G. and Gentile, M. G. (1992). Effect of dietary manipulation on the lipid abnormalities and urinary protein loss in nephrotic patients. *Miner Electrolyte Metab*, 18, 203–6.

D'Amico, G., Gentile, M. G., Manna, G., *et al.* (1992). Effect of vegetarian soy diet on hyperlipidaemia in nephrotic syndrome. *Lancet*, 339, 1131–4.

Davenport, A., Maciver, A. G., Hall, C. L., *et al.* (1994). Do mesangial immune complex deposits affect the renal prognosis in membranous glomerulonephritis? *Clin Nephrol*, 41, 271–6.

Davison, A. M., Cameron, J. S., Kerr, D. N., *et al.* (1984). The natural history of renal function in untreated idiopathic membranous glomerulonephritis in adults. *Clin Nephrol*, 22, 61–7.

Donadio, J. V., Jr., Torres, V. E., Velosa, J. A., *et al.* (1988). Idiopathic membranous nephropathy: the natural history of untreated patients. *Kidney Int*, 33, 708–15.

Dumoulin, A., Hill, G. S., Montseny, J. J., *et al.* (2003). Clinical and morphological prognostic factors in membranous nephropathy: significance of focal segmental glomerulosclerosis. *Am J Kidney Dis*, 41, 38–48.

Dussol, B., Morange, S., Burtey, S., *et al.* (2008). Mycophenolate mofetil monotherapy in membranous nephropathy: a 1-year randomized controlled trial. *Am J Kidney Dis*, 52, 699–705.

Falk, R. J., Hogan, S. L., Muller, K. E., *et al.* (1992). Treatment of progressive membranous glomerulopathy. A randomized trial comparing cyclophosphamide and corticosteroids with corticosteroids alone. The Glomerular Disease Collaborative Network. *Ann Intern Med*, 116, 438–45.

Fervenza, F. C., Abraham, R. S., Erickson, S. B., *et al.* (2010). Rituximab therapy in idiopathic membranous nephropathy: a 2-year study. *Clin J Am Soc Nephrol*, 5, 2188–98.

Fervenza, F. C., Cosio, F. G., Erickson, S. B., *et al.* (2008). Rituximab treatment of idiopathic membranous nephropathy. *Kidney Int*, 73, 117–25.

Franklin, W. A., Jennings, R.B., Earle, D.P. (1973). Membranous glomerulonephritis: long-term serial observations on clinical course and morphology. *Kidney Int*, 4, 36–56.

Fritsche, L., Budde, K., Färber, L., *et al.* (1999). Treatment of membranous glomerulopathy with cyclosporin A: how much patience is required? *Nephrol Dial Transplant*, 14, 1036–8.

Geddes, C. C. and Cattran, D.C. (2000). The treatment of idiopathic membranous nephropathy. *Semin Nephrol*, 20, 299–308.

Gerstoft, J., Balsløv, J. T., Brahm, M., *et al.* (1986). Prognosis in glomerulonephritis. II. Regression analyses of prognostic factors affecting the course of renal function and the mortality in 395 patients. Calculation of a prognostic model. Report from a Copenhagen study group of renal diseases. *Acta Med Scand*, 219, 179–87.

Gluck, M. C., Gallo, G., Lowenstein, J., *et al.* (1973). Membranous glomerulonephritis. Evolution of clinical and pathologic features. *Ann Intern Med*, 78, 1–12.

Harrison, D. J., Thomson, D., and MacDonald, M.K. (1986). Membranous glomerulonephritis. *J Clin Pathol*, 39, 167–7.

Hay, N. M., Bailey, R. R., Lynn, K. L., *et al.* (1992). Membranous nephropathy: a 19 year prospective study in 51 patients. *N Z Med J*, 105, 489–91.

Heaf, J., Lokkegaard, H., and Larsen, S. (1999). The epidemiology and prognosis of glomerulonephritis in Denmark 1985–1997. *Nephrol Dial Transplant*, 14, 1889–97.

Hladunewich, M. A., Cattran, D., Beck, L. H., *et al.* (2014). A pilot study to determine the dose and effectiveness of adrenocorticotrophic hormone (H.P. Acthar® Gel) in nephrotic syndrome due to idiopathic membranous nephropathy. *Nephrol Dial Transplant*, 29(8), 1570–7.

Hladunewich, M. A., Troyanov, S., Calafati, J., *et al.* (2009). The natural history of the non-nephrotic membranous nephropathy patient. *Clin J Am Soc Nephrol*, 4, 1417–22.

Hofstra, J. M., Branten, A. J., Wirtz, J. J., *et al.* (2010). Early versus late start of immunosuppressive therapy in idiopathic membranous nephropathy: a randomized controlled trial. *Nephrol Dial Transplant*, 25, 129–36.

Hofstra, J. M., Deegens, J. K., Willems, H. L., *et al.* (2008). Beta-2-microglobulin is superior to N-acetyl-beta-glucosaminidase in predicting prognosis in idiopathic membranous nephropathy. *Nephrol Dial Transplant*, 23, 2546–51.

Hogan, S. L., Muller, K. E., Jennette, J. C., *et al.* (1995). A review of therapeutic studies of idiopathic membranous glomerulopathy. *Am J Kidney Dis*, 25, 862–75.

Honkanen, E. (1986). Survival in idiopathic membranous glomerulonephritis. *Clin Nephrol*, 25, 122–8.

Honkanen, E., Törnroth, T., Grönhagen-Riska, C., *et al.* (1994). Long-term survival in idiopathic membranous glomerulonephritis: can the course be clinically predicted? *Clin Nephrol*, 41, 127–34.

Hopper, J., Jr., Trew, P.A., and Biava, C.G. (1981). Membranous nephropathy: its relative benignity in women. *Nephron*, 29, 18–24.

Howman, A., Chapman, T. L., Langdon, M. M., *et al.* (2013). Immunosuppression for progressive membranous nephropathy: a UK randomised controlled trial *Lancet*, 381, 744–51.

Jha, V., Ganguli, A., Saha, T. K., *et al.* (2007). A randomized, controlled trial of steroids and cyclophosphamide in adults with nephrotic syndrome caused by idiopathic membranous nephropathy. *Am Soc Nephrol*, 18, 1899–904.

Jindal, K., West, M., Bear, R., *et al.* (1992). Long-term benefits of therapy with cyclophosphamide and prednisone in patients with membranous glomerulonephritis and impaired renal function. *Am J Kidney Dis*, 19, 61–7.

Kida, H., Asamoto, T., Yokoyama, H., *et al.* (1986). Long-term prognosis of membranous nephropathy. *Clin Nephrol*, 25, 64–9.

Kobayashi, Y., Tateno, S., Shigematsu, H., *et al.* (1982). Prednisone treatment of non-nephrotic patients with idiopathic membranous nephropathy. A prospective study. *Nephron*, 30, 210–19.

Kopple, J. D., Levey, A. S., Greene, T., *et al.* (1997). Effect of dietary protein restriction on nutritional status in the Modification of Diet in Renal Disease Study. *Kidney Int*, 52, 778–91.

Kumar, S., Chapagain, A., Nitsch, D., *et al.* (2012). Proteinuria and hypoalbuminemia are risk factors for thromboembolic events in patients with idiopathic membranous nephropathy: an observational study. *BMC Nephrol*, 13, 107.

Laluck, B. J. Jr. and Cattran, D.C. (1999). Prognosis after a complete remission in adult patients with idiopathic membranous nephropathy. *Am J Kidney Dis*, 33, 1026–32.

Lewis, E. J. (1993). Idiopathic membranous nephropathy—to treat or not to treat? *N Engl J Med*, 329, 127–9.

Llach, F. (1985). Hypercoagulability, renal vein thrombosis, and other thrombotic complications of nephrotic syndrome. *Kidney Int*, 28, 429–39.

MacTier, R., Boulton Jones, J. M., Payton, C. D., *et al.* (1986). The natural history of membranous nephropathy in the West of Scotland. *QJM*, 60, 793–802.

Maisonneuve, P., Agodoa, L., Gellert, R., *et al.* (2000). Distribution of primary renal diseases leading to end-stage renal failure in the United States, Europe, and Australia/New Zealand: results from an international comparative study. *Am J Kidney Dis*, 35, 157–65.

Mallick, N. P., Short, C.D., Manos, J. (1983). Clinical membranous nephropathy. *Nephron*, 34, 209–19.

Marx, B. E. and Marx, M. (1997). Prognosis of idiopathic membranous nephropathy: a methodologic meta- analysis. *Kidney Int*, 51, 873–9.

Marx, B. E. and Marx, M. (1999). Prediction in idiopathic membranous nephropathy. *Kidney Int*, 56, 666–73.

Mathieson, P. W., Turner, A. N., Maidment, C. G., *et al.* (1988). Prednisolone and chlorambucil treatment in idiopathic membranous nephropathy with deteriorating renal function. *Lancet*, 2, 869–72.

Miller, G., Zimmerman, R., 3rd, Radhakrishnan, J., *et al.* (2000). Use of mycophenolate mofetil in resistant membranous nephropathy. *Am J Kidney Dis*, 36, 250–6.

Muirhead, N. (1999). Management of idiopathic membranous nephropathy: evidence-based recommendations. *Kidney Int Suppl*, 70, S47–55.

Murphy, B. F., Fairley, K.F., Kincaid-Smith, P.S. (1988). Idiopathic membranous glomerulonephritis: long-term follow-up in 139 cases. *Clin Nephrol*, 30, 175–81.

Neugarten, J., Acharya, A., Silbiger, S.R. (2000). Effect of gender on the progression of nondiabetic renal disease: a meta-analysis. *Am Soc Nephrol*, 11, 319–29.

Noel, L. H., Zanetti, M., Droz, D., *et al.* (1979). Long-term prognosis of idiopathic membranous glomerulonephritis. Study of 116 untreated patients. *Am J Med*, 66, 82–90.

O'Callaghan, C. A., Hicks, J., Doll, H., *et al.* (2002). Characteristics and outcome of membranous nephropathy in older patients. *Int Urol Nephrol*, 33, 157–65.

Ognibene, F. P., Shelhamer, J. H., Hoffman, G. S., *et al.* (1995). Pneumocystis carinii pneumonia: a major complication of immunosuppressive therapy in patients with Wegener's granulomatosis. *Am J Respir Crit Care Med*, 151, 795–9.

Pedrini, M. T., Levey, A. S., Lau, J., *et al.* (1996). The effect of dietary protein restriction on the progression of diabetic and nondiabetic renal diseases: a meta-analysis. *Ann Intern Med*, 124, 627–32.

Pei, Y., Cattran, D., Greenwood, C. (1992). Predicting chronic renal insufficiency in idiopathic membranous glomerulonephritis. *Kidney Int*, 42, 960–6.

Polanco, N., Gutiérrez, E., Covarsí, A., *et al.* (2010). Spontaneous remission of nephrotic syndrome in idiopathic membranous nephropathy. *Am Soc Nephrol*, 21, 697–704.

Ponticelli, C., Altieri, P., Scolari, F., *et al.* (1998). A randomized study comparing methylprednisolone plus chlorambucil versus methylprednisolone plus cyclophosphamide in idiopathic membranous nephropathy. *Am Soc Nephrol*, 9, 444–50.

Ponticelli, C. and Moroni, G. (1998). Renal biopsy in lupus nephritis—what for, when and how often? *Nephrol Dial Transplant*, 13, 2452–4.

Ponticelli, C. and Passerini, P. (1990). The natural history and therapy of idiopathic membranous nephropathy. *Nephrol Dial Transplant*, 5 Suppl 1, 37–41.

Ponticelli, C., Passerini, P., Altieri, P., *et al.* (1992a). Remissions and relapses in idiopathic membranous nephropathy. *Nephrol Dial Transplant*, 7 (Suppl 1), 85–90.

Ponticelli, C., Passerini, P., Salvadori, M., *et al.* (2006). A randomized pilot trial comparing methylprednisolone plus a cytotoxic agent versus synthetic adrenocorticotropic hormone in idiopathic membranous nephropathy. *Am J Kidney Dis*, 47, 233–40.

Ponticelli, C., Zucchelli, P., Imbasciati, E., *et al.* (1984). Controlled trial of methylprednisolone and chlorambucil in idiopathic membranous nephropathy. *N Engl J Med*, 310, 946–50.

Ponticelli, C., Zucchelli, P., Passerini, P., *et al.* (1989). A randomized trial of methylprednisolone and chlorambucil in idiopathic membranous nephropathy. *N Engl J Med*, 320, 8–13.

Ponticelli, C., Zucchelli, P., Passerini, P., *et al.* (1992b). Methylprednisolone plus chlorambucil as compared with methylprednisolone alone for the treatment of idiopathic membranous nephropathy. The Italian Idiopathic Membranous Nephropathy Treatment Study Group. *N Engl J Med*, 327, 599–603.

Ponticelli, C., Zucchelli, P., Passerini, P., *et al.* (1995). A 10-year follow-up of a randomized study with methylprednisolone and chlorambucil in membranous nephropathy. *Kidney Int*, 48, 1600–4.

Praga, M., Barrio, V., Juárez, G. F., *et al.* (2007). Tacrolimus monotherapy in membranous nephropathy: a randomized controlled trial. *Kidney Int*, 71, 924–30.

Rabelink, T. J., Zwaginga, J. J., Koomans, H. A., *et al.* (1994). Thrombosis and hemostasis in renal disease. *Kidney Int*, 46, 287–96.

Radis, C.D., Kahl, L. E., Baker, G. L., *et al.* (1995). Effects of cyclophosphamide on the development of malignancy and on long-term survival of patients with rheumatoid arthritis. A 20-year followup study. *Arthritis Rheum*, 38, 1120–7.

Ramzy, M. H., Cameron, J. S., Turner, D. R., *et al.* (1981). The long-term outcome of idiopathic membranous nephropathy. *Clin Nephrol*, 16, 13–19.

Reichert, L. J., Huysmans, F. T., Assmann, K., *et al.* (1994). Preserving renal function in patients with membranous nephropathy: daily oral chlorambucil compared with intermittent monthly pulses of cyclophosphamide. *Ann Intern Med*, 121, 328–33.

Remuzzi, G., Chiurchiu, C., Abbate, M., *et al.* (2002). Rituximab for idiopathic membranous nephropathy (letter). *Lancet*, 360, 923–4.

Research Group on Progressive Chronic Renal Disease (1999). Nationwide and long-term survey of primary glomerulonephritis in Japan as observed in 1,850 biopsied cases. *Nephron*, 82, 205–13.

Rostoker, G., Belghiti, D., Ben Maadi, A., *et al.* (1993). Long-term cyclosporin A therapy for severe idiopathic membranous nephropathy. *Nephron*, 63, 335–41.

Rostoker, G., Ben Maadi, A., Remy, P., *et al.* (1995). Low-dose angiotensin-converting-enzyme inhibitor captopril to reduce proteinuria in adult idiopathic membranous nephropathy: a prospective study of long-term treatment. *Nephrol Dial Transplant*, 10, 25–29.

Row, P. G., Cameron, J. S., Turner, D. R., *et al.* (1975). Membranous nephropathy. Long-term follow-up and association with neoplasia. *QJM*, 44, 207–39.

Ruggenenti, P., Chiurchiu, C., Abbate, M., *et al.* (2006). Rituximab for idiopathic membranous nephropathy: who can benefit? *Clin J Am Soc Nephrol*, 1, 738–48.

Saag, K. G., Emkey, R., Schnitzer, T. J., *et al.* (1998). Alendronate for the prevention and treatment of glucocorticoid-induced osteoporosis. Glucocorticoid-Induced Osteoporosis Intervention Study Group. *N Engl J Med*, 339, 292–9.

Schieppati, A., Mosconi, L., Perna, A., *et al.* (1993). Prognosis of untreated patients with idiopathic membranous nephropathy. *N Engl J Med*, 329, 85–9.

Senthil Nayagam, L., Ganguli, A., Rathi, M., *et al.* (2008). Mycophenolate mofetil or standard therapy for membranous nephropathy and focal segmental glomerulosclerosis: a pilot study. *Nephrol Dial Transplant*, 23, 1926–30.

Shiiki, H., Saito, T., Nishitani, Y., *et al.* (2004). Prognosis and risk factors for idiopathic membranous nephropathy with nephrotic syndrome in Japan. *Kidney Int*, 65, 1400–7.

Short, C. D., Solomon, L. R., Gokal, R., *et al.* (1987). Methylprednisolone in patients with membranous nephropathy and declining renal function. *QJM*, 65, 929–40.

Simon, P., Ramée, M. P., Autuly, V., *et al.* (1994). Epidemiology of primary glomerular diseases in a French region. Variations according to period and age. *Kidney Int*, 46, 1192–8.

Stirling, C. M., Simpson, K., Boulton-Jones, J.M. (1998). Immunosuppression and outcome in idiopathic membranous nephropathy. *QJM*, 91, 159–64.

Thomas, D. M., Hillis, A. N., Coles, G. A., *et al.* (1991). Enalapril can treat the proteinuria of membranous glomerulonephritis without detriment to systemic or renal hemodynamics. *Am J Kidney Dis*, 18, 38–43.

Tornroth, T., Honkanen, E., Pettersson, E. (1987). The evolution of membranous glomerulonephritis reconsidered: new insights from a study on relapsing disease. *Clin Nephrol*, 28, 107–17.

Torres, A., Domínguez-Gil, B., Carreño, A., *et al.* (2002). Conservative versus immunosuppressive treatment of patients with idiopathic membranous nephropathy. *Kidney Int*, 61, 219–27.

Toth, T. and Takebayashi, S. (1994). Factors contributing to the outcome in 100 adult patients with idiopathic membranous glomerulonephritis. *Int Urol Nephrol*, 26, 93–106.

Trew, P. A., Biava, C. G., Jacobs, R. P., *et al.* (1978). Renal vein thrombosis in membranous glomerulonephropathy: incidence and association. *Medicine (Baltimore)*, 57, 69–82.

Troyanov, S., Roasio, L., Pandes, M., *et al.* (2006). Renal pathology in idiopathic membranous nephropathy: a new perspective. *Kidney Int*, 69, 1641–8.

Troyanov, S., Wall, C. A., Miller, J. A., *et al.* (2004). Idiopathic membranous nephropathy: definition and relevance of a partial remission. *Kidney Int*, 66(3), 1199–205.

Tu, W. H., Petitti, D. B., Biava, C. G., *et al.* (1984). Membranous nephropathy: predictors of terminal renal failure. *Nephron*, 36, 118–24.

United States Renal Data System (2009). *USRDS 2009 Annual Data Report: Atlas of Chronic Kidney Disease and End-Stage Renal Disease in the United States*. Bethesda, MD: National Institutes of Health, National Institute of Diabetes and Digestive and Kidney Diseases.

Wagoner, R. D., Stanson, A. W., Holley, K. E., *et al.* (1983). Renal vein thrombosis in idiopathic membranous glomerulopathy and nephrotic syndrome: incidence and significance. *Kidney Int*, 23, 368–74.

Wakai, S. and Magil, A. B. (1992). Focal glomerulosclerosis in idiopathic membranous glomerulonephritis. *Kidney Int*, 41(2), 428–34.

Warwick, G. L., Geddes, C.G., and Boulton-Jones, J.M. (1994). Prednisolone and chlorambucil therapy for idiopathic membranous nephropathy with progressive renal failure. *QJM*, 87, 223–9.

Wehrmann, M., Bohle, A., Bogenschütz, O., *et al.* (1989). Long-term prognosis of chronic idiopathic membranous glomerulonephritis. An analysis of 334 cases with particular regard to tubulo-interstitial changes. *Clin Nephrol*, 31, 67–76.

Yoshimoto, K., Yokoyama, H., Wada, T., *et al.* (2004). Pathologic findings of initial biopsies reflect the outcomes of membranous nephropathy. *Kidney Int*, 65, 148–53.

Zent, R., Nagai, R., and Cattran, D. C. (1997). Idiopathic membranous nephropathy in the elderly: a comparative study. *Am J Kidney Dis*, 29, 200–6.

Zucchelli, P., Cagnoli, L., Pasquali, S., *et al.* (1986). Clinical and morphologic evolution of idiopathic membranous nephropathy. *Clin Nephrol*, 25, 282–8.

Zucchelli, P., Ponticelli, C., Cagnoli, L., *et al*. (1987). Long-term outcome of idiopathic membranous nephropathy with nephrotic syndrome. *Nephrol Dial Transplant*, 2, 73–8.

Zucchelli, P. and Pasquali, S. (1998). Membranous nephropathy. In A. M. Davison, J. S. Cameron, J. -P. Grunfeld, *et al.* (ed.) *Oxford Textbook of Clinical Nephrology* (2nd ed.), pp. 571–90. Oxford: Oxford University Press.

CHAPTER 63

Secondary membranous glomerulonephritis

Daniel C. Cattran and Heather N. Reich

The approach to distinguishing primary from secondary causes of membranous glomerulonephritis (MGN) is described in Chapter 61. The major causes are considered in more detail here.

The general conservative measures described for the treatment of primary MGN (ACE inhibition, blood pressure control, lipid-lowering therapy, etc.) are, in our opinion, also recommended for the treatment of almost all causes of secondary forms of MGN, despite a lack of clear evidence from controlled trials.

Lupus membranous

A majority of patients with MGN secondary to systemic lupus erythematosus (SLE; see Chapter 161) present with nephrotic-range proteinuria (64%), and preserved renal function (90%) at the time of initial presentation (Kolasinski et al., 2002). Haematuria is variable; a large number of red cell casts should lead the clinician to suspect coexistent proliferative activity, and the possible presence of a mixed lesion on biopsy. Overall, a pure membranous lesion on biopsy is uncommon in SLE, diagnosed in only 14% of biopsies in SLE patients (Gruppo Italiano per lo Studio della Nefrite Lupica, 1992). Furthermore, up to 50% of patients with renal involvement will change histologic classification on later biopsies (Ponticelli and Moroni, 1998).

In the case of MGN secondary to SLE, the prognosis seems very dependent upon the coexistence of proliferative lesions on biopsy. The therapy is therefore usually guided by the degree of proliferation or necrosis seen, or by the presence of systemic features that require therapy. An isolated pure membranous nephropathy in the absence of any systemic features or proliferation on biopsy is relatively rare.

Although patients with pure membranous lesion are generally regarded as having a lower risk of end-stage renal disease (ESRD), morbidity associated with the persistence of nephrotic syndrome has prompted interest in the role of immunosuppression for this scenario (Donadio, 1992). There is evidence of favourable response rates with modified versions of the Italian regimen of combination steroids and cytotoxic agents (Pasquali et al., 1993; Moroni et al., 1998; Chan et al., 1999). When data from two multicentre randomized clinical trials were pooled (Ginzler et al., 2005; Appel et al., 2009), there were no differences in response rate between those treated with induction therapy with mycophenolate versus cyclophosphamide (Radhakrishnan et al., 2010). More recently, evidence regarding the utility of ciclosporin has emerged (Radhakrishnan et al., 1994; Austin et al., 2000). One randomized study of 42 patients with membranous nephropathy compared treatment with ciclosporin for 11 months (with steroids), alternate month IV cyclophosphamide for six doses (with steroids) and alternate day steroids alone (Austin et al., 2009). At 1 year, the cumulative probability of remission was 27% with prednisone, 60% with cyclophosphamide and 83% with ciclosporin; not surprisingly relapse rates were lowest in the group treated with cyclophosphamide. Tacrolimus monotherapy and combination regimens are currently being explored as potential options to minimize steroid exposure, and in resistant disease (Tse et al., 2007; Uchino et al., 2010).

Natural history

The natural history of SLE-related MGN is summarized in a review of the available data, which pooled together studies including 157 patients with SLE and predominant membranous lesion on biopsy (World Health Organization (WHO) class V), with follow-up ranging from 25 to 279 months (Kolasinski et al., 2002). Only 23% of patients with 'pure' MGN had complete resolution of proteinuria at follow-up. Most patients (84.5%) continue to have normal renal function, but the numbers with abnormal renal function (14.6%) and with ESRD (5.1%) are hardly negligible. When data were available, subjects were also examined according to WHO classification subgroups based upon the presence (previously WHO class Vc and Vd) or absence (previously WHO class Va and Vb) of focal segmental changes or diffuse proliferative changes. Those with WHO class Vc and Vd had a higher rate of ESRD (35.7% vs 24.6%), but WHO subclass generally does not appear to be an independent predictor of renal failure when adjusted for renal function at presentation, proteinuria, anaemia, or age (Donadio et al., 1995). Much of the prognosis of SLE-MGN seems related to the subsequent development of proliferative changes on biopsy (Pasquali et al., 1993; Sloan et al., 1996). It is interesting to note that although there are limited data on the subject, there was a rather striking rate of non-renal-related deaths of 8.2% due to a variety of causes, emphasizing the serious prognostic implications of renal involvement in SLE. Although overall survival in SLE patients has improved over time, the 5-year survival of patients with SLE regardless of histologic type is worse if there is renal involvement (not specifically MGN) (Cameron, 1999).

Membranous glomerulonephritis in hepatitis B infection

Hepatitis B (HBV)-associated MGN is usually associated with nephrotic-range proteinuria, and normal renal function (Lin, 1990; Lai et al., 1991; Peña et al., 2001). Clinical evidence of liver disease is not required for the development of MGN, and although the worldwide prevalence of HBV is quite high, the development of MGN in the context of this infection is relatively infrequent. The features of

this condition are described more extensively in paediatric populations, who may have a different natural history compared with adults. Hepatitis and the kidney are further discussed in Chapter 185.

The majority although not all patients with HBV who develop MGN, are also seropositive for hepatitis B e antigen (HBeAg) (Lin, 1990; Lai et al., 1991). The correlation of the presence of HBeAg and clinical outcome is debatable, although remission of MGN with the development of antibodies to this antigen has been reported (Hsu et al., 1989).

Treatment

Data regarding therapy for HBV-related MGN are largely from paediatric populations, in whom spontaneous remissions are relatively common in children. The use of immunosuppressants in this condition carries the risk of allowing uncontrolled viral replication, and possible future exacerbation of hepatitis (Lai et al., 1990), but broadly, treatments that clear the infection improve the nephropathy. The treatment of Hepatitis B in patients with renal disease is reviewed in detail in Chapter 185. Here the results specifically in MGN are discussed.

In children, the utility of alpha-interferon (IFN) following trials of prednisone was been studied, in a relatively large group of approximately 40 patients (Lin, 1995). Twenty received IFN therapy for 12 months, and 20 received supportive treatment only. Both groups had failed steroid therapy. At the end of the study period, all patients in the IFN group were completely free of proteinuria, compared to none in the control group. Disappearance of HBeAg occurred more frequently in the treatment group.

In adults, one study of the natural history of HBV-related MGN documented a poor response to IFN in the 5 patients who received the therapy (Lai et al., 1991). A study assessing the long-term outcome of 15 patients indicated that half of those treated with interferon had a sustained remission in their liver disease with loss of HBV DNA and HbeAg, as well as favourable aminotransferase levels (Conjeevaram et al., 1995). The 'responders', all of whom had MGN as the associated renal lesion, also demonstrated marked improvement in proteinuria. From the limited data available, there appears to be a role for IFN, particularly in the presence of serologic markers of active liver disease.

Patients treated with lamivudine (who also had elevated alanine aminotransferase and HBV DNA titres) had a high rate of remission compared to historical controls (60% vs 25%) and superior renal survival (Tang et al., 2005). Long-term therapy with lamivudine may carry a risk of development of mutations conferring viral resistance to the drug and therefore judicious use of antiviral therapy is warranted.

In endemic regions, vaccination programmes have substantially reduced the incidence of hepatitis B virus-associated membranous nephropathy (Bhimma et al., 2003; Xu et al., 2003); these programmes are an important strategy for minimizing the complications of this infection on a global front.

Natural history

HBV-associated MGN has a variable natural history, depending upon the population studied. The majority of information is available from paediatric populations. In children, the disease generally has a benign course. The cumulative probability of remission at 4 years is 64% (Gilbert and Wiggelinkhuizen, 1994), and is usually associated with younger age, and smaller burden of subepithelial immune deposits (Hsu et al., 1989). The membranous lesion has been documented to actually resolve with the gradual spontaneous remission of the nephrotic syndrome (Gonzalo et al., 1999). The natural history in adults may not be as benign. One study of adults infected in an endemic area (therefore vertical transmission possible) indicated that spontaneous remission was uncommon in this population with progressive renal failure seen in 29%, and 10% reaching ESRD by the end of the average 60-month follow-up period (Lai et al., 1991).

Membranous glomerulonephritis associated with malignancy

The association of MGN with malignancy approaches 22% in patients > 60 years of age (Keur et al., 1989; Burstein et al., 1993). A series of 155 patients indicated that 10% of patients > 60 had MGN in association with a malignancy, versus only 1% of patients < 60 years old (O'Callaghan et al., 2002). However it is not clear whether this reflects a causal relationship versus simply an age-related association. Some studies have included tumours diagnosed long after presentation; many of these are likely to be coincidental in this age group.

Patients with MGN associated with malignancy tend to be older—the mean age was 63 years with all patients over 52, in one study of nine patients (Burstein et al., 1993). The average rate of malignancy in a population or subjects with the nephrotic syndrome is 6–11% (Lee et al., 1966; Row et al., 1975; Cahen et al., 1989; Yamauchi et al., 1985). In the series described by Burstein et al., all patients had nephrotic-range proteinuria, and six out of nine had evidence of renal insufficiency at presentation (one was related to obstruction). Most patients manifested proteinuria prior to or at the time of diagnosis of the malignancy. Reappearance of proteinuria may herald relapse of the malignancy.

Although there is inadequate evidence at this point, it seems possible that the target podocyte antigen in patients with malignancy-associated MGN may be different from that seen in primary MGN (see Chapter 64).

Apart from usual general management, and treatment appropriate for the tumour and the individual patient, there is no evidence on specific approaches to MGN in this setting.

There are several reported cases of resolution of the MGN with treatment of the primary malignancy by resection or medical therapy (Robinson et al., 1984; Coltharp et al., 1991; Burstein et al., 1993).

Drug- and toxin-associated membranous nephropathy

Glomerulonephritis associated with drugs and toxins is considered in Chapter 82.

It is generally held that discontinuation of the causative agent results in a resolution of the nephrotic syndrome in patients with medication-related MGN. This has been documented in a relatively large series of patients with MGN related to gold therapy for rheumatoid arthritis (Hall et al., 1987). All patients had a remission of their proteinuria, although a period of up to 18 months was required for complete resolution. Renal function remained stable during the course of the illness and subsequent resolution of proteinuria.

References

Appel, G. B., Contreras, G., Dooley, M. A., *et al.* (2009). Mycophenolate mofetil versus cyclophosphamide for induction treatment of lupus nephritis. *J Am Soc Nephrol*, 20, 1103–12.

Austin, H. A., Illei, G. G., Braun, M. J., *et al.* (2000). Lupus membranous nephropathy: controlled trial of prednisone, pulse cyclophosphamide, and cyclosporine A (abstract). *J Am Soc Nephrol*, 11, A439.

Austin, H. A., III, Illei, G. G., Braun, M. J., *et al.* (2009). Randomized, controlled trial of prednisone, cyclophosphamide, and cyclosporine in lupus membranous nephropathy. *J Am Soc Nephrol*, 20, 901–11.

Bhimma, R., Coovadia, H. M., Adhikari, M., *et al.* (2003). The impact of the hepatitis B virus vaccine on the incidence of hepatitis B virus-associated membranous nephropathy. *Arch Pediatr Adolesc Med*, 157, 1025–30.

Burstein, D. M., Korbet, S.M., and Schwartz, M.M. (1993). Membranous glomerulonephritis and malignancy. *Am J Kidney Dis*, 22, 5–10.

Cahen, R., Francois, B., Trolliet, P., *et al.* (1989). Aetiology of membranous glomerulonephritis: a prospective study of 82 adult patients. *Nephrol Dial Transplant*, 4, 172–80.

Cameron, J. S. (1999). Lupus nephritis. *Am Soc Nephrol*, 10, 413–24.

Chan, T., Li, F. K., Hao, W. K., *et al.* (1999). Treatment of membranous lupus nephritis with nephrotic syndrome by sequential immunosuppression. *Lupus*, 8, 545–51.

Coltharp, W. H., Lee, S. M., Miller, R. F., *et al.* (1991). Nephrotic syndrome complicating adenocarcinoma of the lung with resolution after resection. *Ann Thorac Surg*, 51, 308–9.

Conjeevaram, H.S., Hoofnagle, J. H., Austin, H. A., *et al.* (1995). Long-term outcome of hepatitis B virus-related glomerulonephritis after therapy with interferon alfa. *Gastroenterology*, 109, 540–6.

Donadio, J. V., Jr. (1992). Treatment of membranous nephropathy in systemic lupus erythematosus. *Nephrol Dial Transplant*, 7 Suppl 1, 97–104.

Donadio, J. V., Jr. Hart, G. M., Bergstralh, E. J., *et al.* (1995). Prognostic determinants in lupus nephritis: a long-term clinicopathologic study. *Lupus*, 4, 109–15.

Gilbert, R. D. and Wiggelinkhuizen, J. (1994). The clinical course of hepatitis B virus-associated nephropathy. *Pediatr Nephrol*, 8, 11–14.

Ginzler, E. M., Dooley, M. A., Aranow, C., *et al.* (2005). Mycophenolate mofetil or intravenous cyclophosphamide for lupus nephritis. *N Engl J Med*, 353, 2219–28.

Gonzalo, A., Mampaso, F., Bárcena, R., *et al.* (1999). Membranous nephropathy associated with hepatitis B virus infection: long-term clinical and histological outcome. *Nephrol Dial Transplant*, 14, 416–18.

Gruppo Italiano per lo Studio della Nefrite Lupica (GISNEL) (1992). Lupus nephritis: prognostic factors and probability of maintaining life- supporting renal function 10 years after the diagnosis. *Am J Kidney Dis*, 19, 473–9.

Hall, C. L., Fothergill, N. J., Blackwell, M. M., *et al.* (1987). The natural course of gold nephropathy: long term study of 21 patients. *Br Med J (Clin Res Ed)*, 295, 745–8.

Hsu, H. C., Wu, C. Y., Lin, C. Y., *et al.* (1989). Membranous nephropathy in 52 hepatitis B surface antigen (HBsAg) carrier children in Taiwan. *Kidney Int*, 36, 1103–7.

Keur, I., Krediet, R.T., and Arisz, L. (1989). Glomerulopathy as a paraneoplastic phenomenon. *Neth J Med*, 34, 270–84.

Kolasinski, S. L., Chung, J. B., and Albert, D. A. (2002). What do we know about lupus membranous nephropathy? An analytic review. *Arthritis and Rheumatism*, 47, 450–5.

Lai, K. N., Li, P. K., Lui, S. F. *et al.* (1991). Membranous nephropathy related to hepatitis B virus in adults. *N Engl J Med*, 324, 1457–63.

Lai, K. N., Tam, J. S., Lin, H. J., *et al.* (1990). The therapeutic dilemma of the usage of corticosteroid in patients with membranous nephropathy and persistent hepatitis B virus surface antigenaemia. *Nephron*, 54, 12–7.

Lee, J. C., Yamauchi, H., Hopper, J., Jr. (1966). The association of cancer and the nephrotic syndrome. *Ann Intern Med*, 64, 41–51.

Lin, C. Y. (1990). Hepatitis B virus-associated membraneous nephropathy: clinical features, immunological profiles and outcome. *Nephron*, 55, 37–44.

Lin, C. Y. (1995). Treatment of hepatitis B virus-associated membranous nephropathy with recombinant alpha-interferon. *Kidney Int*, 47, 225–30.

Moroni, G., Maccario, M., Banfi, G., *et al.* (1998). Treatment of membranous lupus nephritis. *Am J Kidney Dis*, 31, 681–6.

O'Callaghan, C. A., Hicks, J., Doll, H., *et al.* (2002). Characteristics and outcome of membranous nephropathy in older patients. *Int Urol Nephrol*, 33, 157–65.

Pasquali, S., Banfi, G., Zucchelli, A., *et al.* (1993). Lupus membranous nephropathy: long-term outcome. *Clin Nephrol*, 39, 175–82.

Peña, A. Débora, M. J., Melgosa, M., *et al.* (2001). Membranous nephropathy associated with hepatitis B in Spanish children. *Clin Nephrol*, 55, 25–30.

Ponticelli, C. and Moroni, G. (1998). Renal biopsy in lupus nephritis—what for, when and how often? *Nephrol Dial Transplant*, 13, 2452–4.

Radhakrishnan, J., Kunis, C. L., D'Agati, V., *et al.* (1994). Cyclosporine treatment of lupus membranous nephropathy. *Clin Nephrol*, 42, 147–54.

Radhakrishnan, J., Moutzouris, D. A., Ginzler, E. M., *et al.* (2010). Mycophenolate mofetil and intravenous cyclophosphamide are similar as induction therapy for class V lupus nephritis. *Kidney Int*, 77, 152–60.

Robinson, W. L., Mitas, J. A. 2nd, Haerr, R. W., *et al.* (1984). Remission and exacerbation of tumor-related nephrotic syndrome with treatment of the neoplasm. *Cancer*, 54, 1082–4.

Row, P. G., Cameron, J. S., Turner, D. R., *et al.* (1975). Membranous nephropathy. Long-term follow-up and association with neoplasia. *QJM*, 44, 207–39.

Sloan, R. P., Schwartz, M. M., Korbet, S. M., *et al.* (1996). Long-term outcome in systemic lupus erythematosus membranous glomerulonephritis. Lupus Nephritis Collaborative Study Group. *Am Soc Nephrol*, 7, 299–305.

Tang, S., Lai, F. M., Lui, Y. H., *et al.* (2005). Lamivudine in hepatitis B-associated membranous nephropathy. *Kidney Int*, 68(4), 1750–8.

Tse, K. C., Lam, M. F., Tang, S. C., *et al.* (2007). A pilot study on tacrolimus treatment in membranous or quiescent lupus nephritis with proteinuria resistant to angiotensin inhibition or blockade. *Lupus*, 16, 46–51.

Uchino, A., Tsukamoto, H., Nakashima, H., *et al.* (2010). Tacrolimus is effective for lupus nephritis patients with persistent proteinuria. *Clin Exp Rheumatol*, 28, 6–12.

Xu, H., Sun, L., Zhou, L. J., *et al.* (2003). The effect of hepatitis B vaccination on the incidence of childhood HBV-associated nephritis. *Pediatr Nephrol*, 18, 1216–9.

Yamauchi, H., Linsey, M. S., Biava, C. G., *et al.* (1985). Cure of membranous nephropathy after resection of carcinoma. *Arch Intern Med*, 145, 2061–3.

CHAPTER 64

Membranous glomerulonephritis: pathogenesis

Daniel C. Cattran and Heather N. Reich

Heymann nephritis shows that membranous glomerulonephritis is an autoantibody disease

The Heymann model of experimental membranous nephropathy in rats bears many similarities to both the clinical and pathologic findings observed in human membranous glomerulonephritis (MGN). First described over 40 years ago, the immunization of susceptible strains of rats with Freund's adjuvant and 'rat kidney suspension' consisting of material extracted from the brush border of proximal convoluted tubule cells, produces proteinuria and subepithelial deposits identical to that seen in human MGN (Heymann et al., 1959). Much of the focus of research in the Heymann nephritis model has been related to the identification of the responsible antigen(s) and the subsequent immune response (Shankland, 2000). The deposition of immune complexes results in the activation of many mediators of injury, including leucocytes, complement, products of arachidonic acid metabolism, a variety of cytokines, adhesion molecules, and growth factors.

The initial subject of intense investigation involving the Heymann nephritis model was the antigenic target(s) of the immune response stimulated by injection of the nephritogenic kidney membrane extract known as Fx1A. The target has been identified as a large membrane glycoprotein, gp330, which is also known as megalin due to its large size of 515 kD (Kerjaschki and Farquhar, 1982; Saito et al., 1994). The protein is a polyspecific receptor, and is a member of the low-density lipoprotein (LDL) receptor family (Herz et al., 1988; Raychowdhury et al., 1989, Kerjaschki and Neale, 1996). It complexes with a specific epitope of the 44 kD protein known as receptor associated protein, and can be found expressed in the clathrin-coated pits on the bases of podocyte foot processes (Kerjaschki and Farquhar, 1982; Pietromonaco et al., 1990; Kerjaschki et al., 1992; Orlando et al., 1992; Farquhar, 1996). The anatomic location of megalin supports its role in receptor-mediated endocytosis. The receptor-associated protein likely functions as a chaperone assisting in the folding of megalin in the endoplasmic reticulum of the cell, and facilitating its transport to the cell surface.

Circulating antibodies directed against megalin are hypothesized to penetrate the glomerular basement membrane and bind to megalin/RAP forming immune complexes *in situ*. Megalin was confirmed to be the putative antigen in the Heymann model based on the following criteria: (a) active immunization of rats with megalin produces subepithelial immune deposits (active Heymann nephritis), (b) injection of autologous anti-megalin antibodies produces similar deposits (passive Heymann nephritis), and (c) antibodies eluted from affected rat glomeruli recognize only megalin (Kerjaschki and Farquhar, 1982; Farquhar et al., 1995). Furthermore, a binding site for immunoglobulin G has been localized to the LDL-receptor-like domain of megalin, and Heymann nephritis has been reproduced using recombinant segments of this domain (Saito et al., 1996; Raychowdhury et al., 1996).

As megalin is not expressed in human podocytes, and could not be found in human immune deposits, the hunt was on for a comparable target in human disease.

Human antigens

Neutral endopeptidase: rare

One of the first potential human antigens was identified in a case of antenatal membranous glomerulonephritis, which developed following pregnancy-induced immunization of a neutral endopeptidase-deficient mother and subsequent transplacental passage of antibodies directed against fetal neutral endopeptidase, a protein expressed on podocytes (Debiec et al., 2002). While further cases of the antenatal form of the disease were described in families with maternal mutations in the neutral endopeptidase gene, this antigen was not found to be responsible for the development of sporadic idiopathic MGN (Debiec et al., 2004).

M-type PLA2 receptor: common

A breakthrough in the identification of a putative antigen came only recently, as a result of a painstaking graded sieving of protein extracts from pooled human glomeruli from healthy deceased subjects, and use of immunoglobulin (Ig)G-depleted protein fractions in a modified Western blotting protocol (Beck et al., 2009). The serum of a majority (70%) of patients with idiopathic MGN was found to display a 185 kD band detected by Western blotting with a fraction of the glomerular extract, under non-reducing conditions. The glomerular antigen in this band was identified as the M-type phospholipase A2 receptor protein. Antibodies directed against the phospholipase A2 receptor (PLA2R) (predominantly of the IgG4 subtype), were confirmed to be present in the serum of these affected individuals, and while not present in all subjects with idiopathic MGN, were notably absent in subjects with MGN associated with lupus or hepatitis. The PLA2R was also eluted from kidney biopsy immune deposits of patients with idiopathic MGN (Debiec and Ronco, 2011).

A genome-wide association study linked not only a class II antigen (human leucocyte antigen (HLA)-DQ1), a type of association expected in almost any autoimmune disease, but also the *PLA2R1* gene itself with susceptibility to idiopathic MGN (Stanescu et al., 2011). The mechanism of this association is not yet known.

The PLA2R protein is a type 1 transmembrane receptor expressed by human podocytes. Analysis of immune deposits suggests that complement activation is induced despite the fact that the predominant immunoglobulin present in these complexes, IgG4, is not classically thought to activate complement. Further work is required to delineate the mechanism by which anti-PLA2R antibodies may be pathogenic.

Cumulative data suggest that anti-PLA2R antibodies are detectable in the serum of up to 80% of subjects with idiopathic disease when the Western blot technique is applied (Beck et al., 2009; Hofstra et al., 2011; Qin et al., 2011). While assay specificity may account, in part, for variable detection of antibodies, the absence of circulating antibodies does not preclude the presence of anti-PLA2R antigen-antibody complexes in the kidney (Debiec and Ronco, 2011). The level of circulating antibody may be undetectable by the time a patient is referred for kidney biopsy, potentially reflecting clearance of the antibody following resolution of the precipitating immunologic event. A rise in antibody levels may precede the development of proteinuria and conversely, proteinuria may persist following clearing of serum antibodies, given the time required for immune complex reabsorption and basement membrane turn-over (Beck and Salant, 2010) (Fig. 64.1). While further work is required to clarify the relationship between serum antibody detection, proteinuria, and disease activity, multiple studies have suggested correlations (e.g. Beck et al., 2011) and it seems certain that tests for PLA2R antibodies will enter clinical practice soon.

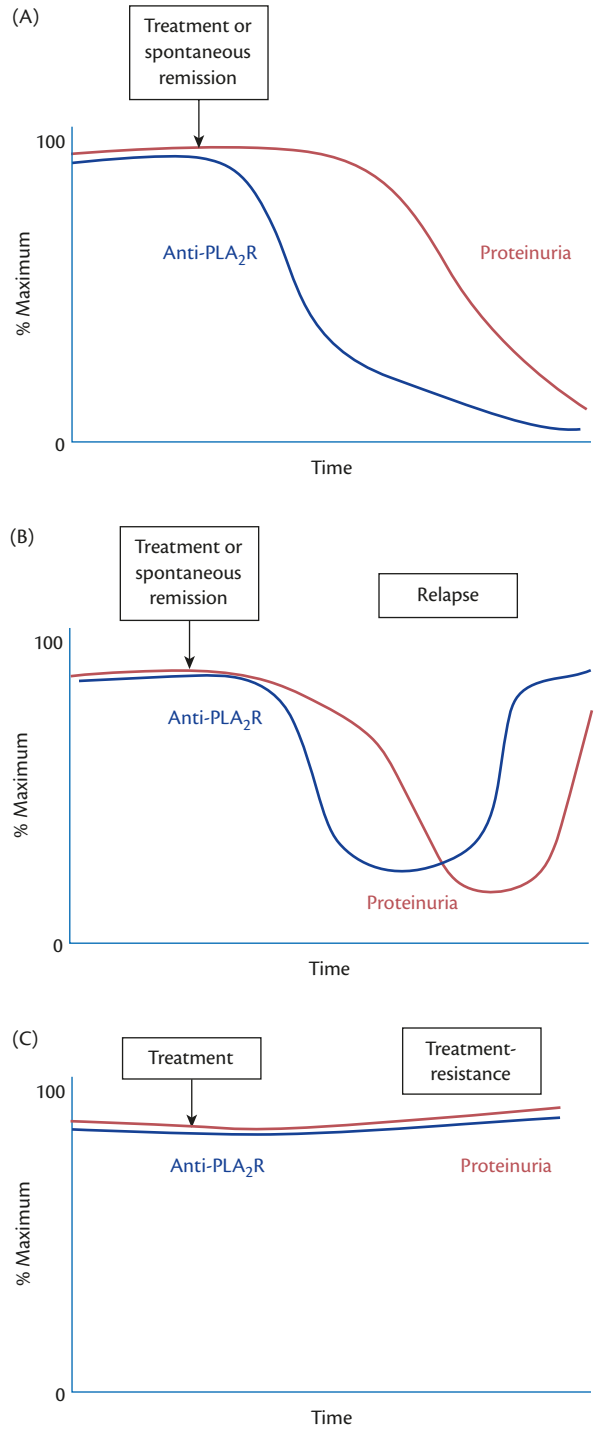

Fig. 64.1 Hypothetical situations relating serum PLA2R antibodies to disease status in idiopathic MGN. (A) In panel A, representing remitting disease, a reduction in PLA2R antibody titre precedes the reduction in proteinuria; this may represent a resolution in immunologic disease activity preceding basement membrane regeneration and healing.
(B) In panel B, representing relapsing disease, the rise in serum PLA2R antibody titre precedes and heralds clinical relapse of proteinuria.
(C) In panel C, representing resistant disease, neither PLA2R antibody titre nor proteinuria demonstrate any change over time or in response to therapy.

Mechanisms of injury

Cytotoxicity

Data derived from the Heymann model suggest that complement is required for the development of tissue injury and proteinuria, as illustrated by the fact that anti-Fx1A antibodies and immune complex deposition in C6- or C8-deficient rats do not result in proteinuria (Salant et al., 1980; Cybulsky et al., 1986; Baker et al., 1989). After the deposition of the antigen–antibody complex in the subepithelial space, the complement system is activated, leading to insertion of the C5b-9 membrane attack complex (MAC) into the podocyte plasma membrane (Couser et al., 1992). The MAC is formed after proteolytic cleavage of C5 and combination with components C6-C9. Its insertion into the cell membranes of nucleated cells results in sublytic activity (Koski et al., 1983), and in combination with other stimuli, is capable of causing cell death. The MAC has been localized to the subepithelial deposits and along the surface of podocytes, particularly in the clathrin-coated pits of glomerular epithelial cells in passive Heymann nephritis. It is likely endocytosed and transported intracellularly in multivesicular bodies, and then exocytosed into the urinary space (Kerjaschki et al., 1987). Podocyte injury and resulting proteinuria has been shown to be dependent upon the presence of the MAC, as depletion of complement with cobra venom factor is associated with lack of proteinuria, despite the formation of immune deposits. The MAC has been documented in the urine of Heymann's nephritis

animals, and may be a marker of disease activity (Schulze et al., 1989; Pruchno et al., 1989).

Although it appears that epithelial cell injury is dependent upon the presence of the MAC, the mechanisms by which the MAC causes injury and the podocyte's maladaptive response to this injury remain areas of ongoing active investigation. Some of the proposed pathways of injury induced by the MAC include those mediated by the generation of reactive oxygen species (ROS), podocyte cytoskeleton modifications, and podocyte apoptosis.

Injury to both the podocyte and the basement membrane is hypothesized to be mediated in part by local generation of reactive oxygen species (ROS) (Adler et al., 1986; Peng et al., 2002). The ROS may in fact be produced locally, as is suggested by *in vitro* experiments in which the application of C5b-9 to cultured epithelial cells results in the production of ROS (Adler et al., 1986). Subsequent injury to the filtration barrier may then be induced by peroxidation of membrane proteins and collagen (Kerjaschki and Neale, 1996). The MAC has also been documented to activate epithelial cell cytosolic phospholipase A2 *in vivo* and *in vitro*, resulting in the production of free arachidonic acid by post-translational regulation of the enzyme (Cybulsky et al., 1995, 2000). Arachidonic acids are known to be precursors of eicosanoids (e.g. prostaglandins, leukotrienes, and thromboxanes), that have potential haemodynamic effects within the glomerulus, and are present in increased levels in the Heymann model. The presence of ROS in tissue has been correlated with the development of proteinuria, and the administration of ROS scavengers (antioxidants) has been documented to cause a decrease in proteinuria (Shah, 1988, 1989). The ROS generated by the MAC are also postulated to mediate activation of a variety of stress-associated pathways, including protein kinase C and the extracellular-signal-related kinase ERK2 (Cybulsky et al., 1998).

Structural effects

Observations from the Heymann model suggest a link between structural changes in the podocyte and interconnecting slit diaphragms with the immune complex formation, permitting the passage of protein through the filtration barrier. Microscopically, foot process effacement is readily appreciable in MGN. This may be related to disruption of the actin cytoskeleton that has been documented to be mediated by MAC exposure (Topham et al., 1999). It has been demonstrated that nephrin dissociates from the actin cytoskeleton in experimental membranous nephropathy, resulting in prominent changes in the morphology of the slit diaphragm (Yuan et al., 2002). Slit diaphragms have also been observed to undergo morphologic and phenotypic changes, confirmed by documentation in human samples of decreased mRNA levels of nephrin, the primary component of the diaphragm (Furness et al., 1999). The C5b-9-mediated glomerular epithelial cell structural and slit diaphragm changes observed in the Heymann model may be modulated in part by cytochrome P450 2B1(CYP2B1) (Liu et al., 2010); cytoskeletal integrity appears to be maintained in cells deficient in this cytochrome protein, and inhibition of CYP2B1 moderates injury in the passive Heymann model.

Podocyte injury and death

Podocyte apoptosis is thought to be mediated both directly and indirectly by C5b-9. The induction of MGN is associated with an overall decrease in podocyte number, and this has been attributed to MAC-induced podocyte apoptosis (Shankland et al., 1997) and podocyte detachment (Petermann et al., 2003). Indirectly the MAC may also potentiate angiotensin II and transforming growth factor beta (TGF-β)-mediated podocyte apoptosis (Ding et al., 2002; Zoja et al., 2003).

The podocyte reaction to injury induced by the MAC is one of hypertrophy, rather than proliferation. It is postulated that interruption of cell cycle regulation may cause the lack of proliferation of podocytes (Shankland et al., 1997, 1999). In addition, an increase in extracellular matrix is observed, and can be appreciated at a microscopic level as thickening of the basement membrane. The accumulation of extracellular matrix may be mediated to a large extent by TGF-β, as is suggested by experiments that indicate that only certain TGF-β/receptor isoforms lead to matrix expansion in the Heymann model (Shankland et al., 1996). The sublytic C5b-9 exposure may also contribute to podocyte elaboration of type I and type IV collagen (Kim et al., 1991; Minto et al., 1993).

Human studies

In human MGN studies, the presence of C5b-9 has been documented within immune complex deposits (Hinglais et al., 1986). In one biopsy study, MAC was found in 50% of biopsies of idiopathic MGN, 75% of MGN secondary to lupus, and a minority of MGN due to hepatitis B (Lai et al., 1989). The presence of MAC on biopsy in one recent study was not, however, found to be correlated with the severity of proteinuria or the presence of the nephrotic syndrome; it was suggested that rather than MAC deposition, glomerular expression of various adhesion molecules may be more reflective of advanced disease (Honkanen et al., 1998; Papagianni et al., 2002). The MAC has also been identified in the urine of human subjects with MGN. The clinical course of the disease has been shown to have some association with the presence of urinary C5b-9 in human studies; two studies have demonstrated that the presence of MAC is correlated with an 'active' clinical course with ongoing proteinuria, and declining function (Coupes et al., 1992, 1993; Kon et al., 1995). One study, however, correlated its presence with a shorter duration of disease and lower creatinine, but more proteinuria (Schulze et al., 1991). The differences may relate to small sample sizes, cross-sectional design, and differing statistical methods used to prove the association.

Other mechanisms of injury

Although much of the focus thus far has been on complement-mediated injury, cellular and humoral-mediated pathways are also thought to be important factors in the ultimate development of proteinuria, at least in the experimental model. A role for cell-mediated injury is supported by the observations that depletion of the cytotoxic CD8+ T-cell reduces the injury and that monoclonal anti-CD4 and anti-CD8 treatment modifies the disease (Penny et al., 1998). However, increased protein permeability was demonstrated in isolated perfused rat glomeruli exposed to antibodies derived from rats with Heymann nephritis, in the complete absence of infiltrating leucocytes (Neale et al., 1982), supporting the notion that the importance of leucocytes in mediating glomerular permeability may differ according to the stage of disease pathogenesis (reviewed by Glassock, 2010).

In addition to injury induced by MAC, pathways of tubulointerstitial injury and subsequent loss of kidney function are likely shared with many forms of progressive proteinuric kidney disease. These may include the loss of microvascular endothelium, and

proteinuria-induced upregulation of renal cytokines and growth factors that promote tubulointerstitial inflammation and fibrosis (Remuzzi and Bertani, 1998; Remuzzi et al., 1994).

Genetics

Both susceptibility to and progression of MGN may have a genetic basis (Vaughan et al., 1995; Kleta, 2008). There is evidence that HLA type may underlie susceptibility to this condition, and potentially account for rare familial variants of the disease (Muller et al., 2008; Stanescu et al., 2011). The HLA type may also be associated with tendency towards progressive disease epidemiology (Abe et al., 1986; Kida et al., 1986; Papiha et al., 1987; Freedman et al., 1994). Advances in laboratory technology that allow a broader look at whole genomes and changes in gene and protein expression may help to elucidate additional pathways responsible for the injury that produces MGN. In addition, further investigation into the factors responsible for an individual's susceptibility to either progression or spontaneous remission in this type of immunologic injury is required.

References

Abe, S., Amagasaki, Y., Konishi, K., *et al.* (1986). Idiopathic membranous glomerulonephritis: aspects of geographical differences. *J Clin Pathol*, 39, 1193–8.

Adler, S., Baker, P. J., Johnson, R. J., *et al.* (1986). Complement membrane attack complex stimulates production of reactive oxygen metabolites by cultured rat mesangial cells. *J Clin Invest*, 77, 762–7.

Baker, P. J., Ochi, R. F., Schulze, M., *et al.* (1989). Depletion of C6 prevents development of proteinuria in experimental membranous nephropathy in rats. *Am J Pathol*, 135, 185–94.

Beck, L. H., Jr., Bonegio, R. G., Lambeau, G., *et al.* (2009). M-type phospholipase A2 receptor as target antigen in idiopathic membranous nephropathy. *N Engl J Med*, 361, 11–21.

Beck, L. H., Jr. and Salant, D. J. (2010). Membranous nephropathy: recent travels and new roads ahead. *Kidney Int*, 77, 765–70.

Coupes, B., Brenchley, P. E., Short, C. D., *et al.* (1992). Clinical aspects of C3dg and C5b-9 in human membranous nephropathy. *Nephrol Dial Transplant*, 7 (Suppl 1), 32–4.

Coupes, B. M., Kon, S. P., Brenchley, P. E., *et al.* (1993). The temporal relationship between urinary C5b-9 and C3dg and clinical parameters in human membranous nephropathy. *Nephrol Dial Transplant*, 8, 397–401.

Couser, W. G., Schulze, M., and Pruchno, C. J. (1992). Role of C5b-9 in experimental membranous nephropathy. *Nephrol Dial Transplant*, 7 Suppl 1, 25–31.

Cybulsky, A. V., Monge, J. C., Papillon, J., *et al.* (1995). Complement C5b-9 activates cytosolic phospholipase A2 in glomerular epithelial cells. *Am J Physiol*, 269, F739–49.

Cybulsky, A. V., Papillon, J., and McTavish, A.J. (1998). Complement activates phospholipases and protein kinases in glomerular epithelial cells. *Kidney Int*, 54, 360–72.

Cybulsky, A. V., Rennke, H. G., Feintzeig, I. D., *et al.* (1986). Complement-induced glomerular epithelial cell injury. Role of the membrane attack complex in rat membranous nephropathy. *J Clin Invest*, 77, 1096–107.

Cybulsky, A. V., Takano, T., Papillon, J., *et al.* (2000). Complement-induced phospholipase A2 activation in experimental membranous nephropathy. *Kidney Int*, 57, 1052–62.

Debiec, H., Guigonis, V., Mougenot, B., *et al.* (2002). Antenatal membranous glomerulonephritis due to anti-neutral endopeptidase antibodies. *N Engl J Med*, 346, 2053–60.

Debiec, H., Nauta, J., Coulet, F., *et al.* (2004). Role of truncating mutations in MME gene in fetomaternal alloimmunisation and antenatal glomerulopathies. *Lancet*, 364, 1252–9.

Debiec, H. and Ronco, P. (2011). PLA2R autoantibodies and PLA2R glomerular deposits in membranous nephropathy. *N Engl J Med*, 364, 689–90.

Ding, G., Reddy, K., Kapasi, A. A., *et al.* (2002). Angiotensin II induces apoptosis in rat glomerular epithelial cells. *Am J Physiol Renal Physiol*, 283, F173–80.

Farquhar, M. G., Saito, A., Kerjaschki, D., *et al.* (1995). The Heymann nephritis antigenic complex: megalin (gp330) and RAP. *Am Soc Nephrol*, 6, 35–47.

Freedman, B. I., Spray, B. J., Dunston, G. M., *et al.* (1994). HLA associations in end-stage renal disease due to membranous glomerulonephritis: HLA-DR3 associations with progressive renal injury. Southeastern Organ Procurement Foundation. *Am J Kidney Dis*, 23, 797–802.

Furness, P. N., Hall, L. L., Shaw, J. A., *et al.* (1999). Glomerular expression of nephrin is decreased in acquired human nephrotic syndrome. *Nephrol Dial Transplant*, 14, 1234–7.

Glassock, R. J. (2010). The pathogenesis of idiopathic membranous nephropathy: a 50-year odyssey. *Am J Kidney Dis*, 56, 157–67.

Herz, J., Hamann, U., Rogne, S., *et al.* (1988). Surface location and high affinity for calcium of a 500-kd liver membrane protein closely related to the LDL-receptor suggest a physiological role as lipoprotein receptor. *EMBO J*, 7, 4119–27.

Heymann, W., Hackel, D. B., Harwood, S., *et al.* (1959). Production of nephrotic syndrome in rats by Freund's adjuvants and rat kidney suspensions. 1951. *Proc Soc Exp Biol Med*, 100, 600–7.

Hinglais, N., Kazatchkine, M. D., Bhakdi, S., *et al.* (1986). Immunohistochemical study of the C5b-9 complex of complement in human kidneys. *Kidney Int*, 30, 399–410.

Hofstra, J. M., Beck, L. H. Jr, Beck, D. M., *et al.* (2011). Anti-phospholipase A(2) receptor antibodies correlate with clinical status in idiopathic membranous nephropathy. *Clin J Am Soc Nephrol*, 6, 1286–91.

Honkanen, E., von Willebrand, E., Teppo, A. M., *et al.* (1998). Adhesion molecules and urinary tumor necrosis factor-alpha in idiopathic membranous glomerulonephritis. *Kidney Int*, 53, 909–17.

Kerjaschki, D. and Farquhar, M. G. (1982). The pathogenic antigen of Heymann nephritis is a membrane glycoprotein of the renal proximal tubule brush border. *Proc Natl Acad Sci U S A*, 79, 5557–81.

Kerjaschki, D. and Neale, T.J. (1996). Molecular mechanisms of glomerular injury in rat experimental membranous nephropathy (Heymann nephritis). *Am Soc Nephrol*, 7, 2518–26.

Kerjaschki, D., Miettinen, A., and Farquhar, M.G. (1987). Initial events in the formation of immune deposits in passive Heymann nephritis. gp330-anti-gp330 immune complexes form in epithelial coated pits and rapidly become attached to the glomerular basement membrane. *J Exp Med*, 166, 109–28.

Kerjaschki, D., Ullrich, R., Diem, K., *et al.* (1992). Identification of a pathogenic epitope involved in initiation of Heymann nephritis. *Proc Natl Acad Sci U S A*, 89, 11179–83.

Kida, H., Asamoto, T., Yokoyama, H., *et al.* (1986). Long-term prognosis of membranous nephropathy. *Clin Nephrol*, 25, 64–9.

Kim, Y., Butkowski, R., Burke, B., *et al.* (1991). Differential expression of basement membrane collagen in membranous nephropathy. *Am J Pathol*, 139, 1381–8.

Kleta, R. (2008). Fanconi or not Fanconi? Lowe syndrome revisited. *Clin J Am Soc Nephrol*, 3, 1244–5.

Kon, S. P., Coupes, B., Short, C. D., *et al.* (1995). Urinary C5b-9 excretion and clinical course in idiopathic human membranous nephropathy. *Kidney Int*, 48, 1953–8.

Koski, C. L., Ramm, L. E., Hammer, C. H., *et al.* (1983). Cytolysis of nucleated cells by complement: cell death displays multi- hit characteristics. *Proc Natl Acad Sci U S A*, 80, 3816–20.

Lai, K. N., Lo, S.T., Lai, F.M. (1989). Immunohistochemical study of the membrane attack complex of complement and S-protein in idiopathic and secondary membranous nephropathy. *Am J Pathol*, 135, 469–76.

Liu, H., Tian, N., Arany, I., *et al.* (2010). Cytochrome P450 2B1 mediates complement-dependent sublytic injury in a model of membranous nephropathy. *J Biol Chem*, 285, 40901–10.

Minto, A. W., Fogel, M. A., Natori, Y., *et al.* (1993). Expression of type I collagen mRNA in glomeruli of rats with passive Heymann nephritis. *Kidney Int*, 43, 121–7.

Muller, C., Alenabi, F., Chantrel, F., *et al.* (2008). Familial membranous glomerulopathy, toxic exposure and/or genetic sensibility? *Clin Nephrol*, 70, 422–3.

Neale, T. J., Couser, W. G., Salant, D. J., *et al.* (1982). Specific uptake of Heymann's nephritic kidney eluate by rat kidney: studies in vivo and in isolated perfused kidneys. *Lab Invest*, 46, 450–3.

Orlando, R. A., Kerjaschki, D., Kurihara, H., *et al.* (1992). gp330 associates with a 44-kDa protein in the rat kidney to form the Heymann nephritis antigenic complex. *Proc Natl Acad Sci U S A*, 89, 6698–702.

Papagianni, A. A., Alexopoulos, E., Leontsini, M., *et al.* (2002). C5b-9 and adhesion molecules in human idiopathic membranous nephropathy. *Nephrol Dial Transplant*, 17, 57–63.

Papiha, S. S., Pareek, S. K., Rodger, R. S., *et al.* (1987). HLA-A, B, DR and Bf allotypes in patients with idiopathic membranous nephropathy (IMN). *Kidney Int*, 31, 130–4.

Peng, H., Takano, T., Papillon, J., *et al.* (2002). Complement activates the c-Jun N-terminal kinase/stress-activated protein kinase in glomerular epithelial cells. *J Immunol*, 169, 2594–601.

Penny, M. J., Boyd, R.A., and Hall, B.M. (1998). Mycophenolate mofetil prevents the induction of active Heymann nephritis: association with Th2 cytokine inhibition. *Am Soc Nephrol*, 9, 2272–82.

Petermann, A. T., Krofft, R., Blonski, M., *et al.* (2003). Podocytes that detach in experimental membranous nephropathy are viable. *Kidney Int*, 64, 1222–31.

Pietromonaco, S., Kerjaschki, D., Binder, S., *et al.* (1990). Molecular cloning of a cDNA encoding a major pathogenic domain of the Heymann nephritis antigen gp330. *Proc Natl Acad Sci U S A*, 87, 1811–15.

Pruchno, C. J., Burns, M. W., Schulze, M., *et al.* (1989). Urinary excretion of C5b-9 reflects disease activity in passive Heymann nephritis. *Kidney Int*, 36, 65–71.

Qin, W., Beck, L. H. Jr., Zeng, C., *et al.* (2011). Anti-phospholipase A2 receptor antibody in membranous nephropathy. *Am Soc Nephrol*, 22, 1137–43.

Raychowdhury, R., Niles, J. L., McCluskey, R. T., *et al.* (1989). Autoimmune target in Heymann nephritis is a glycoprotein with homology to the LDL receptor. *Science*, 244, 1163–5.

Raychowdhury, R., Zheng, G., Brown, D., *et al.* (1996). Induction of Heymann nephritis with a gp330/megalin fusion protein. *Am J Pathol*, 148, 1613–23.

Remuzzi, G., Schieppati, A., Garattini, S. (1994). Treatment of idiopathic membranous glomerulopathy. *Curr Opin Nephrol Hypertens*, 3, 155–63.

Remuzzi, G. and Bertani, T. (1998). Pathophysiology of progressive nephropathies. *N Engl J Med* 339, 1448–56.

Saito, A., Pietromonaco, S., Loo, A. K., *et al.* (1994). Complete cloning and sequencing of rat gp330/ 'megalin,' a distinctive member of the low density lipoprotein receptor gene family. *Proc Natl Acad Sci U S A*, 91, 9725–9.

Saito, A., Yamazaki, H., Rader, K., *et al.* (1996). Mapping rat megalin: the second cluster of ligand binding repeats contains a 46-amino acid pathogenic epitope involved in the formation of immune deposits in Heymann nephritis. *Proc Natl Acad Sci U S A*, 93, 8601–5.

Salant, D. J., Belok, S., Madaio, M. P., *et al.* (1980). A new role for complement in experimental membranous nephropathy in rats. *J Clin Invest*, 66, 1339–50.

Schulze, M., Baker, P. J., Perkinson, D. T., *et al.* (1989). Increased urinary excretion of C5b-9 distinguishes passive Heymann nephritis in the rat. *Kidney Int*, 35, 60–8.

Schulze, M., Donadio, J. V. Jr., Pruchno, C. J., *et al.* (1991). Elevated urinary excretion of the C5b-9 complex in membranous nephropathy. *Kidney Int*, 40, 533–8.

Shah, S. V. (1988). Evidence suggesting a role for hydroxyl radical in passive Heymann nephritis in rats. *Am J Physiol*, 254, F337–44.

Shah, S. V. (1989). Role of reactive oxygen metabolites in experimental glomerular disease. *Kidney Int*, 35, 1093–106.

Shankland, S. J. (2000). New insights into the pathogenesis of membranous nephropathy. *Kidney Int*, 57, 1204–5.

Shankland, S. J., Floege, J., Thomas, S. E., *et al.* (1997). Cyclin kinase inhibitors are increased during experimental membranous nephropathy: potential role in limiting glomerular epithelial cell proliferation in vivo. *Kidney Int*, 52, 404–13.

Shankland, S. J., Pippin, J.W., Couser, W.G. (1999). Complement (C5b-9) induces glomerular epithelial cell DNA synthesis but not proliferation in vitro. *Kidney Int*, 56, 538–48.

Shankland, S. J., Pippin, J., Pichler, R. H., *et al.* (1996). Differential expression of transforming growth factor-beta isoforms and receptors in experimental membranous nephropathy. *Kidney Int*, 50, 116–24.

Stanescu, H. C., Arcos-Burgos, M., Medlar, A., *et al.* (2011). Risk HLA-DQA1 and PLA(2)R1 alleles in idiopathic membranous nephropathy. *N Engl J Med*, 364, 616–26.

Topham, P. S., Haydar, S. A., Kuphal, R., *et al.* (1999). Complement-mediated injury reversibly disrupts glomerular epithelial cell actin microfilaments and focal adhesions. *Kidney Int*, 55, 1763–75.

Vaughan, R. W., Tighe, M. R., Boki, K., *et al.* (1995). An analysis of HLA class II gene polymorphism in British and Greek idiopathic membranous nephropathy patients. *Eur J Immunogenet*, 22, 179–86.

Yuan, H., Takeuchi, E., Taylor, G. A., *et al.* (2002). Nephrin dissociates from actin, and its expression is reduced in early experimental membranous nephropathy. *Am Soc Nephrol*, 13, 946–56.

Zoja, C., Morigi, M., and Remuzzi, G. (2003). Proteinuria and phenotypic change of proximal tubular cells. *Am Soc Nephrol*, 14 Suppl 1, S36–41.

CHAPTER 65

Immunoglobulin A nephropathy: overview

Kar Neng Lai and Sydney C. W. Tang

Immunoglobulin A (IgA) nephropathy is the most common primary glomerulonephritis. It runs a slow and sometimes relentless clinical course (see Chapter 66) with consequent end-stage renal failure in 35–40% of patients 25–30 years after first clinical presentation (see Chapter 68). The pathology is characterized by deposition of macromolecular (polymeric) IgA1 in the glomerular mesangium, proliferation of mesangial cells, increased synthesis of extracellular matrix, and infiltration of macrophages, monocytes, and T cells (Fig. 65.1) (see Chapter 67). The severity of glomerular and tubulointerstitial damage in IgA nephropathy correlates with the rate of renal function decline and long-term renal outcome. However, IgA deposition is a common incidental finding at autopsy and in some patients is associated with minimal or no overt renal disease.

The kidney is believed to be an innocent bystander in IgA nephropathy (see Chapter 69). The primary defect appears to be aberrant glycosylation of *O*-linked glycans in the hinge region of a fraction of IgA1 molecules. Rather than terminating with galactose, the aberrant galactose-deficient *O*-glycans end with *N*-acetylgalactosamine or sialylated acetylgalactosamine. The absence of galactose in *O*-glycans reduces their uptake by the liver and reticuloendothelial system by asialoglycoprotein receptor. The terminal *N*-acetylgalactosamine moiety on the aberrantly glycosylated IgA1 may in turn be recognized by antiglycan antibodies. The aberrant IgA1 or nephritogenic immune complexes may induce kidney injury. The aberrant underglycosylation of macromolecular IgA1 explains the recurrence of IgA nephropathy in transplanted kidney. Serum galactose-deficient IgA1 levels appear to be heritable in a dominant pattern in IgA nephropathy, although most relatives with high levels have no clinical manifestation of renal injury.

Familial IgA nephropathy is uncommon but may be under-recognized. It may have a poorer prognosis. This supports that genetic factors are involved in the pathogenesis of IgA nephropathy and specific candidate genes have been detected in selected ethnic groups.

Blockade of the renin–angiotensin system and blood pressure control remain the mainstay of treatment (see Chapter 68). Courses (months) of high-dose corticosteroids have antiproteinuric effects and seem to preserve glomerular filtration rate in selected patients. The efficacy of other immunosuppressive agents remains debatable.

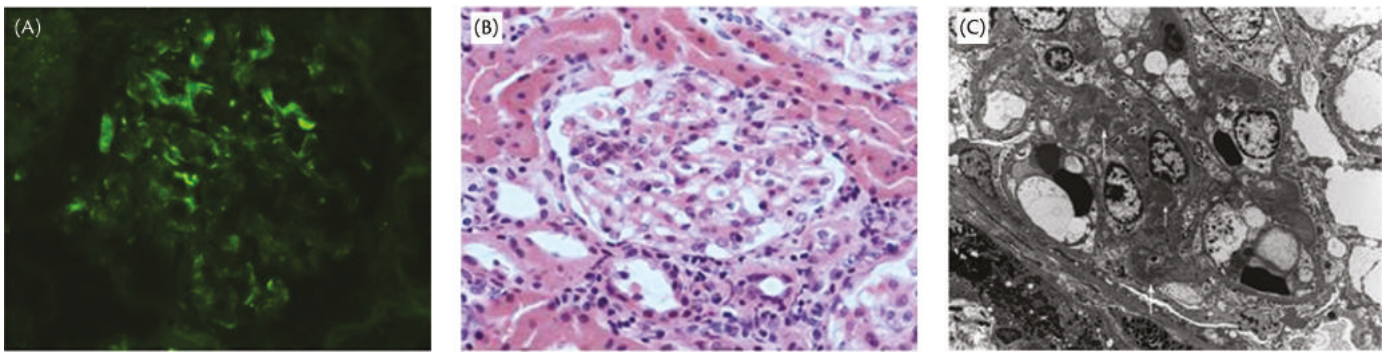

Fig. 65.1 (A) Immunofluorescent staining for IgA deposits in the mesangium (×500).
(B) Moderate mesangial matrix expansion with increased cell number (H&E ×400).
(C) Electron micrograph showing mesangial expansion with electron-dense deposits (arrows) (×9600).

CHAPTER 66

Immunoglobulin A nephropathy: clinical features

Kar Neng Lai and Sydney C. W. Tang

Introduction

Primary IgA nephropathy is the commonest form of idiopathic (primary) glomerulonephritis in the developed world and it is an important cause of end-stage kidney failure. In 1967, Drs Jean Berger and Nicole Hinglais at the Paris Necker Hospital first described a new glomerulopathic entity that they subsequently called mesangial IgA/IgG deposition with IgA predominance following the application of new technique of immunofluorescence staining (Berger and Hinglais, 1968). Levy et al. (1972) first used in print the term 'Berger's disease'. By 1975, Berger disease became an established glomerular entity: a condition with moderate proliferative glomerular changes, usually mesangial but often focal or segmental in distribution, associated with microscopic haematuria and about 15–20% with macroscopic haematuria.

IgA nephropathies are characterized by the presence of diffuse mesangial deposition of IgA in the glomeruli in selected pathological entities such as Berger disease, Henoch–Schönlein purpura, and systemic lupus erythematosus. Secondary IgA nephropathy may occur in several systemic diseases when associated with an abnormal response of the IgA immune system. Cirrhosis and heavy alcohol intake may induce nephritis with IgA deposits. The association between staphylococcal infection and IgA-predominant or co-dominant glomerulonephritis (see Chapter 78) was first reported in Japan, and subsequently in other regions. Distinction of this entity from primary IgA nephropathy is important to avoid immunosuppressive treatment.

Epidemiology

Geographical distribution

Mesangial IgA deposits are also present in 4–16% of normal, healthy adults, living and cadaveric donors. Thus, the biopsy-proven IgA nephropathy cases represent a very small fraction of the total individuals with disease in the population as a whole (Levy and Berger, 1988). The systematic screening of urinary abnormalities could have influenced the higher prevalence reported both in Japan and in Singapore and different clinical policies for diagnostic renal biopsy may account for lower detection of IgA nephropathy (Schena et al., 1990). However, the finding of familial aggregation of IgA nephropathy has hinted that genetic factors are important in the aetiology of IgA nephropathy.

The clinical onset of IgA nephropathy is usually in the second and third decade of life but may occur at any age. Males are affected from two- to sixfold more frequently than females in North America and Europe but from 1.5- to twofold in Asia. It is more frequent in white people and Asians than in African Americans, and rarely reported in black people of direct African descent. The difference is still unexplained.

Most of the worldwide studies report prevalence rates as a percentage of cases of primary glomerulonephritides or as a percentage of a total series of renal biopsies, while few epidemiologic studies focused on the real incidence of primary IgA nephropathy in various populations (Table 66.1).

Familial studies

The best evidence for a genetic effect comes from reports of familial aggregation of the disease, sometimes recognized when screening for prospective kidney donors (Lavigne et al., 2010). As a result of the requirement for renal biopsy for diagnosis, and the intermittence of urinary abnormalities, no systematic study has reported the prevalence of familial IgA nephropathy or sibling-recurrence risk. A good family history should document occurrence of kidney disease or unexplained haematuria in first-degree and second-degree relatives, any childhood deaths, age of onset of disease in all cases, gender distribution, ethnic origin, presence of consanguinity, and potential environmental exposures.

Familial aggregation of biopsy-confirmed IgA nephropathy was independently reported in two families in the late 1970s. Since then, this has been described with increasing frequency. Reports from the United Kingdom and Germany indicated that 4–10% of patients with IgA nephropathy had a family history of renal disease (Rambausek et al., 1987; Johnston et al., 1992). Schena et al. (1993) detected urinary abnormalities in 61 of 269 asymptomatic first-degree relatives from 48 families of IgA nephropathy patients. In another Italian survey lasting 25 years, IgA nephropathy was diagnosed in 185 patients; 26 of these (14%) were related to at least one other IgA nephropathy patient, forming ten multiplex pedigrees (Scolari et al., 1999). Levy (1989) discovered 40 families with two or three members who had biopsy-documented IgA nephropathy. The majority of families were of European origin, but there were also families from Asia and North America (Tam et al., 2009; Lavigne et al., 2010). These data indicate that familial IgA nephropathy is quite common, but probably underdiagnosed as urinalysis is not routinely performed and microhaematuria is intermittent. In addition to multiplex kindreds, epidemiologic investigations have identified an increased prevalence of IgA nephropathy in some isolated populations in geographically isolated areas. In such populations,

Table 66.1 Frequency of IgA nephropathy amongst renal biopsies of primary glomerulonephritis

	Frequency (%)	Number of renal biopsy
America		
Brazil (Bahiense-Oliveira et al., 2004)	21.4 (A)	943
Peru (Hurtado et al., 2000)	0.9 (A)	1263
USA (Swaminathan et al., 2006)	21.4 (A)	5586
Asia		
China (Lai et al., 2004)	45.3 (A)	13519
Hong Kong (Lai et al., 1994)	35.0 (A)	961
India (Chandrika, 2007)	14.3 (A)	1544
Japan (Research Group on Progressive Chronic Renal Disease, 1999)	47.4 (A) 22.1 (A)	1045 4514
Korea (Choi et al., 2001)	10.3 (P)	3555
Singapore (Sinniah, 1983)	34.0 (A)	
Thailand (Parichatikanond et al., 2006)	17.9 (A)	
Australia		
(Briganti et al., 2001)	34.1 (A)	2030
Europe		
Croatia (Batinic et al., 2007)	18.1 (A) 20.0 (P)	565
France (Simon et al., 2004)	29.7 (A)	600
Germany (Werner et al., 2009)	26 (A)	359
Italy (Schena et al., 1997)	21.5 (A) 18.8 (P)	32862
Netherlands (Tiebosch et al., 1987)	22.0 (A)	
Portugal (Carvalho et al., 2006)	31.2 (A)	
Romania (Covic et al., 2006)	28.9 (A)	635
Spain (Rivera et al., 2002)	17.2 (A) 19.5 (P)	7016
United Kingdom (Ballardie et al., 1987)	31.0 (A)	

A = adult, P = paediatric.

individuals with sporadic IgA nephropathy have been shown to share common ancestors as many as seven to eight generations (Julian et al., 1985; Izzi et al., 2006). No familial clustering in nearby villages with similar population histories and lifestyles strongly indicates an inherited rather than environmental mechanism.

There is little difference between familial and sporadic forms of IgA nephropathy with respect to clinical presentations. Additionally, histologic findings, frequency of the immunoglobulin isotype and presence of complement C3 in renal tissue do not differ between the two forms. Two studies reported a worse renal prognosis and a more severe histopathology for familial IgA nephropathy 20 years after apparent onset of the disease, resulting in 41% renal survival rate compared with 94% in patients with sporadic IgA nephropathy (Schena et al., 2002; Tam et al., 2010).

Abnormalities of IgA glycosylation have been reported in relatives of patients (see 'Aberrant structure of the IgA molecule' in Chapter 69). Families in which IgA nephropathy aggregate can be associated with other forms of glomerular disease including IgM nephropathy, thin basement membrane disease, and most importantly, Henoch–Schönlein purpura (Levy, 1989; Frasca et al., 2004).

Immunogenetic association

Most IgA nephropathies are 'sporadic' rather than familial. IgA nephropathy is generally considered to be a complex disorder, that is, it is a multifactorial disease with both genetic and environmental factors likely contributing in the majority. However, in some families the disease segregates in an obviously autosomal dominant fashion. It therefore seems likely that the genetic contribution to the disease is heterogeneous, and can lie anywhere in the spectrum from monogenic, through oligogenic to polygenic, differing in individual cases and families. Recent studies suggest genes responsible for sporadic and familial IgA nephropathy could well be different.

Genes responsible for, or predisposing to, IgA nephropathy

Familial IgA nephropathy and linked loci

2q36 locus

The most clear-cut report of familial IgA nephropathy and linkage is a study of a four- generation Canadian family of German-Austrian origin with 14 affected and 11 unaffected members (Paterson et al., 2007). The pedigree is consistent with autosomal dominant inheritance. Parametric and non-parametric linkage analysis produced significant logarithm of the ratio of odds (LOD) scores according to standard criteria for Mendelian disease.

6q22–q23 (IGAN1) and 3p24–p23 loci

These were the first loci identified in linkage analysis of IgA nephropathy with a LOD score of 5.6 to 6q22–q23, which was named *IGAN1* (Gharavi et al., 2000). The study analysed 24 Italian and six American families suggesting locus at 3p24–p23 with maximum LOD score of 2.8.

4q26–q31 (IGAN2) and 17q12–q22 (IGAN3) loci

The European IgAN consortium identified suggestive loci at 4q26–q31 (LOD score 1.8) and 17q12–q22 (LOD score 2.6), in 22 Italian families with 59 affected and 127 unaffected members. The loci were named *IGAN2* and *IGAN3* (Bisceglia et al., 2006).

These four loci have not been revealed in familial IgA nephropathy of other ethnicity (Karnib et al., 2007).

Genome-wide association study in sporadic IgA nephropathy

By genome-wide association study (GWAS), a strong signal of association on chromosome 6p in the region of the major histocompatibility complex ($P = 1 \times 10^{-9}$) was identified in a cohort of 533 European patients with IgA nephropathy recruited from the United Kingdom Glomerulonephritis DNA Bank (Feehally et al., 2010). Human leucocyte antigen (HLA) imputation analysis showed the strongest association signal from a combination of DQ loci with some support for an independent HLA-B signal. These results suggest that the HLA region contains the strongest common susceptibility alleles that predispose to IgA nephropathy in the European

population. Using similar approach in 1194 patients of Chinese Han ethnicity, Gharavi et al. (2011) identified three independent loci in the major histocompatibility complex, as well as a common deletion of *CFHR1* and *CFHR3* at chromosome 1q32 and a locus at chromosome 22q12 that each surpassed genome-wide significance (P values for association between 1.59×10^{-26} and 4.84×10^{-9} and minor allele odds ratios of 0.63–0.80). However, these five loci explain 4–7% of the disease variance and up to a tenfold variation in interindividual risk. IgA nephropathy risk allele frequencies closely parallel the variation in disease prevalence among Asian, European, and African populations, suggesting complex selective pressures. In a GWAS replication study and geospatial risk analysis (Kiryluk et al., 2012), a seven-single nucleotide polymorphism genetic risk score, which explained 4.7% of overall IgA nephropathy risk, increased sharply with Eastward and Northward distance from Africa ($r = 0.30$, $P = 3 \times 10^{-128}$). This model paralleled the known East–West gradient in disease burden. In another GWAS of 1434 Han Chinese, the genes encoding tumour necrosis factor (TNFSF13) and α-defensin (DEFA) were identified as susceptibility genes, and the association at 22q12 was confirmed (Yu et al., 2011).

Susceptibility or disease progression genes in non-familial IgA nephropathy

Numerous association studies have reported different frequencies of genetic variants but none of these candidate genes is unique to IgA nephropathy (Tables 66.2 and 66.3). In an analysis of 123 candidate-gene association studies, Kiryluk et al. (2010) found 31%, 32%, and 35% were associated with disease susceptibility, disease progression, or both respectively. One-third of the studies involved polymorphism of the renin–angiotensin system. Cohorts

Table 66.2 Genes in which variants have been reportedly associated with susceptibility to IgA nephropathy

Immune system genes
PIGR (polymeric immunoglobulin receptor)
IGHMBP2 (immunoglobulin mu binding protein 2)
TRAC (T cell receptor alpha constant; T-cell receptor constant alpha chain)
FCAR (Fc fragment of IgA, receptor for; CD89; FcalphaR, Fcalpha receptor)
HLA-DRA (major histocompatibility complex, class II, DR α)
FCGR3B (Fc fragment of IgG, low affinity IIIb, receptor; CD16b; *FcgRIIIb*)
FCGR2B (Fc region of IgG)
FCRLB (Fc receptor-like protein expressed on B linkage cells)
Cytokine coding genes
TNF-α (tumour necrosis factor alpha)
IFNG (interferon gamma)
TGF-β1 (transforming growth factor, beta1; TGF-beta1)
IL10 (interleukin 10)
IL5RA (interleukin 5 receptor, alpha; IL5RA)
TNFRSF6B (tumour necrosis factor receptor superfamily, member 6b, decoy)
Adhesion molecule genes
SELE (selectin E; endothelial adhesion molecule 1)
SELL (selectin L; lymphocyte adhesion molecule 1)
Renin–angiotensin system genes
ACE (angiotensin I converting enzyme 1)
Glycosylation-related gene
ST6GALNAC2 (ST6 alpha-*N*-acetyl-neuraminyl-2,3-beta-galactosyl-1,3-*N*-acetylgalactosaminide alpha-2, 6-sialyltransferase 2)
C1GALT1 (core 1 synthase, glycoprotein-*N*-acetylgalactosamine 3-beta-galactosyltransferase, 1)
Others
EDN1 (endothelin 1; ET-1)
NPHS2 (nephrosis 2, idiopathic, steroid-resistant; podocin)
GNB3 (G protein beta polypeptide 3; G protein beta 3 subunit)
SERPINB7 (serpin peptidase inhibitor, clade B, member 7; *MEGSIN*)
SCGB1A1 (secretoglobin, family 1A, member 1; uteroglobin; UG)
PAI-1 (plasminogen activator inhibitor-1)
BMP2 (bone morphogenetic protein 2)

Adapted and modified from Maxwell, P. H. and Wang, Y. (2009). Genetic contribution to IgA nephropathy. In K.N. Lai (ed.) Recent Advances in IgA nephropathy, pp 21–36. Copyright © World Scientific Press.

Table 66.3 Genes in which variants have been reportedly associated with progression of IgA nephropathy

Immune system genes
FCGR3B (Fc fragment of IgG, low affinity IIIb, receptor; CD16b; *FcgRIIIb*)
FCGR2A (Fc fragment of IgG, low affinity IIa, receptor; CD32; *FcgammaRIIa*)
CD14 (CD14 molecule)
Cytokine coding genes
TNF-α (tumour necrosis factor alpha)
IL10 (interleukin-10)
IL4 (interleukin-4)
TGF-β1 (transforming growth factor beta 1; *TGF-beta1*)
CCL2 (chemokine C-C motif ligand 2; Monocyte chemoattractant protein -1; *MCP-1*)
CCR5 (chemokine C-C motif receptor 5; CC-chemokine receptor five; chemokine receptor 5)
Adhesion molecules
SELE (selectin E; endothelial adhesion molecule 1, E-selectin)
SELL (selectin L, lymphocyte adhesion molecule 1, L-selectin)
Renin–angiotensin system genes
ACE (angiotensin I converting enzyme 1)
AGT (angiotensinogen; serpin peptidase inhibitor, clade A, member 8)
Others
SCGB1A1 (secretoglobin, family 1A, member 1; uteroglobin; Clara cell secretory protein; *CC16*)
PON1 (paraoxonase 1)
NPHS1 (nephrosis 1, congenital, Finnish type; *Nephrin*)
NPHS2 (nephrosis 2, idiopathic, steroid-resistant; *Podocin*)
VEGFA (Vascular endothelial growth factor A; *VEGF*)
SERPINE1 (Serpin pepdase inhibitor, clade E, member 1; Plasminogen activator inhibitor-1; *PAI-1*)
SERPINB7 (Serpin peptidase inhibitor, clade B, member 7; *MEGSIN*)
PPARG (Peroxisome proliferator-activated receptor gamma)
ACSM3 (Acyl-CoA synthetase medium-chain family member 3; SA)
MUC20 (Mucin 20, cell surface associated)
KLOTHO

Adapted and modified from Genetic contribution to IgA nephropathy. Maxwell, P. H. and Wang, Y. (2009). In K.N. Lai (ed.) Recent Advances in IgA nephropathy, pp 21–36. Copyright © World Scientific Press.

Table 66.4 Age and presenting clinical features on the onset of 356 subjects with primary IgA nephropathy (IGAN-STET-CO: prospective cohort diagnosed from 1990 to 1999)

	Male (N = 255)	Female (N = 101)
	No. (%)	No. (%)
Age at onset		
Mean (SD) years	35.2 (15.4)	36.5 (15.2)
Median (range)	34.4 (5.1–76.6)	36.2 (2.7–71.6)
Modalities at the onset		
Isolated microhaematuria	54 (21.2 %)	40 (39.6 %)
Macrohaematuria ± microhaematuria	24 (9.4 %)	11 (10.9 %)
Isolated proteinuria	34 (13.3 %)	9 (8.9 %)
Proteinuria ± (microhaematuria/ macrohaematuria)	73 (28.6 %)	20 (19.8 %)
Hypertension ± (proteinuria/ microhaematuria/ macrohaematuria)	40 (15.7 %)	12 (11.9 %)
Renal failure (acute/chronic) ± (proteinuria/ microhaematuria/ macrohaematuria)	30 (11.8 %)	9 (8.9 %)

Adapted from Clinical course of IgA nephropathy. Berthoux, F. C. and Mohey, H. (2009). In K. N. Lai (ed.) *Recent Advances in IgA Nephropathy*, pp. 107–19. Copyright © 2009 World Scientific Press.

in these studies were underpowered with inadequate cover of single nucleotide polymorphisms. It is therefore unclear which of the reported genes truly confers susceptibility.

Clinical manifestations

Clarkson et al. (1977) remarked that 'Berger disease' was a syndrome of uniform morphology, diverse clinical features, and uncertain prognosis. It is now recognized that primary IgA nephropathy has diverse clinical presentations (Table 66.4) but it still has uncertain prognosis.

Among Caucasians, there is a male predominance. In a French analysis of 356 patients, synpharyngitic macrohaematuria, isolated microhaematuria, and arterial hypertension occur in 20%, 26%, and 22% of patients, respectively (Berthoux and Mohey, 2009). In East Asian subjects, the male-to-female ratio is close to unity (Lai et al., 1985; Yoshikawa et al., 1994). The clinical presentation, however, is not different between the two genders.

Haematuria

The first episode of macroscopic haematuria generally occurs between 15 and 30 years of age—often a decade earlier than a biopsy diagnosis is made. The true onset of the glomerulopathy is likely in the teens or even earlier as inflammatory processes will take time to develop following mesangial IgA deposition. Not infrequently, patients may first present with macroscopic haematuria complicating mucosal infection (respiratory or gastrointestinal), and the former is often described as 'synpharyngitic macrohaematuria'. Macrohaematuria occurs shortly (12–72 hours) following the pharyngitic episode in contrast to 1–3 weeks in postinfectious glomerulonephritis. The macrohaematuria is sometimes accompanied by flank and loin pain. The urine colour is red or brown, but never contains clots.

Asymptomatic microscopic haematuria is a more common presentation than macrohaematuria especially in Asian population often detected with health screening. Urine microscopy reveals dysmorphic red blood cells and red cell casts. Despite the early controversy between the prognostic value of microhaematuria versus macrohaematuria, multivariate Cox analysis fails to demonstrate any difference (Coppo and D'Amico, 2005).

Proteinuria

Asymptomatic proteinuria may be found in 9–13% of cases (Table 66.5). The degree of proteinuria tends to fluctuate within a narrow range for most patients. Proteinuria is usually not heavy and < 30% have proteinuria exceeding 1 g/day. A transient increase of

Table 66.5 Characteristics at diagnosis (first renal biopsy) in 356 subjects with primary IgA nephropathy (IGAN-STET-CO: prospective cohort diagnosed from 1990 to 1999)

	Male (N = 255)	Female (N = 101)
	No. (%)	No. (%)
Hypertension		
Yes (independent of treatment)	98 (38.4 %)	28 (27.7 %)
Haematuria		
Macrohaematuria	57 (22.4%)	21 (20.8 %)
Microhaematuria	175 (68.6 %)	79 (78.2 %)
Proteinuria (g/day)		
Mean (SD)	1.08 (1.50)	0.57 (1.04)
Median (range)	0.50 (0–9.04)	0.08 (0–7.26)
0–0.30 g/day	92 (36.1 %)	60 (59.4 %)
0.31–0.99 g/day	77 (30.2 %)	24 (23.8 %)
1.00–2.99 g/day	58 (22.7 %)	13 (12.9 %)
> 3 g/day	28 (11.0 %)	4 (4.0 %)
GFR (MDRD): mL/min/1.73 m^2		
Mean (SD)	76.3 (28.9)	74.6 (25.2)
Median (range)	81.6 (5.8–157)	76.7 (5.6–16.8)
CKD stage 1	89 (34.9 %)	27 (26.7 %)
CKD stage 2	103 (40.4 %)	52 (51.5 %)
CKD stage 3	40 (15.7 %)	15 (14.9 %)
CKD stage 4	12 (4.7 %)	6 (5.9 %)
CKD stage 5	11 (4.3 %)	1 (1.0 %)
End-stage renal failure/dialysis	4 (1.6 %)	0 (0 %)

Adapted from Clinical course of IgA nephropathy. Berthoux, F. C. and Mohey, H. (2009). In K. N. Lai (ed.) *Recent Advances in IgA Nephropathy*, pp. 107–19. Copyright © 2009 World Scientific Press.

CKD = chronic kidney disease; GFR = glomerular filtration rate; MDRD = Modification of Diet in Renal Disease Study Group.

proteinuria occurs with gross haematuria complicating mucosal infection or urinary tract infection. In a small group of nephrotic patients, the renal biopsy shows histological and ultrastructural features of minimal change nephropathy but with mesangial IgA deposits. These are now referred to as 'an overlapping syndrome of IgA nephropathy and lipoid nephrosis' (Lai et al., 1986). This entity occurs more frequently in children. Clinically, this entity behaves like minimal change nephropathy with good response to corticosteroids. In contrast, for nephrotic patients with advanced glomerular pathology, response to corticosteroid is poor with high probability of renal deterioration.

Other presentations

Acute kidney injury is an uncommon presentation and the pathology is frequently associated with extensive crescentic formation (Lai et al., 1987). Occasionally, acute kidney injury may be a complication from bouts of macroscopic haematuria in IgA nephropathy. Biopsy reveals prominent tubular red blood cell casts, acute tubular necrosis, and interstitial extravasation of red cells (Gutiérrez et al., 2007). The abundance of interstitial macrophages expressing the haemoglobin scavenger receptor CD163 and oxidative stress markers suggests a pathogenetic role for free haemoglobin-induced tubulointerstitial renal injury (Martín-Cleary et al., 2010). Malignant hypertension in the setting of IgA nephropathy may lead to rapidly progressive renal failure. In advanced disease, it may be associated with thrombotic microangiopathy as a renovascular complication of IgA nephropathy. Renal prognosis is as poor as in patients with primary malignant hypertension (Chen et al., 2005). Fifteen per cent of patients have significant renal impairment (chronic kidney disease stage 3 or higher) at first presentation (Table 66.5). Occasionally, IgA nephropathy may present as end-stage renal failure requiring dialytic treatment at presentation, more commonly seen in population with under-privileged healthcare. Fifteen per cent of IgA nephropathy is diagnosed in patients undergoing investigation for hypertension. A renal cause is suggested by abnormal urinalysis, elevated serum creatinine, or raised serum IgA level.

References

Bahiense-Oliveira, M., Saldanha, L. B., Mota, E. L., *et al.* (2004). Primary glomerular diseases in Brazil (1979-1999): is the frequency of focal and segmental glomerulosclerosis increasing? *Clin Nephrol*, 61, 90–7.

Ballardie, F. W., O'Donoghue, D. J., and Feehally, J. (1987). Increasing frequency of adult IgA nephropathy in the UK? *Lancet*, 2, 1205.

Batinic, D., Scukanec-Spoljar, M., Milosevic, D., *et al.* (2007). [Clinical and histopathological characteristics of biopsy-proven renal diseases in Croatia]. *Acta Med Croatica*, 61, 361–4.

Berger, J. and Hinglais, N. (1968). [Intercapillary deposits of IgA-IgG]. *J Urol Nephrol*, 74, 694–5.

Berthoux, F. C. and Mohey, H. (2009). Clinical course of IgA nephropathy. In K. N. Lai (ed.) *Recent Advances in IgA Nephropathy*, pp. 107–19. Singapore: World Scientific Press.

Bisceglia, L., Cerullo, G., Forabosco, P., *et al.* (2006). Genetic heterogeneity in Italian families with IgA nephropathy: suggestive linkage for two novel IgA nephropathy loci. *Am J Hum Genet*, 79, 1130–4.

Briganti, E. M., Dowling, J., Finlay, M., *et al.* (2001). The incidence of biopsy-proven glomerulonephritis in Australia. *Nephrol Dial Transplant*, 16, 1364–7.

Carvalho, E., do Sameiro, F. M., Nunes, J.P., *et al.* (2006). Renal diseases: a 27-year renal biopsy study. *J Nephrol*, 19, 500–7.

Chandrika, B. K. (2007). Non-neoplastic renal diseases in Kerala, India—analysis of 1592 cases, a two year retrospective study. *Indian J Pathol Microbiol*, 50, 300–2.

Chen, Y., Tang, Z., Yang, G., *et al.* (2005). Malignant hypertension in patients with idiopathic IgA nephropathy. *Kidney Blood Press Res*, 28, 251–8.

Choi, I. J., Jeong, H. J., Han, D. S., *et al.* (2001). An analysis of 4,514 cases of renal biopsy in Korea. *Yonsei Med J*, 42, 247–54.

Clarkson, A. R., Seymour, A. E., Thompson, A. J., *et al.* (1977). IgA nephropathy: a syndrome of uniform morphology, diverse clinical features and uncertain prognosis. *Clin Nephrol*, 8, 459–71.

Coppo, R. and D'Amico, G. (2005). Factors predicting progression of IgA nephropathies. *J Nephrol*, 18, 503–12.

Covic, A., Schiller, A., Volovat, C., *et al.* (2006). Epidemiology of renal disease in Romania: a 10 year review of two regional renal biopsy databases. *Nephrol Dial Transplant*, 21, 419–24.

Feehally, J., Farrall, M., Boland, A., *et al.* (2010). HLA has strongest association with IgA nephropathy in genome-wide analysis. *J Amer Soc Nephrol*, 21, 1791–7.

Frasca, G. M., Soverini, L., Gharavi, A. G., *et al.* (2004). Thin basement membrane disease in patients with familial IgA nephropathy. *J Nephrol*, 17, 778–85.

Gharavi, A. G., Kiryluk, K., Choi, M., *et al.* (2011). Genome-wide association study identifies susceptibility loci for IgA nephropathy. *Nat Genet*, 43, 321–7.

Gharavi, A. G., Yan, Y., Scolari, F., *et al.* (2000). IgA nephropathy, the most common cause of glomerulonephritis, is linked to 6q22–23. *Nat Genet*, 26, 354–7.

Gutiérrez, E., Gonzalez, E., Hernandez, E., *et al.* (2007). Factors that determine an incomplete recovery of renal function in macrohematuria-induced acute renal failure of IgA nephropathy. *Clin J Am Soc Nephrol*, 2, 51–7.

Hurtado, A., Escudero, E., Stromquist, C. S., *et al.* (2000). Distinct patterns of glomerular disease in Lima, Peru. *Clin Nephrol*, 53, 325–32.

Izzi, C., Sanna-Cherchi, S., Prati, E., *et al.* (2006). Familial aggregation of primary glomerulonephritis in an Italian population isolate: Valtrompia study. *Kidney Int*, 69, 1033–40.

Johnston, P. A., Brown, J. S., Braumholtz, D. A., *et al.* (1992). Clinico-pathological correlations and long-term follow-up of 253 United Kingdom patients with IgA nephropathy. A report from the MRC Glomerulonephritis Registry. *QJM*, 84, 619–27.

Julian, B. A., Quiggins, P. A., Thompson, J. S., *et al.* (1985). Familial IgA nephropathy. Evidence of an inherited mechanism of disease. *N Engl J Med*, 312, 202–8.

Karnib, H. H., Sanna-Cherchi, S., Zalloua, P. A., *et al.* (2007). Characterization of a large Lebanese family segregating IgA nephropathy. *Nephrol Dial Transplant*, 22, 772–7.

Kiryluk, K., Julian, B. A., Wyatt, R. J. *et al.* (2010). Genetic studies of IgA nephropathy: past, present, and future. *Pediatr Nephrol*, 25, 2257–68.

Kiryluk, K., Li, Y., Sanna-Cherchi, S., *et al.* (2012). Geographic differences in genetic susceptibility to IgA nephropathy: GWAS replication study and geospatial risk analysis. *PLoS Genet*, 8, e1002765.

Lai, K. N., Chan, L. Y., Tang, S. C., *et al.* (2004). Mesangial expression of angiotensin II receptor in IgA nephropathy and its regulation by polymeric IgA1. *Kidney Int*, 66, 1403–16.

Lai, K. N., Ho, C. P., Chan, K. W., *et al.* (1985). Nephrotic range proteinuria—a good predictive index of disease in IgA nephropathy? *QJM*, 57, 677–88.

Lai, K. N., Ho, R. T., Lai, C. K., *et al.* (1994). Increase of both circulating Th1 and Th2 T lymphocyte subsets in IgA nephropathy. *Clin Exp Immunol*, 96, 116–21.

Lai, K. N., Lai, F. M., Chan, K. W., *et al.* (1986). An overlapping syndrome of IgA nephropathy and lipoid nephrosis. *Am J Clin Pathol*, 86, 716–23.

Lai, K. N., Lai, F. M., Leung, A. C., *et al.* (1987). Plasma exchange in patients with rapidly progressive idiopathic IgA nephropathy: a report of two cases and review of literature. *Am J Kidney Dis*, 10, 66–70.

Lavigne, K. A., Woodford, S. Y., Barker, C. V., *et al.* (2010). Familial IgA nephropathy in southeastern Kentucky. *Clin Nephrol*, 73, 115–21.

Levy, M. (1989). Familial cases of Berger's disease and anaphylactoid purpura: more frequent than previously thought. *Am J Med*, 87, 246–8.

Levy, M., Beaufils, H., Gubler, M.C., *et al.* (1972). Idiopathic recurrent macroscopic hematuria and mesangial IgA-IgG deposits in children (Berger's disease). *Clin Nephrol*, 1, 63–9.

Levy, M. and Berger, J. (1988). Worldwide perspective of IgA nephropathy. *Am J Kidney Dis*, 12, 340–7.

Martín-Cleary, C., Moreno, J. A., Fernandez, B., *et al.* (2010). Glomerular haematuria, renal interstitial haemorrhage and acute kidney injury. *Nephrol Dial Transplant*, 25, 4103–6.

Parichatikanond, P., Chawanasuntorapoj, R., Shayakul, C., *et al.* (2006). An analysis of 3,555 cases of renal biopsy in Thailand. *J Med Assoc Thai*, 89 Suppl 2, S106–11.

Paterson, A. D., Liu, X. Q., Wang, K., *et al.* (2007). Genome-wide linkage scan of a large family with IgA nephropathy localizes a novel susceptibility locus to chromosome 2q36. *J Am Soc Nephrol*, 18, 2408–15.

Rambausek, M., Hartz, G., Waldherr, R., *et al.* (1987). Familial glomerulonephritis. *Pediatr Nephrol*, 1, 416–18.

Research Group on Progressive Chronic Renal Disease (1999). Nationwide and long-term survey of primary glomerulonephritis in Japan as observed in 1,850 biopsied cases. *Nephron*, 82, 205–13.

Rivera, F., Lopez-Gomez, J. M., and Perez-Garcia, R. (2002). Frequency of renal pathology in Spain 1994–1999. *Nephrol Dial Transplant*, 17, 1594–602.

Schena, F. P. (1997). Survey of the Italian Registry of Renal Biopsies. Frequency of the renal diseases for 7 consecutive years. The Italian Group of Renal Immunopathology. *Nephrol Dial Transplant*, 12, 418–26.

Schena, F. P., Cerullo, G., Rossini, M., *et al.* (2002). Increased risk of end-stage renal disease in familial IgA nephropathy. *J Am Soc Nephrol*, 13, 453–60.

Schena, F. P., Scivittaro, V., Di Cillo, M., *et al.* (1990). Is Berger's disease a hereditary nephritis? *Contrib Nephrol*, 80, 118–25.

Schena, F. P., Scivittaro, V., and Ranieri, E. (1993). IgA nephropathy: pros and cons for a familial disease. *Contrib Nephrol*, 104, 36–45.

Scolari, F., Amoroso, A., Savoldi, S., *et al.* (1999). Familial clustering of IgA nephropathy: further evidence in an Italian population. *Am J Kidney Dis*, 33, 857–65.

Simon, P., Ramee, M. P., Boulahrouz, R. *et al.* (2004). Epidemiologic data of primary glomerular diseases in western France. *Kidney Int*, 66, 905–8.

Sinniah, R. (1983). Occurrence of mesangial IgA and IgM deposits in a control necropsy population. *J Clin Pathol*, 36, 276–9.

Swaminathan, S., Leung, N., Lager, D. J., *et al.* (2006). Changing incidence of glomerular disease in Olmsted County, Minnesota: a 30-year renal biopsy study. *Clin J Am Soc Nephrol*, 1, 483–7.

Tam, K. Y., Leung, J. C., Chan, L. Y., *et al.* (2009). Macromolecular IgA1 taken from patients with familial IgA nephropathy or their asymptomatic relatives have higher reactivity to mesangial cells in vitro. *Kidney Int*, 75, 1330–9.

Tam, K. Y., Leung, J. C., Chan, L. Y., *et al.* (2010). In vitro enhanced chemotaxis of CD25+ mononuclear cells in patients with familial IgAN through glomerulotubular interactions. *Am J Physiol Renal Physiol*, 299, F359–68.

Tiebosch, A. T., Wolters, J., Frederik, P. F., *et al.* (1987). Epidemiology of idiopathic glomerular disease: a prospective study. *Kidney Int*, 32, 112–6.

Werner, T., Brodersen, H. P., and Janssen, U. (2009). [Analysis of the spectrum of nephropathies over 24 years in a West German center based on native kidney biopsies]. *Med Klin (Munich)*, 104, 753–9.

Yoshikawa, N., Nakamura, H., and Ito, H. (1994). IgA nephropathy in children and adults. *Springer Semin Immunopathol*, 16, 105–20.

Yu, X. Q., Li, M., Zhang, H., *et al.* (2011).A genome-wide association study in Han Chinese identifies multiple susceptibility loci for IgA nephropathy. *Nat Genet*, 44,178–82.

CHAPTER 67

Immunoglobulin A nephropathy: diagnosis

Kar Neng Lai and Sydney C. W. Tang

Urinalysis

The presence of red cell casts and dysmorphic red cells (see Chapter 6), though less often undertaken by many nephrologists now, indicates glomerular bleeding. This should spare the patient from unnecessary urologic procedures such as cystoscopy or retrograde pyelography (see Chapter 46). Other slowly evolving glomerular disorders that are particularly likely to present in a similar way include hereditary nephritis (see Chapter 321) and thin basement membrane disease (see Chapter 325). Varying degrees of proteinuria may also be present.

Renal biopsy

The diagnosis cannot be made with certainty without a renal biopsy, and this can offer additional prognostic information.

Light microscopy

A typical biopsy of immunoglobulin A (IgA) nephropathy is characterized by an increase in mesangial cells with matrix expansion and normal glomerular basement membranes (GBMs) (Fig. 67.1B). However, light microscopic changes are highly variable, ranging from normal or minimal lesions in the glomerular architecture, diffuse mesangial proliferative changes to focal segmental glomerulosclerosis (FSGS), and rarely focal segmental necrotizing lesions with crescent formation.

It is likely that the presence of focal segmental or global sclerosis represents the disease that has already been ongoing for some time. Indeed, capsular adhesions without underlying abnormalities in the tuft, often the first sign of FSGS, are also frequent in IgA nephropathy. A recent French study showed that some form of lesion resembling FSGS, including classical, prehilar, cellular, tip and collapsing variants, was present in 101 out of 128 biopsies IgA nephropathy (El Karoui et al., 2011). It was proposed that most cases of IgA nephropathy can therefore be interpreted as representing a variant of FSGS, highlighting the notion that podocytopathy plays a role in the pathogenesis and progression of IgA nephropathy (see Chapter 139). The presence of aggressive crescentic lesions may represent a different category of disease and may associate with the presence of antineutrophil cytoplasmic autoantibodies (ANCAs). In the overlapping syndrome of IgA nephropathy and lipoid nephrosis, the light microscopy is normal with mesangial IgA deposits.

Apart from changes in the glomerulus, the tubulointerstitium and periglomerular arterioles may also display pathological changes, though these are often non-specific for IgA nephropathy and signify a final common pathway of renal damage in a variety of glomerulopathies in the advanced stage. Variable degrees of tubular atrophy/interstitial fibrosis can occur during the different stages of IgA nephropathy, and its severity carries prognostic implications (Chan et al., 2004; Coppo and D'Amico, 2005). In addition, excessive inflammatory cells may populate the cortical interstitium leading to interstitial inflammation, another feature of the chronic kidney irrespective of the original disease. The presence of granule membrane protein of 17 kDa (GMP-17)-positive cytotoxic T-lymphocytes in intact renal tubules serves as a marker of disease progression in early

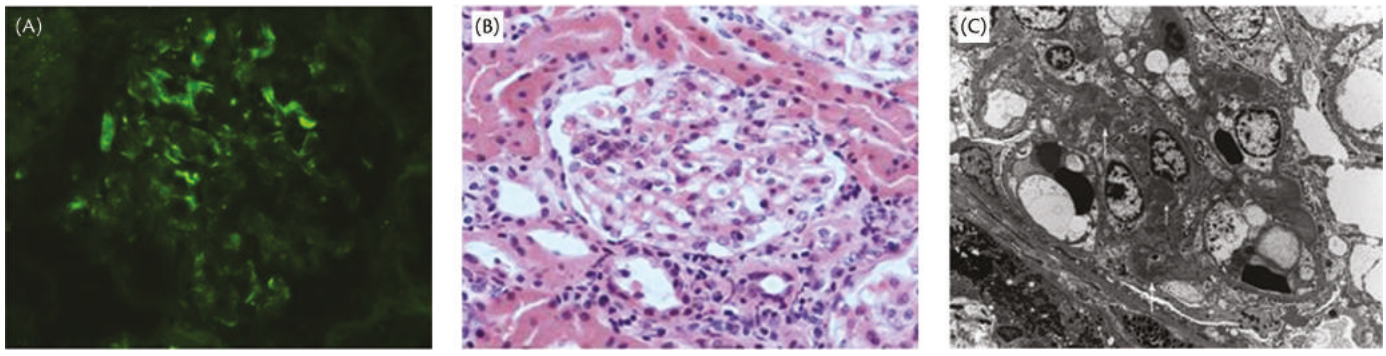

Fig. 67.1 (A) Immunofluorescent staining for IgA deposits in the mesangium (×500).
(B) Moderate mesangial matrix expansion with increased cell number (H&E ×400).
(C) Electron micrograph showing mesangial expansion with electron-dense deposits (arrows) (×9600).

stage (Van Es et al., 2008). Arteriolar lesions are characterized by wall hyalinosis, intimal thickening, and subintimal fibrosis. These changes often accompany the presence of hypertension.

Immune deposits

The defining histological hallmark is the presence of dominant or co-dominant deposition of IgA in the glomerular mesangium (Fig. 67.1A). Mesangial IgA deposits are predominantly polymeric in nature with λ-light chain (Lai et al., 1988). These consist mostly of underglycosylated polymeric IgA1 with the absence of the secretory component and the presence of the J chain. Apart from primary IgA nephropathy, a variety of systemic conditions may also be associated with secondary IgA nephropathy (Table 67.1).

IgG, IgM, and C3 may co-distribute with IgA. C3 is detectable in up to 70–90% of cases often with same distribution as IgA. IgG is present in about 50–70%, and often assumes co-dominance with the IgA staining. IgM deposits are less common and are found in 31–66%. The early components of complement, such as C1q and C4, are infrequently present. Occasionally, C5b-9 with properdin is found, indicating activation of the alternative pathway.

Fibrin/fibrinogen deposits have been reported in 30–40% of biopsies, mainly locate in the mesangium and the glomerular endothelium. Von Willebrand factor may be present in the endothelium, and together with platelet aggregates activates intraglomerular coagulation and contributes to glomerular sclerosis. The extent of fibrin deposition correlates with mesangial proliferation, indicating that the coagulation/fibrinolysis system participates in renal damage of IgA nephropathy.

Electron microscopy

Typically, there are electron-dense deposits that are confined to mesangial or paramesangial areas (Fig. 67.1C). Occasionally, they may be found in the subepithelial or subendothelial space in 10–20% of cases, but are generally small and segmental in nature (Woodrow et al., 1989). The GBM is usually of normal thickness, but may display focal thinning in up to a third of patients. In the overlapping IgA nephropathy/lipoid nephrosis variant, there is extensive effacement of the foot processes.

Pathology-based classification and grading for IgA nephropathy

There have been numerous attempts by pathologists to develop and refine different grading systems based on the extent of pathological lesions to predict clinical outcome (Table 67.2). A common feature of all these systems is that they are all based solely upon light microscopic findings of the renal biopsy which are the most highly variable pathological footprint in IgA nephropathy. These classifications, mostly favouring injury in glomeruli over that in the interstitium and vasculature, have individual strengths and limitations in predicting prognosis. Conflicting results may arise from differences in patient selection, cohort size, treatment, and clinical outcome measures. In general, studies that employ end-stage renal disease (ESRD) as an endpoint have shown chronic pathologic lesions, such as tubular atrophy, and glomerulosclerosis, to predict outcome. In contrast, studies that use rate of GFR decline or responsiveness to immunosuppressive treatment have shown active glomerular lesions, such as mesangial, endocapillary, or extracapillary hypercellularity, to have predictive power.

Table 67.1 Systemic diseases associated with predominant mesangial IgA deposition with probable pathogenetic association with secondary IgA nephropathy

Diseases	Common	Rare
Gastrointestinal	Coeliac disease	Crohn disease Ulcerative colitis
Hepatic	Alcoholic liver disease Non-alcoholic liver cirrhosis	
Infections	HIV Hepatitis B Schistosomiasis Staphylococcal PIGN	Brucellosis Leprosy
Malignancies	Renal cell carcinoma	Monoclonal lymphoproliferative diseases Mixed cryoglobulinaemia Carcinoma (lung, larynx, pancreas) Mycosis fungoides
Rheumatic diseases	Ankylosing spondylitis Rheumatoid arthritis	
Autoimmune diseases		Systemic lupus erythematosus Wegener's granulomatosis Sjögren disease Hashimoto's thyroiditis
Dermatological disorders	Dermatitis herpetiformis	Psoriasis
Others	Diabetes mellitus/ metabolic syndrome	Idiopathic pulmonary haemosiderosis, sarcoidosis

PIGN: post-infectious glomerulonephritis

None of these pathological grading systems has gained widespread acceptance and the debates continue as to whether pathological findings may provide additional prognostic indicators beyond those from clinical features (Bartosik et al., 2001). In 2004, the International IgA Nephropathy Network (<http://wwww.IgAN-world.org>) and the International Society of Nephrology/Renal Pathology Society formed a working group to devise an international consensus on a new classification for IgA nephropathy. Renal biopsy materials from 206 adult and 59 paediatric patients were assessed objectively. The report is duly called 'The Oxford Classification of IgA Nephropathy' (Cattran et al., 2009). After extensive iterative work by five pathologists confined to histological features exclusively on periodic acid–Schiff (PAS)-stained sections on light microscopy, the working group concluded that four glomerular and parenchymal parameters possess reproducible and independent predictive value on renal outcome: mesangial hypercellularity, segmental glomerulosclerosis, endocapillary proliferation, and tubular atrophy/interstitial fibrosis. A unique feature of this system is that it recommends the individual reporting on these four features without artificially clustering into different 'grading' in pathology reports. Thus, the nephrologist would interpret

Table 67.2 Grading and classification systems for IgA nephrology from different eras

Classification (author/year)	Grade 1	Grade 2	Grade 3	Grade 4	Grade 5
(Southwest Pediatric Nephrology Study Group, 1982)	Minimal glomerular changes	Mesangial proliferation only	Any focal or sclerotic lesion		
(Haas, 1997)	Minimal or no mesangial hypercellularity; without sclerosis or crescents	Focal segmental glomerulosclerosis; minimal increase in mesangial hypercellularity; no crescents	Focal proliferative: changes, < 50% of glomeruli are hypercellular	Diffuse proliferative: > 50% of glomeruli are hypercellular	Advanced sclerotic changes: ≥ 40% of glomeruli are globally sclerotic and/or ≥ 40% tubular atrophy or loss of cortex
(To et al., 2000)	Mean glomerular sclerosis < 25% Tubular atrophy and interstitial fibrosis < 5%	Mean glomerular sclerosis 25–49% Tubular atrophy and interstitial fibrosis 5–49%	Mean glomerular sclerosis ≥ 50% Tubular atrophy and interstitial fibrosis ≥ 50%		
(Lee et al., 2005)	Normal or focal mesangial cellular proliferation	Diffuse mesangial cellular proliferation, or < 25% of glomeruli with crescents, segmental /global sclerosis	25–49% of glomeruli with crescents, segmental or global sclerosis	50–75% of glomeruli with crescents, segmental or global sclerosis	> 75% of glomeruli with crescents, segmental or global sclerosis
(Wakai et al., 2006)	Slight mesangial cell proliferation and increased matrix	Slight mesangial cell proliferation and increased matrix. Glomerulosclerosis, crescent formation or adhesion to Bowman's capsule in < 10% of glomeruli	Moderate diffuse mesangial cell proliferation and increased matrix. Glomerulosclerosis, crescent formation or adhesion to Bowman's capsule in 10–30% of glomeruli	Severe diffuse mesangial cell proliferation and increased matrix. Glomerulosclerosis, crescent formation or adhesion to Bowman's capsule in > 30% of glomeruli	
(Manno et al., 2007)	Normal glomeruli or slight increase in mesangial matrix and/or cellularity	Moderate or diffuse mesangial proliferation and/or focal segmental sclerosis and/or endocapillary proliferative and/or cellular crescents ≤ 0% of glomeruli	Cellular crescents in > 50% of glomeruli and/or global sclerosis and fibrous crescents involving > 1/3 of glomeruli and/or diffuse segmental sclerosis		
Oxford (Roberts et al., 2009)	Forgoes arbitrary classification into different grades Emphasizes on the following four reproducible histologic elements, each independently predictive of renal outcome: (1) mesangial hypercellularity (M0 or M1)[a] (2) segmental glomerulosclerosis (S0 or S1)[b] (3) endocapillary proliferation (E0 or E1)[c] (4) tubular atrophy/interstitial fibrosis (T0, T1 or T2)[d]				

[a] Mesangial score should be assessed on PAS-stained sections; M0 ≤ 50% of the glomeruli showing hypercellularity (> 3 cells in a mesangial area), M1 > 50% of the glomeruli having hypercellularity.

[b] Any amount of sclerosis in the glomerular tuft; S0 = absent, S1 = present.

[c] Hypercellularity within glomerular capillary lumina causing its narrowing; E0 = absent, E1 = present.

[d] Percentage cortical area involved; T0 = 0–25%, T1 = 26–50%, T2 > 50%.

Adapted and modified with permission from Clinicopathologic findings. Julian, B. A. and Wyatt, R. J. (2009). In K. N. Lai (ed.) *Recent Advances in IgA Nephropathy*, pp. 83–106. Copyright © 2009 World Scientific Press.

these four features separately on top of the salient clinical features known to impact upon prognosis. It must be borne in mind that this classification suffers the same drawback as being a retrospective observational review, and the histopathological specimens and biochemical data were not uniformly collected in a standardized fashion at source. Nevertheless, a number of validation studies have supported this classification. A French study (Alamartine et al., 2011) supported the validity of the Oxford classification in predicting outcome and also showed that categorizing the type of FSGS lesion carries prognostic value. Similar data have also been generated from Chinese (Shi et al., 2011) and Japanese (Shima et al., 2012) patients.

Other investigations

Renal function

Patients with estimated GFR (eGFR) < 60 mL/min/1.73 m^2 at the time of renal biopsy have worse outcomes than those with normal eGFR. However, the overall rate of decline of eGFR is difficult to predict as some patients with mild-to-moderate renal impairment may remain stable for years, particularly when treated with renin–angiotensin blockade. In the Oxford classification of IgA nephropathy study cohort (Cattran et al., 2009), the rate of GFR decline correlated with segmental glomerulosclerosis and tubular atrophy/interstitial fibrosis, whereas mesangial hypercellularity and tubular atrophy/interstitial fibrosis are predictive of the composite endpoints of 50% reduction in eGFR or ESRD.

Serum IgA

Serum IgA levels have been much studied but are not diagnostically very helpful. Levels are elevated in 33–50% of patients, but this is neither sensitive nor specific for IgA nephropathy. Serum IgA level also bears no prognostic value and the evolution of serum IgA during the course of the disease remains undefined. IgA is unique in its ability to form multimers. Elevated circulating levels of polymeric IgA occur in 25% of patients. The predominant subclass is IgA1, although IgA2 is also elevated in African American patients with IgA nephropathy. This finding is consistent with the mesangial deposition of IgA2 in 56% of these patients.

Galactose-deficient IgA1 is implicated in pathogenesis (see Chapter 69). Serum levels determined by a lectin-based enzyme-linked immunosorbent assay were reported to have 90% specificity and 76% sensitivity for diagnosing sporadic IgA nephropathy in a large cohort of Caucasians from the United States (Moldoveanu et al., 2007). Interestingly, a high serum galactose-deficient IgA1 level was present in the majority of index cases in this cohort, as well as among their parents (39%), siblings (28%), and children (30%). Levels in spouses were indistinguishable from controls, ruling out an environmental effect (Gharavi et al., 2008). Segregation analysis of galactose-deficient IgA1 suggested inheritance of a major dominant gene with an additional polygenic component. Inheritance of galactose-deficient IgA1 has been confirmed in Chinese patients with familial and sporadic adult IgAN (Lin et al., 2009; Tam et al., 2009). These data demonstrate that an elevated serum galactose-deficient IgA1 level is antecedent to disease but, because most family members with elevated levels are asymptomatic, IgA1 glycosylation abnormalities are not sufficient to produce IgA nephropathy, and additional cofactors must trigger formation of immune complexes.

Complement

Serum complement levels are generally normal and their main value is in excluding other conditions. IgA deposits are commonly associated with the deposition of complement components, most notably C3, the membrane attack complex (C5b-9), and properdin (Couser et al., 1985; Rauterberg et al., 1987; Schena, 1992), suggesting the participation of complement activation in disease pathogenesis. Two Japanese groups (Komatsu et al., 2004; Nakayama et al., 2008) reported that the serum IgA/C3 ratio may reflect the histological severity of IgA nephropathy and could serve as a marker for disease progression, and may be useful for prediction of diagnosis of IgA nephropathy and distinguishing it from other glomerulonephritides. Another German study (Zwirner et al., 1997) indicated that only alternative pathway complement was activated in IgA nephropathy and its activation was associated with more severe renal disease. Serum complement levels of C3, C4, and factor B are usually normal, although small amounts of neoantigens of C3, such as iC3b and C3b, can be detected using sensitive methods. Increased activated plasma C3 levels are present in 30% of patients, particularly in those with proteinuria and haematuria, and these correlated with renal deterioration on follow-up. Deficiencies in certain complement components, such as factor H, and C9, have been described in some subjects.

Immune complexes

Circulating IgA immune complex (IgA-IC) formation is thought to be universal in IgA nephropathy (see Chapter 69), but again this is not a diagnostically useful test.

Antineutrophil cytoplasmic autoantibodies

Circulating ANCAs are not usually found but are described in some case reports (Bantis et al., 2010). ANCAs against myeloperoxidase are more commonly encountered than those against proteinase 3. When ANCAs are present, the disease often takes a more aggressive form with active urine sediments, rapidly progressive glomerulonephritis, and renal biopsies often reveal necrotizing and crescentic lesions. Therapeutic response to timely immunosuppressive treatment is usually good.

Advanced urinalysis

A number of techniques show promise as predictors of disease or disease activity, but are mainly research tools at present.

Cytokines and chemokines in urine are elevated in IgA nephropathy, particularly in patients with moderately advanced renal injury. Urinary monocyte chemoattractant protein-1 (MCP-1) levels correlate with proteinuria (Wasilewska et al., 2011). Together with urinary interleukin-6 and epidermal growth factor, these cytokines may act as predictors of renal outcome (Stangou et al., 2009). Urinary interleukin-8 levels have also been correlated with disease activity. Urinary angiotensinogen is a powerful tool for determining intrarenal renin–angiotensin system activity and is associated renal derangement (Nishiyama et al., 2011).

Urinary complement factor H is shown to correlate closely with disease activity (Zhang et al., 2009). In crescentic IgA nephropathy, fractional excretion of IgG in relation to the degree of nephron loss predicts disease progression (Bazzi et al., 2009).

Urinary secretory IgA (sIgA) has been associated with high-grade histological changes and proteinuria, and might be used as a non-invasive biomarker to evaluate kidney injury in IgA nephropathy (Tan et al., 2009).

Urinary podocytes or their fragments can be identified by immunohistochemical approaches, or by mRNA analysis, or by protein analysis. Podocyte loss may be a marker for progressive renal disease (see Chapter 139).

Urine uromodulin fragment has been suggested as a biomarker for the non-invasive diagnosis of IgA nephropathy (Wu et al., 2010). Several urinary microRNAs (miR-200a, miR-200b, and miR-429) are downregulated in patients with IgA nephropathy, and the degree of reduction suggested to correlate with disease severity and rate of progression (Wang et al., 2010).

References

Alamartine, E., Sauron, C., Laurent, B., *et al.* (2011). The use of the Oxford classification of IgA nephropathy to predict renal survival. *Clin J Am Soc Nephrol*, 6, 2384–88.

Bantis, C., Stangou, M., Schlaugat, C., *et al.* (2010). Is presence of ANCA in crescentic IgA nephropathy a coincidence or novel clinical entity? A case series. *Am J Kidney Dis*, 55, 259–68.

Bartosik, L. P., Lajoie, G., Sugar, L., *et al.* (2001). Predicting progression in IgA nephropathy. *Am J Kidney Dis*, 38, 728–35.

Bazzi, C., Rizza, V., Raimondi, S., *et al.* (2009). In crescentic IgA nephropathy, fractional excretion of IgG in combination with nephron loss is the best predictor of progression and responsiveness to immunosuppression. *Clin J Am Soc Nephrol*, 4, 929–35.

Cattran, D. C., Coppo, R., Cook, H. T., *et al.* (2009). The Oxford classification of IgA nephropathy: rationale, clinicopathological correlations, and classification. *Kidney Int*, 76, 534–45.

Chan, L. Y., Leung, J. C., and Lai, K. N. (2004). Novel mechanisms of tubulointerstitial injury in IgA nephropathy: a new therapeutic paradigm in the prevention of progressive renal failure. *Clin Exp Nephrol*, 8, 297–303.

Coppo, R., Amore, A., Chiesa, M., *et al.* (2007). Serological and genetic factors in early recurrence of IgA nephropathy after renal transplantation. *Clin Transplant*, 21, 728–37.

Couser, W. G., Baker, P. J., and Adler, S. (1985). Complement and the direct mediation of immune glomerular injury: a new perspective. *Kidney Int*, 28, 879–90.

El Karoui, K., Hill, G. S., Karras, A., *et al.* (2011). Focal segmental glomerulosclerosis plays a major role in the progression of IgA nephropathy. II. Light microscopic and clinical studies. *Kidney Int*, 79, 643–54.

Gharavi, A. G., Moldoveanu, Z., Wyatt, R. J., *et al.* (2008). Aberrant IgA1 glycosylation is inherited in familial and sporadic IgA nephropathy. *J Amer Soc Nephrol*, 19, 1008–14.

Haas, M. (1997). Histologic subclassification of IgA nephropathy: a clinicopathologic study of 244 cases. *Am J Kidney Dis*, 29, 829–42.

Julian, B. A. and Wyatt, R. J. (2009). Clinicopathologic findings. In K. N. Lai (ed.) *Recent Advances in IgA Nephropathy*, pp. 83–106. Singapore: World Scientific Press.

Komatsu, H., Fujimoto, S., Hara, S., *et al.* (2004). Relationship between serum IgA/C3 ratio and progression of IgA nephropathy. *Intern Med*, 43, 1023–8.

Lai, K. N., Chui, S. H., Lai, F. M., *et al.* (1988). Predominant synthesis of IgA with lambda light chain in IgA nephropathy. *Kidney Int*, 33, 584–9.

Lee, H. S., Lee, M. S., Lee, S. M., *et al.* (2005). Histological grading of IgA nephropathy predicting renal outcome: revisiting H. S. Lee's glomerular grading system. *Nephrol Dial Transplant*, 20, 342–8.

Lin, X., Ding, J., Zhu, L., *et al.* (2009). Aberrant galactosylation of IgA1 is involved in the genetic susceptibility of Chinese patients with IgA nephropathy. *Nephrol Dial Transplant*, 24, 3372–5.

Manno, C., Strippoli, G. F., D'Altri, C., *et al.* (2007). A novel simpler histological classification for renal survival in IgA nephropathy: a retrospective study. *Am J Kidney Dis*, 49, 763–75.

Moldoveanu, Z., Wyatt, R. J., Lee, J. Y., *et al.* (2007). Patients with IgA nephropathy have increased serum galactose-deficient IgA1 levels. *Kidney Int*, 71, 1148–54.

Nakayama, K., Ohsawa, I., Maeda-Ohtani, A., *et al.* (2008). Prediction of diagnosis of immunoglobulin A nephropathy prior to renal biopsy and correlation with urinary sediment findings and prognostic grading. *J Clin Lab Anal*, 22, 114–18.

Nishiyama, A., Konishi, Y., Ohashi, N., *et al.* (2011). Urinary angiotensinogen reflects the activity of intrarenal renin-angiotensin system in patients with IgA nephropathy. *Nephrol Dial Transplant*, 26, 170–7.

Rauterberg, E. W., Lieberknecht, H. M., Wingen, A. M., *et al.* (1987). Complement membrane attack (MAC) in idiopathic IgA-glomerulonephritis. *Kidney Int*, 31, 820–9.

Roberts, I. S., Cook, H. T., Troyanov, S., *et al.* (2009). The Oxford classification of IgA nephropathy: pathology definitions, correlations, and reproducibility. *Kidney Int*, 76, 546–56.

Schena, F. P. (1992). IgA nephropathies. In S. Cameron, A. M. Davison, J. P. Grunfield, *et al.* (eds.) *Oxford Textbook of Clinical Nephrology*, pp. 339–69. Oxford: Oxford University Press.

Schena, F. P. (1997). Survey of the Italian Registry of Renal Biopsies. Frequency of the renal diseases for 7 consecutive years. The Italian Group of Renal Immunopathology. *Nephrol Dial Transplant*, 12, 418–26.

Shi, S. F., Wang, S. X., Jiang, L., *et al.* (2011). Pathologic predictors of renal outcome and therapeutic efficacy in IgA nephropathy: validation of the oxford classification. *Clin J Am Soc Nephrol*, 6, 2175–84.

Shima, Y., Nakanishi, K., Hama, T., *et al.* (2012). Validity of the Oxford classification of IgA nephropathy in children. *Pediatr Nephrol*, 27,783–92.

Southwest Pediatric Nephrology Study Group (1982). A multicenter study of IgA nephropathy in children. A report of the Southwest Pediatric Nephrology Study Group. *Kidney Int*, 22, 643–52.

Stangou, M., Alexopoulos, E., Papagianni, A., *et al.* (2009). Urinary levels of epidermal growth factor, interleukin-6 and monocyte chemoattractant protein-1 may act as predictor markers of renal function outcome in immunoglobulin A nephropathy. *Nephrology*, 14, 613–20.

Tam, K. Y., Leung, J. C., Chan, L. Y., *et al.* (2009). Macromolecular IgA1 taken from patients with familial IgA nephropathy or their asymptomatic relatives have higher reactivity to mesangial cells in vitro. *Kidney Int*, 75, 1330–9.

Tan, Y., Zhang, J. J., Liu, G., *et al.* (2009). The level of urinary secretory immunoglobulin A (sIgA) of patients with IgA nephropathy is elevated and associated with pathological phenotypes. *Clin Exp Immunol*, 156, 111–16.

Tiebosch, A. T., Wolters, J., Frederik, P. F., *et al.* (1987). Epidemiology of idiopathic glomerular disease: a prospective study. *Kidney Int*, 32, 112–16.

To, K. F., Choi, P. C., Szeto, C. C., *et al.* (2000). Outcome of IgA nephropathy in adults graded by chronic histological lesions. *Am J Kidney Dis*, 35, 392–400.

Van Es, L. A., de, Heer, E., Vleming, L. J., *et al.* (2008). GMP-17-positive T-lymphocytes in renal tubules predict progression in early stages of IgA nephropathy. *Kidney Int*, 73, 1426–33.

Wakai, K., Kawamura, T., Endoh, M., *et al.* (2006). A scoring system to predict renal outcome in IgA nephropathy: from a nationwide prospective study. *Nephrol Dial Transplant*, 21, 2800–8.

Wang, G., Kwan, B. C., Lai, F. M., *et al.* (2010). Expression of microRNAs in the urinary sediment of patients with IgA nephropathy. *Dis Markers*, 28, 79–86.

Wasilewska, A., Zoch-Zwierz, W., Taranta-Janusz, K., *et al.* (2011). Urinary monocyte chemoattractant protein-1 excretion in children with glomerular proteinuria. *Scand J Urol Nephrol*, 45, 52–9.

Woodrow, D. F., Shore, I., Moss, J., *et al.* (1989). Immunoelectron microscopic studies of immune complex deposits and basement membrane components in IgA nephropathy. *J Pathol*, 157, 47–57.

Wu, J., Wang, N., Wang, J., *et al.* (2010). Identification of a uromodulin fragment for diagnosis of IgA nephropathy. *Rapid Commun Mass Spectrom*, 24, 1971–8.

Zhang, J. J., Jiang, L., Liu, G., *et al.* (2009). Levels of urinary complement factor H in patients with IgA nephropathy are closely associated with disease activity. *Scand J Immunol*, 69, 457–64.

Zwirner, J., Burg, M., Schulze, M., *et al.* (1997). Activated complement C3: a potentially novel predictor of progressive IgA nephropathy. *Kidney Int*, 51, 1257–64.

CHAPTER 68

Immunoglobulin A nephropathy: treatment and outcome

Kar Neng Lai and Sydney C. W. Tang

Clinical course

The clinical course of IgA nephropathy is highly variable. In general, the glomerulopathy usually runs an indolent but slowly progressive course leading to ESRD in 20–50% of patients over 30 years. The symptoms and prevalence vary between regions due to ethnic difference and biopsy criteria. Some asymptomatic patients are diagnosed after incidental finding of microscopic haematuria, low-grade proteinuria, or hypertension. Patients with these isolated features may not be biopsied in many centres. Some patients present with episodic synpharyngitic macrohaematuria, with or without significant proteinuria or hypertension. Some patients present with advanced renal failure, and a subset presents with features of rapidly progressive glomerulonephritis.

The natural history of primary IgA nephropathy is progression to CKD and eventually ESRD at variable rates, which means some patients may not develop CKD in their lifetime, while some patients may develop ESRD shortly after diagnosis. Clearly, these patients differ in terms of clinical as well as histologic features in the kidney biopsy. Even patients with the most benign clinical features must be monitored at least yearly for life. Among 72 patients in Hong Kong who presented with isolated microhaematuria and were found to have minimal proteinuria of < 0.4 g/day, normal renal function and blood pressure, 33% developed proteinuria of > 1 g/day, 26% became hypertensive, and 7% developed impaired renal function after a median observation period of 7 years (Szeto et al., 2001). Similar rates of progression among clinically early disease patients are reproduced in a recent analysis of 177 patients from Shanghai (Shen et al., 2008).

Outcome prognostic markers

In assessing prognosis, most studies have examined features at the time of renal biopsy. Due to the indolent clinical course in most instances, using the definitive end-point of ESRD is often beyond the scope of prospective studies. This has led to the use of alternative markers including time to doubling of serum creatinine, and slope of 1/creatinine, creatinine clearance, or eGFR against time. Some studies employ the absolute or percentage change in proteinuria as a surrogate marker as proteinuria per se indicates the degree of glomerular injury and correlates strongly with outcome (Chapter 50).

Predicting clinical outcome

Predicting clinical outcome for IgA nephropathy remains an imprecise process. There are clinicopathological features that are generally, but not universally, accepted as indicating a less favourable prognosis in patients with preserved clearance function at diagnosis (Table 68.1). Clinical predictors of progression include raised serum creatinine at the time of diagnosis, arterial hypertension, significant proteinuria (> 1 g/day), male gender, and persistent microhaematuria. Clearly patients with raised serum creatinine at diagnosis are likely to have progressive loss of renal function. It is likely that impaired renal function at diagnosis simply reflects belated discovery of an indolent disease process that has progressed over a substantial period of time before the diagnosis was made. Up to 50% of patients have a chronic course, characterized by a slowly declining renal function over 10–20 years, eventually developing renal failure. The percentage of patients who will be in ESRD is roughly the same as the duration of the disease in years from the time of diagnosis (Johnston et al., 1992). Life-table analysis of several series from Asia, Australia, Europe, and North America shows a 10-year renal survival of 80–90% (D'Amico et al., 1993).

Proteinuria

Traditionally, the severity of proteinuria upon presentation carries prognostic implication. More recently, a strong correlation is found between proteinuria at the time of biopsy and the degree of histologic lesions (Cattran et al., 2009). While early studies suggested that the cut-off value for poor prognosis was 2 g/day, subsequent reports showed a continuous effect, with an adverse impact on outcome starting at 500 mg/day. More importantly, rather than a single measurement upon presentation, the change in proteinuria over time is being regarded as a better yardstick for prognosis. A Canadian study has showed that proteinuria during follow-up was the most important predictor of the rate of GFR decline (Reich et al., 2007). Patients with sustained proteinuria > 3 g/day lost GFR 25 times faster than those with proteinuria maintained < 1 g/day. Furthermore, patients with proteinuria > 3 g/day upon presentation who achieved a partial remission (< 1 g/day) during follow-up had a similar clinical course to patients with proteinuria ≤ 1 g/day throughout, and fared better than patients who never achieved remission. Another study in Hong Kong (Tang et al.,

Table 68.1 Commonly accepted markers of a worse prognosis for patients with IgA nephropathy

Demographic
◆ Male sex
◆ Older age at diagnosis
◆ Obesity.
Clinical
◆ No history of macroscopic haematuria
◆ Persistent microscopic haematuria
◆ Hypertension, persistent.
Laboratory
◆ Proteinuria persistently > 1000 mg/day
◆ Hyperuricaemia
◆ Hypertriglyceridemia.
Histological (see Chapter 67)
◆ Light microscopy:
• Mesangial hypercellularity
• Focal segmental glomerular sclerosis
• Endocapillary cellular proliferation
• Capillaritis
• Interstitial fibrosis/tubular atrophy
• Thrombotic microangiopathy
• Loss of podocytes
◆ Immunofluorescence microscopy:
• IgG in mesangial deposits
◆ Electron microscopy:
• Electron-dense deposits in capillary loops.

2010) demonstrated change in the urine albumin-to-creatinine ratio at 1 year to be an independent predictor of progression to ESRD upon a 6-year follow-up period. Using multivariate Cox analysis, age and mean proteinuria at follow-up are powerful independent prognostic predictors in a cohort of Italian patients (Coppo and D'Amico, 2005).

Other factors

The mechanisms leading to progression for some of the markers are not well understood (e.g. the apparent benefit of a history of macroscopic haematuria). Other markers, such as obesity and hyperuricaemia, may exert some of their adverse effects remotely. Light microscopic findings of the renal biopsy are the basis for several classifications that have correlated specific features with a progressively worse prognosis (Table 68.2; see Chapter 67). The traditional histologic predictors of poor renal outcome include glomerulosclerosis, interstitial fibrosis, tubular atrophy, and crescent formation. The Oxford classification (see Chapter 67) has redefined some of these histologic predictors in which mesangial hypercellularity and segmental glomerulosclerosis/adhesions predicted a 50% reduction in eGFR or ESRD and rate of eGFR decline, respectively, whereas tubular atrophy/interstitial fibrosis predicted both. Finally, endocapillary proliferation, though not predictive of any these outcome parameters, was associated with treatment responsiveness to immunosuppressive agents (Cattran et al., 2009; Roberts et al., 2009). These findings were recently validated in a retrospective cohort of 128 adult patients in France in which mesangial hypercellularity, endocapillary proliferation, segmental glomerulosclerosis, and tubular atrophy/interstitial fibrosis each predicted bad outcome, defined as doubling of serum creatinine or need of dialysis after a follow up of 80 months. More importantly, the presence of FSGS lesions, particularly the collapsing and cellular forms, significantly worsened renal survival (El Karoui et al., 2011).

Predictive equations

Several individual centres have derived formulae to predict the risk of ESRD or rate of GFR loss for an individual patient, using commonly measured laboratory and/or histological features (Table 68.3). To date, there has been no consensus as to which of the components of the formula or even the end-points bear the most significant prognostic implication. Some components are unique to a given population in a region. Furthermore, the relative weights of shared components (e.g. serum creatinine) differ between formulae. It is disappointing that these calculations often yield disparate estimates under commonly encountered clinical scenarios. Only one formula from Finland (Rauta et al., 2002) has been validated in a second cohort of patients. Another formula from Japan (Wakai et al., 2006) was validated in the same cohort of subjects with additional patients after an extension of follow-up (Goto et al., 2009).

Genetic factors

Finally, some studies suggest that gene polymorphisms also make a prognostic difference. None are yet ready for use in the clinic. The angiotensin-converting enzyme (ACE) DD genotype is associated with an increased rate of progressive renal disease in Caucasians (Harden et al., 1995) and Japanese (Hunley et al., 1996). More recently, the PREDICT-IgAN study group from Japan identified from a hundred atherosclerotic disease-related gene polymorphisms that glycoprotein Ia and intercellular adhesion molecule-1 polymorphisms are significantly associated with progression (Yamamoto et al., 2009). Among the Chinese population, the 2093C-2180T haplotype of the *MEGSIN* gene, which is predominantly expressed in the mesangial cell regulating its matrix metabolism, proliferation, and apoptosis and is upregulated in IgA nephropathy, is shown to be associated with more severe forms of IgA nephropathy and more rapid progression (Xia et al., 2006). This finding, however, cannot be reproduced in a smaller cohort of Czech patients (Maixnerova et al., 2008).

Treatment

There is a lack of large randomized controlled trials (RCTs) that provide a definitive immunosuppressive protocol for IgA nephropathy

Non-immunosuppressive treatment

Blockade of the renin–angiotensin system remains the mainstay of treatment for IgA nephropathy. Cheng et al. (2009a) analysed 11 RCTs involving 585 patients with seven trials using placebo/no treatment as controls and four trials used other antihypertensive agents as controls. They reported angiotensin-converting enzyme inhibitors (ACEIs)/angiotensin receptor blockers (ARBs) had statistically significant effects on protecting renal function and reduction of proteinuria when compared with control group. A recent

Table 68.2 Formulae to predict clinical outcome in an individual patient with IgA nephropathy

Authors, year (number of subjects, country of origin)	Component of score clinical factors	Histology	Endpoint measurement	Comment	Validated
Beukhof et al., 1986 75 patients, the Netherlands	History of macroscopic haematuria, microscopic haematuria, creatinine clearance, and 24-hr proteinuria.	None	ESRD at 5 years	Requires control of hypertension.	No
Alamartine et al., 1991 (282 patients, France)	Proteinuria, hypertension, and HLA B35	Global optical score (mesangial, tubular, interstitial, and vascular components)	Renal insufficiency (serum creatinine ≥ 1.5 mg/dL (135μmol/L)) at 10 and 20 years	Proteinuria was strongest factor	No
Radford et al., 1997 (206 patients, Midwest USA)	Serum creatinine and age	Total glomerular score	ESRD at 5 and 10 years	Serum creatinine dominates. Older age confers better score. No accounting for treatment.	No
Bartosik et al., 2001 (298 patients, Canada)	Time-averaged MAP and proteinuria	None	Slope of GFR (C-G) after 2–3 years	No demographic, clinical, laboratory or histological parameter at biopsy significantly predicted progressive loss of GFR. MAP and proteinuria accounted for only one third of the variability in loss of renal function.	No
Rauta et al., 2002 (161 patients, Finland)	Microscopic haematuria and hypertension	Arteriosclerosis and glomerular score >2	ESRD at 10 years	Starting GFR (C-G) > 85 mL/min. Some patients had been treated.	No
Magistroni et al., 2006 (310 patients, Italy)	Serum creatinine >1.4 mg/dL, proteinuria >1g/d, hypertension and age >30 yr.	None	ESRD at 10 years	Serum creatinine was strongest risk factor.	Yes
Wakai et al., 2006 (1754 patients, Japan)	Sex, age, systolic BP, proteinuria (dipstick), haematuria (microscopic), serum total protein, and serum creatinine.	Total histological grade (glomerular + interstitial/vascular)	ESRD at 7 years	Serum creatinine dominates, better score with older age; no accounting for treatment.	No
Goto et al., 2009 (2283 patients, Japan)	Sex, age, systolic BP, proteinuria (dipstick), haematuria (microscopic), serum albumin, and serum creatinine.	Total histological grade (glomerular + interstitial/vascular)	ESRD at 10 years	An extended observation of the study reported by Wakai et al. (2006)	Yes

BP = blood pressure; C-G = Cockcroft-Gault formula; ESRD = end-stage renal disease; GFR = glomerular filtration rate; MAP = mean arterial pressure.

meta-analysis of 27 RCTs (1577 participants) using ACEIs, ARBs, or a combination of both, versus other antihypertensives, other agents or placebo reveals renin–angiotensin blockade appears to potentially outweigh the harms in patients with IgA nephropathy (Reid et al., 2011). The benefits are largely manifested as a reduction in proteinuria, a surrogate outcome. There is no evidence that treatment with any of the antihypertensive agents evaluated affects major renal and/or cardiovascular endpoints or long-term mortality risk beyond the benefit that arises from controlling hypertension. The RCT evidence is insufficiently robust to demonstrate efficacy for any of the other non-immunosuppressive therapies including fish oils, anticoagulants, and tonsillectomy.

Immunomodulatory treatment

Research is hampered by the slowly progressive nature of the disease, with 10-year renal survival rates exceeding 85%, marked patient heterogeneity, and the lack of a good animal model that closely resembles human IgA nephropathy.

Corticosteroids: effective in those at high risk

A relatively large quantity of data on corticosteroid was contributed by Japanese researchers in early years. Kobayashi et al. (1986) reported that steroid treatment in 14 patients with proteinuria 1–2 g/day versus 29 control subjects was effective in lowering proteinuria, particularly in patients with baseline GFR > 70 mL/min. After

Table 68.3 Recurrence rate of IgA nephropathy

Authors, year	Follow-up duration (mean) (months)	No. of allograft	Recurrence rate[a] No. (%)	Graft loss due to recurrence No. (%)
Moroni et al., 2013	*113.1 (60.5–165)	190	42 (22.1%)	12 (6.3%)
Coppo et al., 2007	*62.4 (45.6–114)	116	36 (31%)	NA
Moriyama et al., 2005	67.8 ± 19.9	49	13(26.5%)	5 (10%)
Choy et al., 2003	100.0 ± 5.8	75	14(18.7%)	3 (4.0 %)
Briganti et al., 2002	12–120	532	NA	15 (2.8%)
Andresdottir et al., 2001	67.2 ± 54	79	17(21.5%)	1 (1.3 %)
Ponticelli et al., 2001	70.4 ± 50.5	106	37(35%)	4 (3.8 %)
Wang et al., 2001	*52 (18–155)	48	14(29.2%)	4 (8.3 %)
Kim et al., 2001	2–164	90	19(21.1%)	2 (2.2 %)
Freese et al., 1999	*67 (11–159)	104	13(12.5%)	6 (5.8 %)
Bumgardner et al., 1998	61 ± 37	61	18(29.5%)	7 (11.5 %)
Ohmacht et al., 1997[c]	54 (7–127)	61	20(29.9%)	10 (16.4 %)
Frohnert et al., 1997	*78 (3–156)	53	10(19%)	3 (5.7 %)
Kessler et al., 1996[b]	68.1 ± 37.2	84	13(15.5%)	4 (4.8 %)
Hartung et al., 1995	45.9 ± 10	128	47(36.7%)	9 (7.0 %)
Odum et al., 1994	3–183	51	17(33.3%)	5 (9.8 %)
Bachman et al., 1986	20 ± 13	13	6 (46.2%)	1 (7.6 %)

* = median; % = percentage was calculated from number of graft loss due to recurrent IgA nephropathy / total number of patients with primary IgA nephropathy.

[a] Recurrence rate in patients with clinical symptoms of proteinuria/haematuria/renal impairment.

[b] Included 13 patients suffered from underlying Henoch–Schönlein purpura.

[c] Included four patients suffered from underlying Henoch–Schönlein purpura.

Updated and adopted from Recurrent IgA nephropathy in transplant. Choy, B. Y. and Lai, K. N. (2009). In K. N. Lai (ed.) *Recent Advances in IgA Nephropathy*, pp. 149–59.

10 years' follow-up, renal survival was 80% versus 34% (Kobayashi et al., 1996). These results were reproduced in 86 Italian patients when Pozzi et al. (1999) reported renoprotective effects of a 6-month course of steroid treatment. At 10 years, renal survival was better in the steroid group (Pozzi et al., 2004). On the other hand, Lai et al. (1986) found no therapeutic value of short-term (6-month) corticosteroid treatment in Chinese patients. A meta-analysis of seven RCTs involving 386 subjects suggests that corticosteroids have statistically significant effects on protecting renal function and reduction of proteinuria, but gastrointestinal tract reaction is a concern (Cheng et al., 2009b). Another meta-analysis (including 15 controlled, quasi-randomized controlled and non-controlled trials with 1542 participants) suggests corticosteroid therapy is associated with a decrease of proteinuria and with a statistically significant reduction of the risk in ESRD. Moreover, subgroup analysis also suggested that long-term steroid therapy had a higher efficiency than standard and short-term therapy (Zhou et al., 2011). A more recent meta-analysis (including 536 patients who had urinary protein excretion >1 g/day and normal renal function from nine relevant trials) suggested that high-dose and short-term therapy produced significant renal protection, whereas low-dose, long-term steroid use did not (Lv et al., 2012). The KDIGO Clinical Practice Guideline for Glomerulonephritis (Kidney Disease: Improving Global Outcomes (KDIGO) Glomerulonephritis Work Group, 2012) suggested that there is low-quality evidence for corticosteroids to provide additional benefits on top of optimized supportive care, and suggested that patients with persistent proteinuria > 1 g/day despite adequate ACEI or ARB and blood pressure control and GFR > 50 mL/min/1.73 m^2 receive a 6-month course of steroid therapy. Trials studying the efficacy and safety of steroid treatment in IgA nephropathy are currently underway.

Cyclophosphamide plus corticosteroids: uncertain

Evidence that pulse corticosteroid plus intravenous or oral cyclophosphamide slow the progression of advanced IgA nephropathy was provided by several groups worldwide.

Ballardie and Roberts (2002) showed in 38 patients with progressive renal deterioration that renal survival in cyclophosphamide-treated patients was considerably better at 5 years (72% compared with 6% in controls). Proteinuria and erythrocyturia reduced from 12 and 6 months of treatment, respectively. This study may be faulted, however, for suboptimal blood pressure control and insufficient use of medications that block the angiotensin system, the unusually poor survival rate of the placebo group, and the small number of patients. In another prospective, uncontrolled, open-label trial, 12 patients with crescentic IgA

nephropathy received three doses of methylprednisolone at 15 mg/kg/day, followed by intravenous cyclophosphamide at 0.5 g/m^2/month for 6 months (Tumlin et al., 2003). Serum creatinine fell from 2.7 to 1.5 mg/dL, and proteinuria decreased from 4 to 1.3 g/day after treatment, suggesting a beneficial role of cyclophosphamide in crescentic glomerulonephritis. In another observational, uncontrolled study, similar results were reported in 21 patients with advanced IgA nephropathy treated with intravenous pulse cyclophosphamide (0.75 g/m^2/month) for 6 months together with low-dose oral prednisolone (Rasche et al., 2003). More recently, Mitsuiki et al. (2007) retrospectively examined the outcome of 35 patients with histologically advanced IgA nephropathy, in whom 27 received prednisolone for 2 years and oral cyclophosphamide (50 mg/day) for 6 months, and the remaining eight received supportive treatment. Renal prognosis was significantly better in the treatment group.

Overall, these studies suggest that combined cyclophosphamide/steroid therapy may benefit patients at very high risk of renal failure, namely those with a progressive decline in GFR and/or crescentic lesions before randomization. Due to the side effects, it is reasonable to use short-term cyclophosphamide with corticosteroids for IgA nephropathy patients with true crescentic or rapidly progressive glomerulonephritis. The KDIGO Clinical Practice Guideline for Glomerulonephritis (Kidney Disease: Improving Global Outcomes (KDIGO) Glomerulonephritis Work Group, 2012) suggested a similar approach (low-quality evidence).

Tonsillectomy: doubtful

For a long time, tonsillectomy was considered a treatment option for IgA nephropathy, aimed at removing a relevant source of pathogens, which can multiply in tonsil crypts, and also in macrophages and B cells in lymphoid tonsil follicles. This specific antigen challenge was thought to induce a supernormal IgA synthesis, as tonsil lymphocytes from IgA nephropathy patients showed a higher production of dimeric and undergalactosylated IgA1 than control subjects. In Japan, tonsillectomy-steroid pulse therapy has frequently been used for treatment of early IgA nephropathy, and showed favourable outcomes (Moriyama and Nitta, 2011). A recent meta-analysis of seven studies (six from Japan and one from China) comprising 858 patients (534 underwent tonsillectomy and 324 did not) showed that tonsillectomy combined with either normal steroid or steroid pulse treatment, but not tonsillectomy or steroid treatment alone, resulted in higher remission rates with favourable long-term efficacy at both 5- and 10-year follow-up (Wang et al., 2011).

Elsewhere, the benefits of tonsillectomy have been less impressive. A retrospective review of 61 Caucasian patients showed that tonsillectomy was not associated with a different rate of disease progression after 20 years of follow-up (Piccoli et al., 2010). From the available evidence, it seems unlikely that a dysregulated mucosal immune system in IgA nephropathy could be substantially controlled by tonsillectomy alone. The role of tonsillectomy remains controversial. It is presently encouraged and practised only in Japan and certain parts of Asia but opposed in the rest of the world except where there are clear ENT indications. Randomized, controlled trials are needed to resolve this conflict. At present, the KDIGO Clinical Practice Guideline for Glomerulonephritis (Kidney Disease: Improving Global Outcomes (KDIGO) Glomerulonephritis Work Group, 2012) suggested that tonsillectomy should not be performed for IgA nephropathy (low-quality evidence).

Calcineurin inhibitors: no evidence of long term benefit

There is minimal evidence for calcineurin inhibitors, though they may reduce proteinuria in the short term. Lai et al. (1987) conducted a randomized prospective single blind study of ciclosporin in 19 patients with proteinuria > 1.5 g/day. Patients who received the drug had significant reduction of proteinuria, serum IgA, and increase of plasma albumin concentration compared with placebo. However, there was transient deterioration of renal function during treatment, despite within-range trough drug levels. The authors discourage indiscriminate use of ciclosporin in IgA nephropathy due to lack of efficacy and nephrotoxicity. A more recent study suggested that tacrolimus could induce remission of proteinuria in 14 patients with refractory IgA nephropathy, possibly by stabilizing podocyte cytoskeleton (Zhang, et al., 2012) as in other conditions (see Chapters 45, 58).

Azathioprine: ineffective

A retrospective analysis of 74 IgA nephropathy patients followed for 10 years shows that long-term azathioprine combined with low-dose prednisone did not alter the clinical course compared to untreated controls (Goumenos et al., 2003). However, in a subgroup of patients with heavy proteinuria > 3 g/day and baseline serum creatinine between 1.4 and 2.5 mg/dL, this immunosuppressive regimen reduced the risk of doubling serum creatinine compared to controls (27% versus 78%) and delayed progression to end-stage renal failure (17% versus 55%). The Japanese Paediatric IgA Nephropathy Treatment Study Group randomized 78 children with newly diagnosed early IgA nephropathy to receive either prednisolone, azathioprine, heparin-warfarin, and dipyridamole or the combination of heparin-warfarin, and dipyridamole only (Yoshikawa and Ito, 1999). The study was flawed by a lack of data on baseline proteinuria and creatinine clearance as well as blood pressure control in both groups. A recent prospective randomized study of 207 subjects showed that the addition of azathioprine to corticosteroids did not provide additional benefits in terms of renal survival versus corticosteroids alone in patients with proteinuria ≥ 1 g/day and plasma creatinine ≤ 2.0 mg/dL (Pozzi et al., 2010). Current data therefore suggest that the addition of azathioprine was ineffective and may even be potentially toxic.

Mycophenolate mofetil: probably ineffective

To date, four randomized clinical trials have been published on the use of mycophenolate mofetil (MMF) in IgA nephropathy, which add more controversy than consensus. Although these trials have produced conflicting results, they differ significantly in patient selection and treatment duration and deserve attention.

The first randomized study was conducted 62 Chinese patients with severe IgA nephropathy and urinary protein > 2.0 g/day receiving MMF or oral prednisone for at least 12 months (Chen et al., 2002). After 18 months' follow-up, the MMF group showed significant improvement in proteinuria and serum lipids than the prednisone group.

In a study of 34 Belgian patients with impaired renal function, histologic unfavourable criteria and arterial hypertension, after instituting salt restriction and ACEI therapy in all, MMF failed

to demonstrate a better beneficial effect after 3 years of evaluation (Maes et al., 2004). In a similar study that recruited patients with even more advanced renal insufficiency using MMF as a 'salvage' therapy, a worse outcome occurred in the MMF group (Frisch et al., 2005).

Tang et al. (2005) treated 40 Chinese patients with mild tubulointerstitial lesions with persistent proteinuria > 1 g/day despite full angiotensin blockade for 6 months. Twelve months after stopping MMF, the overall remission rate was significantly higher in MMF-treated patients whose proteinuria dropped to 62% of baseline, whereas urine protein in control patients increased to 120% of baseline. Serum interleukin-6 levels and, more intriguingly, *in vitro* binding of IgA to mesangial cells were elevated at baseline in both groups compared with normal healthy subjects; however, after MMF treatment these parameters were comparable to those of the healthy subjects. Serum interleukin-6 concentration and mesangial binding of IgA in patients who did not receive MMF showed no change. After 6 years' follow-up, the difference in proteinuria between the two groups was lost, but renal survival was significantly better in the MMF group (Tang et al., 2010). In another Italian study, a subset of IgA nephropathy patients with florid glomerular changes treated with MMF and steroid showed remission of proteinuria and reversal of progressive renal failure (Roccatello et al., 2012).

Overall, MMF appears to be effective in reducing proteinuria in Chinese but not Caucasoid subjects. Therefore, ethnic differences may be one possible reason to account for the differences observed in these studies. Another possibility is the mild histologic grade in Tang's study versus the moderate-to-severe grades in the studies by Maes and Frisch. Further observation and studies are needed to provide more definitive answers on the efficacy of MMF in IgA nephropathy. The KDIGO Clinical Practice Guideline for Glomerulonephritis (Kidney Disease: Improving Global Outcomes (KDIGO) Glomerulonephritis Work Group, 2012) suggested not using MMF in IgA nephropathy (low-quality evidence).

New therapies

Chapter 136 gives an overview of hypotheses of long term progression of renal disease, and chapter 99 lists recommendations for management.

In vitro studies shows that peroxisome proliferator-activated receptor-γ (PPAR-γ) agonist attenuates inflammatory response in activated tubular epithelial cells in IgA nephropathy by downregulating the expression of ATR1 (Xiao et al., 2009). Dual treatment of PPAR-γ and ARB provide synergistic effect in reducing inflammation and angiotensin II signalling in renal tubular epithelial cells and the therapeutic benefit is confirmed in an animal model of IgA nephropathy (Lai et al., 2011). It is possible that this drug acts at the level of the podocyte (Chapter 45).

Aliskiren, a direct renin inhibitor, confers an antiproteinuric effect in 25 IgA nephropathy patients with significant residual proteinuria despite receiving the adequate ARB treatment (Tang et al., 2012). Treatment for 12 months lowered the mean urinary protein:creatinine ratio by 26.3% with significant reductions in plasma renin activity, and serum interleukin-6 and transforming growth factor-β levels. The antiproteinuric efficacy of aliskiren on top of an ACEI/ARB has been confirmed in a randomized cross-over study of 22 patients (Szeto et al., 2013). However, combining aliskiren with an ARB has not been found to confer renoprotection and has been hailed to be potentially harmful due to the high incidence of hyperkalaemia and hypotension among type 2 diabetic subjects (Parving et al., 2012). Its application in the treatment of IgA nephropathy, therefore, remains to be investigated.

Enteric budesonide targeted to the Peyer's patches in the ileocaecal region has been shown to reduce proteinuria by 23% and modestly augment eGFR by 8% in 16 patients who received this new formulation for 6 months followed by 3 months' further observation (Smerud et al., 2011). Based on these encouraging results, a multicentre phase IIb trial is currently being planned in Europe.

Immunoglobulin A nephropathy after renal transplantation

Recurrence of mesangial IgA deposits in the renal allografts was first described by Berger et al. (1975). Subsequent studies reported a recurrence rate ranging from 13% to 60% of patients (Table 68.1) (Bachman et al., 1986; Odum et al., 1994; Hartung et al., 1995; Kessler et al., 1996; Frohnert et al., 1997; Ohmacht et al., 1997; Bumgardner et al., 1998; Freese et al., 1999; Andresdottir et al., 2001; Kim et al., 2001; Ponticelli et al., 2001; Wang et al., 2001; Briganti et al., 2002; Moriyama et al., 2005; Choy et al., 2003, 2006, 2007; Moroni et al., 2013). Great variation can partly be explained by the difference in biopsy policy of different transplant centres and the duration of follow-up. Most centres performed renal biopsy only when patients presented with clinical symptoms. This would potentially underestimate the rate of recurrence as patients who were clinically asymptomatic but with immunohistological changes in the graft kidneys would remain undiagnosed. In a Canadian epidemiologic study comprising 2026 sequential renal transplant recipients without loss to follow-up, IgA nephropathy was found to recur in 25.3% of patients after 15 years (Chailimpamontree et al., 2009). The cumulative risk of graft loss following the diagnosis of post-transplant glomerulonephritis (recurrent and *de novo* forms summed) was over sevenfold.

Recurrent disease after transplantation is also discussed in Chapter 289.

Clinical course

Recurrent disease exhibits considerable clinical similarities with primary IgA nephropathy. Microscopic haematuria and proteinuria are common presenting symptoms followed by slow decline in renal function. With increasing long-term data, it is apparent that recurrent disease is not as benign as had been reported previously (Berger et al., 1975). Graft loss from recurrence with histological features of diffuse mesangial proliferative expansion and glomerular sclerosis were reported between 2% and 16% depending on duration of follow up. Briganti et al. (2002) reported an estimated 10-year incidence of graft loss due to recurrent IgA nephropathy of 9.7% basing on data from the Australia and New Zealand Dialysis and Transplant Registry (ANZDATA) which contains 532 allograft recipients with primary IgA nephropathy.

The renal allograft survival of recurrent IgA nephropathy for the first 5 years post-transplant is better compared to patients with other primary diseases. Lim et al. (1993) reported a superior 5-year graft survival rate in patients with IgA nephropathy as compared to patients with other primary diseases. The superior graft survival of IgA nephropathy patients for the early post-transplant period

is no longer observed on longer follow-up. Ponticelli et al. (2001) reported a comparable 10-year graft survival for patient with IgA nephropathy. Choy et al. (2006) reported an inferior graft survival for primary IgA nephropathy patients with follow up beyond 12 years. At 15 years, IgA nephropathy had a higher cumulative incidence of graft failure with non-IgA nephropathy controls (Moroni et al., 2013). These observations suggest that impact of other factors including recurrent disease on graft survival becomes more apparent on long-term follow up and recurrent IgA nephropathy runs an indolent course similar to primary disease with favourable outcome in the initial 10 years post transplant and thereafter its contribution to graft loss becomes more significant (Kim et al., 2001; Ponticelli et al., 2001; Choy et al., 2003). Patients with prior graft loss due to recurrent IgA nephropathy have higher risk of recurrence in the second transplant (20–100%).

Potential risk factors for recurrence

Donor type

Pooling all available data from literature that contained information on graft recurrence and graft loss in relation to donor type showed a higher risk of disease recurrence amongst transplant recipients with related donors (common odds ratio 2.29, $P < 0.001$) but the risk of graft loss was not increased (common odds ratio 1.95, $P = 0.24$) (Choy and Lai, 2009). Given the fact that the graft survival of patients with primary IgA nephropathy is excellent for the first decade post transplant, it is inappropriate to refrain from living related donor transplantation even though there may be a slight risk of recurrence. In contrast, familial IgA nephropathy should be rigorously excluded in potential living related donors since this may be associated with high risk of development of the nephropathy in affected members with more severe pathology (Tam et al., 2010).

Human leucocyte antigens and degree of mismatch

No specific type of HLA has been identified to be predictive of recurrence. An analysis by ANZDATA studying 1306 patients with primary IgA nephropathy reported a higher risk of recurrent disease only in patients who received zero HLA mismatch living donor grafts (McDonald and Russ, 2006).

Latent IgA deposition from donor kidney

Incidental finding of glomerular mesangial IgA deposits in donor kidneys has been reported in 4–24 % of patients (Suzuki et al., 2003; Ji et al., 2004). These deposits usually disappear within 6 months post transplant if recipients do not have primary IgA nephropathy.

Serological and genetic factors

High level of aberrantly glycosylated IgA1 in recipients did not predict recurrence and no association of ACE gene (insertion/deletion) polymorphism was detected with recurrence (Coppo et al., 2007).

Prevention and management

Apparently, no effective therapy for prevention or treatment of recurrent IgA nephropathy is available at the moment. There is no evidence that any particular immunosuppressive regime alters the incidence or clinical course of recurrent disease. Systemic hypertension, glomerular hyperfiltration, and heavy proteinuria secondary to recurrent IgA nephropathy are detrimental to the graft function. Adequate blockade of the renin–angiotensin system provides a better graft survival for recurrent IgA nephropathy (Oka et al., 2000; Courtney et al., 2006). However, a recent retrospective study from Italy revealed a significant reduction of recurrence of IgA nephropathy from 1981 to 2010 correlating with mycophenolate treatment and triple immunosuppressive therapy (Moroni et al., 2013).

References

Alamartine, E., Sabatier, J. C., Guerin, C., *et al.* (1991). Prognostic factors in mesangial IgA glomerulonephritis: an extensive study with univariate and multivariate analyses. *Am J Kidney Dis*, 18, 12–19.

Andresdottir, M. B., Hoitsma, A. J., Assmann, K. J., *et al.* (2001). Favorable outcome of renal transplantation in patients with IgA nephropathy. *Clin Nephrol*, 56, 279–88.

Bachman, U., Biava, C., Amend, W., *et al.* (1986). The clinical course of IgA-nephropathy and Henoch-Schonlein purpura following renal transplantation. *Transplantation*, 42, 511–15.

Ballardie, F. W. and Roberts, I. S. (2002). Controlled prospective trial of prednisolone and cytotoxics in progressive IgA nephropathy. *J Amer Soc Nephrol*, 13, 142–8.

Bartosik, L. P., Lajoie, G., Sugar, L., *et al.* (2001). Predicting progression in IgA nephropathy. *Am J Kidney Dis*, 38, 728–35.

Berger, J., Yaneva, H., Nabarra, B., *et al.* (1975). Recurrence of mesangial deposition of IgA after renal transplantation. *Kidney Int*, 7, 232–41.

Beukhof, J. R., Kardaun, O., Schaafsma, W., *et al.* (1986). Toward individual prognosis of IgA nephropathy. *Kidney Int*, 29, 549–56.

Briganti, E. M., Russ, G. R., McNeil, J. J., *et al.* (2002). Risk of renal allograft loss from recurrent glomerulonephritis. *N Engl J Med*, 347, 103–9.

Bumgardner, G. L., Amend, W. C., Ascher, N. L., *et al.* (1998). Single-center long-term results of renal transplantation for IgA nephropathy. *Transplantation*, 65, 1053–60.

Cattran, D. C., Coppo, R., Cook, H. T., *et al.* (2009). The Oxford classification of IgA nephropathy: rationale, clinicopathological correlations, and classification. *Kidney Int*, 76, 534–45.

Cheng, J., Zhang, W., Zhang, X. H., *et al.* (2009a). ACEI/ARB therapy for IgA nephropathy: a meta analysis of randomised controlled trials. *Int J Clin Pract*, 63, 880–8.

Cheng, J., Zhang, X., Zhang, W., *et al.* (2009b). Efficacy and safety of glucocorticoids therapy for IgA nephropathy: a meta-analysis of randomized controlled trials. *Am J Nephrol*, 30, 315–22.

Choy, B. Y., Chan, T. M., and Lai, K. N. (2006). Recurrent glomerulonephritis after kidney transplantation. *Am J Transplant*, 6, 2535–42.

Choy, B. Y., Chan, T. M., Lo, S. K., *et al.* (2003). Renal transplantation in patients with primary immunoglobulin A nephropathy. *Nephrol Dial Transplant*, 18, 2399–404.

Choy, B. Y. and Lai, K. N. (2009). Recurrent IgA nephropathy in transplant. In K. N. Lai (ed.) *Recent Advances in IgA Nephropathy*, pp. 149–59. Singapore: World Scientific Press.

Coppo, R., Amore, A., Chiesa, M., *et al.* (2007). Serological and genetic factors in early recurrence of IgA nephropathy after renal transplantation. *Clin Transplant*, 21, 728–37.

Coppo, R. and D'Amico, G. (2005). Factors predicting progression of IgA nephropathies. *J Nephrol*, 18, 503–12.

Courtney, A. E., McNamee, P. T., Nelson, W. E., *et al.* (2006). Does angiotensin blockade influence graft outcome in renal transplant recipients with IgA nephropathy? *Nephrol Dial Transplant*, 21, 3550–4.

D'Amico, G., Ragni, A., Gandini, E., *et al.* (1993). Typical and atypical natural history of IgA nephropathy in adult patients. *Contrib Nephrol*, 104, 6–13.

El Karoui, K., Hill, G. S., Karras, A., *et al.* (2011). Focal segmental glomerulosclerosis plays a major role in the progression of IgA nephropathy. II. Light microscopic and clinical studies. *Kidney Int*, 79, 643–54.

Freese, P., Svalander, C., Norden, G., *et al.* (1999). Clinical risk factors for recurrence of IgA nephropathy. *Clin Transplant*, 13, 313–17.

Frohnert, P. P., Donadio, J. V., Jr., Velosa, J. A., *et al.* (1997). The fate of renal transplants in patients with IgA nephropathy. *Clin Transplant*, 11, 127–33.

Goto, M., Wakai, K., Kawamura, T., *et al.* (2009). A scoring system to predict renal outcome in IgA nephropathy: a nationwide 10-year prospective cohort study. *Nephrol Dial Transplant*, 24, 3068–74.

Harden, P. N., Geddes, C., Rowe, P. A., *et al.* (1995). Polymorphisms in angiotensin-converting-enzyme gene and progression of IgA nephropathy. *Lancet*, 345, 1540–2.

Hartung, R., Livingston, B., Excell, L., *et al.* (1995). Recurrence of IgA deposits/disease in grafts. An Australian Registry Survey 1980-1990. *Contrib Nephrol*, 111, 13–16.

Hunley, T. E., Julian, B. A., Phillips, J. A., *et al.* (1996). Angiotensin converting enzyme gene polymorphism: potential silencer motif and impact on progression in IgA nephropathy. *Kidney Int*, 49, 571–7.

Ji, S., Liu, M., Chen, J., *et al.* (2004). The fate of glomerular mesangial IgA deposition in the donated kidney after allograft transplantation. *Clin Transplant*, 18, 536–40.

Johnston, P. A., Brown, J. S., Braumholtz, D. A., *et al.* (1992). Clinico-pathological correlations and long-term follow-up of 253 United Kingdom patients with IgA nephropathy. A report from the MRC Glomerulonephritis Registry. *QJM*, 84, 619–27.

Kessler, M., Hiesse, C., Hestin, D., *et al.* (1996). Recurrence of immunoglobulin A nephropathy after renal transplantation in the cyclosporine era. *Am J Kidney Dis*, 28, 99–104.

Kidney Disease: Improving Global Outcomes (KDIGO) Glomerulonephritis Work Group (2012). KDIGO clinical practice guideline for glomerulonephritis. Kidney Int Suppl, 2(2), 139–274.

Kim, Y. S., Moon, J. I., Jeong, H. J., *et al.* (2001). Live donor renal allograft in end-stage renal failure patients from immunoglobulin A nephropathy. *Transplantation*, 71, 233–8.

Kobayashi, Y., Fujii, K., Hiki, Y., *et al.* (1986). Steroid therapy in IgA nephropathy: a prospective pilot study in moderate proteinuric cases. *QJM*, 61, 935–43.

Kobayashi, Y., Hiki, Y., Kokubo, T., *et al.* (1996). Steroid therapy during the early stage of progressive IgA nephropathy. A 10-year follow-up study. *Nephron*, 72, 237–42.

Lai, K. N., Chan, L. Y., Guo, H., *et al.* (2011). Additive effect of PPAR-gamma agonist and ARB in treatment of experimental IgA nephropathy. *Pediatr Nephrol*, 26, 257–66.

Lai, K. N., Lai, F. M., Ho, C. P., *et al.* (1986). Corticosteroid therapy in IgA nephropathy with nephrotic syndrome: a long-term controlled trial. *Clin Nephrol*, 26, 174–80.

Lai, K. N., Lai, F. M., Li, P. K., *et al.* (1987). Cyclosporin treatment of IgA nephropathy: a short term controlled trial. *Br Med J (Clin Res Ed)*, 295, 1165–8.

Lai, K. N., To, W. Y., Li, P. K., *et al.* (1996). Increased binding of polymeric lambda-IgA to cultured human mesangial cells in IgA nephropathy. *Kidney Int*, 49, 839–45.

Lv, J., Xu, D., Perkovic, V., *et al.* (2012). TESTING Study Group. Corticosteroid therapy in IgA nephropathy. *J Am Soc Nephrol*, 23, 1108–16.

Magistroni, R., Furci, L., Leonelli, M., *et al.* (2006). A validated model of disease progression in IgA nephropathy. *J Nephrol*, 19, 32–40.

Maixnerova, D., Merta, M., Reiterova, J. *et al.* (2008). The influence of two megsin polymorphisms on the progression of IgA nephropathy. *Folia Biol (Praha)*, 54, 40–5.

McDonald, S. P. and Russ, G. R. (2006). Recurrence of IgA nephropathy among renal allograft recipients from living donors is greater among those with zero HLA mismatches. *Transplantation*, 82, 759–62.

Mitsuiki, K., Harada, A., Okura, T., *et al.* (2007). Histologically advanced IgA nephropathy treated successfully with prednisolone and cyclophosphamide. *Clin Exp Nephrol*, 11, 297–303.

Moriyama, T., Nitta, K., Suzuki, K., *et al.* (2005). Latent IgA deposition from donor kidney is the major risk factor for recurrent IgA nephropathy in renal transplantation. *Clin Transplant*, 19 Suppl 14, 41–8.

Moriyama, T. and Nitta, K. (2011). Tonsillectomy and steroid pulse therapy for IgA nephropathy. *Tohoku J Exp Med*, 224, 243–50.

Moroni, G., Longhi, S., Quaglini, S., *et al.* (2013). The long-term outcome of renal transplantation of IgA nephropathy and the impact of recurrence on graft survival. *Nephrol Dial Transplant* , 28, 1305–14.

Odum, J., Peh, C. A., Clarkson, A. R., *et al.* (1994). Recurrent mesangial IgA nephritis following renal transplantation. *Nephrol Dial Transplant*, 9, 309–12.

Ohmacht, C., Kliem, V., Burg, M., *et al.* (1997). Recurrent immunoglobulin A nephropathy after renal transplantation: a significant contributor to graft loss. *Transplantation*, 64, 1493–6.

Oka, K., Imai, E., Moriyama, T., *et al.* (2000). A clinicopathological study of IgA nephropathy in renal transplant recipients: beneficial effect of angiotensin-converting enzyme inhibitor. *Nephrol Dial Transplant*, 15, 689–95.

Parving, H. H., Brenner, B. M., McMurray, J. J., *et al.* (2012). ALTITUDE Investigators. Cardiorenal end points in a trial of aliskiren for type 2 diabetes. *N Engl J Med*, 367, 2204–13.

Piccoli, A., Codognotto, M., Tabbi, M. G. *et al.* (2010). Influence of tonsillectomy on the progression of mesangioproliferative glomerulonephritis. *Nephrol Dial Transplant*, 25, 2583–9.

Ponticelli, C., Traversi, L., Feliciani, A., *et al.* (2001). Kidney transplantation in patients with IgA mesangial glomerulonephritis. *Kidney Int*, 60, 1948–54.

Pozzi, C., Andrulli, S., Pani, A., *et al.* (2010). Addition of Azathioprine to Corticosteroids Does Not Benefit Patients with IgA Nephropathy. *J Am Soc Nephrol*, 21, 1783–90.

Pozzi, C., Bolasco, P. G., Fogazzi, G. B. *et al.* (1999). Corticosteroids in IgA nephropathy: a randomised controlled trial. *Lancet*, 353, 883–7.

Radford, M. G., Jr., Donadio, J. V., Jr., Bergstralh, E. J., *et al.* (1997). Predicting renal outcome in IgA nephropathy. *J Am Soc Nephrol*, 8, 199–207.

Rasche, F. M., Klotz, C. H., Czock, D., *et al.* (2003). Cyclophosphamide pulse therapy in advanced progressive IgA nephropathy. *Nephron Clin Pract*, 93, c131–c136.

Rauta, V., Finne, P., Fagerudd, J., *et al.* (2002). Factors associated with progression of IgA nephropathy are related to renal function—a model for estimating risk of progression in mild disease. *Clin Nephrol*, 58, 85–94.

Reich, H. N., Troyanov, S., Scholey, J. W., *et al.* (2007). Remission of proteinuria improves prognosis in IgA nephropathy. *J Amer Soc Nephrol*, 18, 3177–83.

Reid, S., Cawthon, P. M., Craig, J. C., *et al.* (2011). Non-immunosuppressive treatment for IgA nephropathy. *Cochrane Database Syst Rev*, 3, CD003962.

Roberts, I. S., Cook, H. T., Troyanov, S., *et al.* (2009). The Oxford classification of IgA nephropathy: pathology definitions, correlations, and reproducibility. *Kidney Int*, 76, 546–56.

Samuels, J.A., Strippoli, G.F., Craig, J.C., Schena, F.P., and Molony, D.A. (2004). Immunosuppressive treatments for immunoglobulin A nephropathy: a meta-analysis of randomized controlled trials. *Nephrology*, 9, 177–85.

Shen, P., He, L., and Huang, D. (2008). Clinical course and prognostic factors of clinical early IgA nephropathy. *Neth J Med*, 66, 242–7.

Smerud, H. K., Barany, P., Lindstrom, K., *et al.* (2011). New treatment of IgA nephropathy: enteric budesonide targeted to the ileocecal region ameliorates proteinuria. *Nephrol Dial Transplant*, 26, 3237–42.

Suzuki, K., Honda, K., Tanabe, K., *et al.* (2003). Incidence of latent mesangial IgA deposition in renal allograft donors in Japan. *Kidney Int*, 63, 2286–94.

Szeto, C. C., Lai, F. M., To, K. F., *et al.* (2001). The natural history of immunoglobulin a nephropathy among patients with hematuria and minimal proteinuria. *Am J Med*, 110, 434–7.

Szeto, C. C., Kwan, B. C., Chow, K. M., *et al.* (2013). The safety and short-term efficacy of aliskiren in the treatment of immunoglobulin a nephropathy—a randomized cross-over study. *PLoS One*, 8, e62736.

Tam, K. Y., Leung, J. C., Chan, L. Y., *et al.* (2010). In vitro enhanced chemotaxis of CD25+ mononuclear cells in patients with familial IgAN

through glomerulotubular interactions. *Am J Physiol Renal Physiol*, 299, F359–68.

Tang, S., Leung, J. C., Chan, L. Y. *et al.* (2005). Mycophenolate mofetil alleviates persistent proteinuria in IgA nephropathy. *Kidney Int*, 68, 802–12.

Tang, S. C. and Lai, K. N. (2009). The ubiquitin-proteasome pathway and IgA nephropathy: a novel link? *Kidney Int*, 75, 457–9.

Tang, S. C., Lin, M., Tam, S., *et al.* (2012). Aliskiren combined with losartan in immunoglobulin A nephropathy: an open-labeled pilot study. *Nephrol Dial Transplant*, 27, 613–18.

Tang, S. C., Tang, A. W., Wong, S. S., *et al.* (2010). Long-term study of mycophenolate mofetil treatment in IgA nephropathy. *Kidney Int*, 77, 543–9.

Tumlin, J. A., Lohavichan, V., and Hennigar, R. (2003). Crescentic, proliferative IgA nephropathy: clinical and histological response to methylprednisolone and intravenous cyclophosphamide. *Nephrol Dial Transplant*, 18, 1321–9.

Wakai, K., Kawamura, T., Endoh, M., *et al.* (2006). A scoring system to predict renal outcome in IgA nephropathy: from a nationwide prospective study. *Nephrol Dial Transplant*, 21, 2800–8.

Wang, A. Y., Lai, F. M., Yu, A. W., *et al.* (2001). Recurrent IgA nephropathy in renal transplant allografts. *Am J Kidney Dis*, 38, 588–96.

Wang, Y., Chen, J., Wang, Y., *et al.* (2011). A meta-analysis of the clinical remission rate and long-term efficacy of tonsillectomy in patients with IgA nephropathy. *Nephrol Dial Transplant*, 26, 1923–31.

Xia, Y., Li, Y., Du, Y., *et al.* (2006). Association of MEGSIN 2093C-2180T haplotype at the 3' untranslated region with disease severity and progression of IgA nephropathy. *Nephrol Dial Transplant*, 21, 1570–4.

Xiao, J., Leung, J. C., Chan, L. Y., *et al.* (2009). Crosstalk between peroxisome proliferator-activated receptor-gamma and angiotensin II in renal tubular epithelial cells in IgA nephropathy. *Clin Immunol*, 132, 266–76.

Yamamoto, R., Nagasawa, Y., Shoji, T., *et al.* (2009). A candidate gene approach to genetic prognostic factors of IgA nephropathy—a result of Polymorphism REsearch to DIstinguish genetic factors Contributing To progression of IgA Nephropathy (PREDICT-IgAN). *Nephrol Dial Transplant*, 24, 3686–94.

Zhang, Q., Shi, S.F., Zhu, L., *et al.* (2012). Tacrolimus improves the proteinuria remission in patients with refractory IgA nephropathy. *Am J Nephrol*, 35, 312–20.

Zhou, Y. H., Tang, L. G., Guo, S. L., *et al.* (2011). Steroids in the treatment of IgA nephropathy to the improvement of renal survival: a systematic review and meta-analysis. *PLoS One*, 6, e18788.

CHAPTER 69

Immunoglobulin A nephropathy: pathogenesis

Kar Neng Lai and Sydney C. W. Tang

Aberrant structure of the immunoglobulin A molecule

The primary defect of IgA nephropathy seems to lie in the structure of IgA molecule. In humans, IgA1 represents one of the two structurally and functionally distinct subclasses of IgA. Unlike IgA2, IgM, and IgG, IgA1 has heavy chains that contain a unique hinge-region segment between the first and second constant-region domains, which is the site of attachment of three to five *O*-linked glycan chains. *O*-glycans on circulatory IgA1 consist of *N*-acetylgalactosamine (GalNAc) with a β1,3-linked galactose; both residues may be sialylated. Carbohydrate composition of *O*-linked glycans on normal serum IgA1 is variable (Figs. 69.1, 69.2). Prevailing forms include the galactose-GalNAc disaccharide and its mono- and disialylated forms. Galactose-deficient variants with terminal GalNAc or sialylated GalNAc are more common in IgA nephropathy patients. These aberrantly glycosylated galactose-deficient forms predominate in glomerular IgA deposits

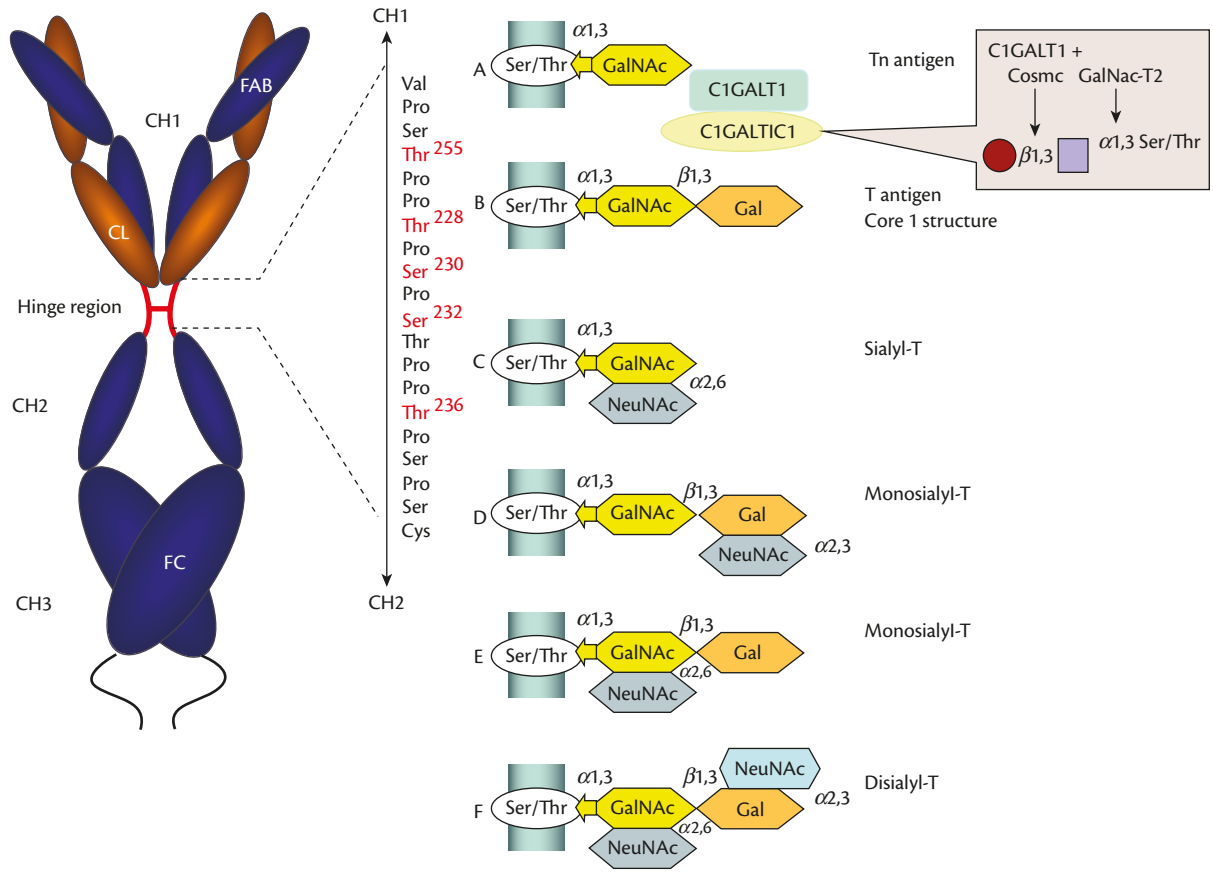

Fig. 69.1 The microheterogeneity of the *O*-glycans at the hinge region of IgA1 molecule. *O*-Glycosylation of protein is initiated by the addition of GalNAc to serine or threonine residues through the activity of UDP-*N*-acetyl-α-D-galactosamine:polypeptide *N*-acetylgalactosaminyltransferases (pp-GalNAc-Ts), which is followed by β-galactosylation through core 1 synthase 1, also called glycoprotein-*N*-acetylgalactosamine 3-β-galactosyltransferase 1, core 1 β3-Gal-T, or T-synthase (C1GALT1). The moiety is galactosylated in the β1,3 configuration by core-1 β1-3 galactosyltransferase-1 (C1Gal-T1), which requires the presence of the core-1-β3-Gal-T-specific molecular chaperone (Cosmc). Cosmc is encoded by the *CIGALT1C1* gene.

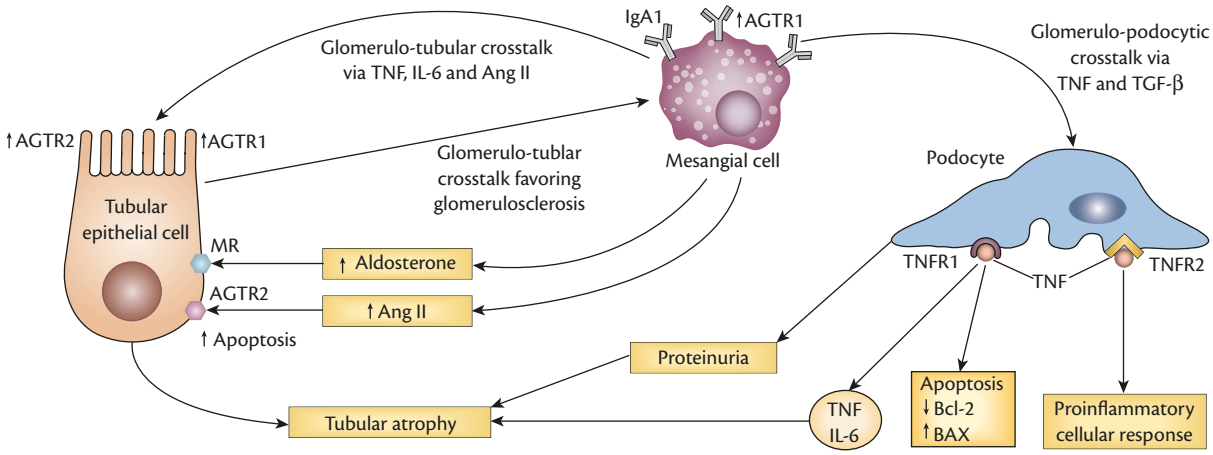

Fig. 69.2 Proposed pathways leading to glomerular damage, podocyte dysfunction, and tubulointerstitial injury in IgAN. Mesangial deposition of IgA-IC leads to activation of mesangial cells (HMC), triggering mesangial cell proliferation and release of proinflammatory and profibrotic mediators including tumour necrosis factor alpha (TNF-α), transforming growth factor beta (TGF-β), interleukin 6 (IL-6), and angiotensin II (AngII). There is insignificant binding of IgA-IC to podocytes or tubular epithelial cells (TEC). Tumour necrosis factor-α released from mesangium after IgA deposition induces TNF-α synthesis by podocytes. Podocyte-derived TNF-α further upregulates the TNF-α production in an autocrine manner. TNF-α upregulates the expression of TNF-α receptors. The binding to TNF-α receptor 1 (TNFR1) leads to IL-6 synthesis and apoptosis, while binding to TNF-α receptor 2 (TNFR2) maintains proinflammatory cellular responses. Podocytes enhance interstitial damage in IgAN by amplifying the TEC activation with enhanced TNF-α synthesis. In the renal tubulointerstitium the interaction of AngII and angiotensin receptor subtype-1 (AGTR1) will lead to inflammatory responses through the upregulation of PKC and MAPK pathways. The activation of angiotensin receptor subtype 2 (AGTR2) leads to apoptosis through downregulation of the MAPK pathway. The aldosterone released from HMC following IgA-IC deposition acts synergistically with AngII to induce apoptosis in renal tubular epithelial cells. The mesangial-derived AngII maintains the tubulointerstitial injury. MR represents mineralocorticoid receptor.
From Lai (2012) with permission with permission from Nature Publishing Group.

and circulating complexes in IgA nephropathy (Tomana et al., 1997, 1999; Hiki et al., 2001).

The synthesis of *O*-linked glycans of circulatory IgA1 follows a step-wise manner. *O*-Glycosylation of protein is initiated by the addition of GalNAc to serine or threonine residue through the activity of UDP-*N*-acetyl-α-D-galactosamine: polypeptide *N*-acetylgalactosaminyltransferases (pp-GalNAc-Ts), which is followed by β-galactosylation through core 1 synthase 1, also called glycoprotein-*N*-acetyl-galactosamine 3-β-galactosyltransferase 1, core 1 β3-Gal-T, or T-synthase (C1GALT1). The moiety is galactosylated in the β1,3 configuration by core-1 β1-3 galactosyltransferase-1 (C1Gal-T1), which requires the presence of the core-1-β3-Gal-T-specific molecular chaperone (Cosmc). The formation of the glycan structure is accomplished by the α-2,6-sialyltransferase 2 (ST6GalNAc2) and α-2,3-sialyltransferase (ST3Gal) that attach sialic acid to the GalNAc and galactose residues, respectively. Sialic acid may also be added to terminal GalNAc by ST6GalNAc2. This represent a terminal step of *O*-glycan formation as the addition prevents any further modification of the molecule (Raska et al., 2007; Suzuki et al., 2008).

Genes encoding C1GALT1, Cosmc (C1GALT1C1), and ST6GalNAc2 are located in chromosome 7p13–14, chromosome Xq24, and chromosome 17q25.1, respectively. Earlier Chinese and Italian studies reported risk haplotypes in ST6GALNAc2 and C1GALT1 for IgAN (Li et al., 2007; Pirulli et al., 2009; Zhu et al., 2009). However, later studies revealed that raised serum galactose-deficient IgA1 was derived from IgA1-producing cells and the serum level correlated with that in supernatant of cultured IgA1-producing cells isolated from peripheral blood of the same IgAN patients (Suzuki et al., 2008). These data suggest that the aberrant glycosylation in IgAN is an intrinsic and specific defect originated from IgA1-producing cells instead of attribution to modification of IgA1 during the immune complexes formation. The expression and enzymatic activity of C1GALT1 appear to be dissociated from *O*-glycosylation in IgAN (Buck et al., 2008). Such aberrant glycosylation does not exist in other glycoproteins with *O*-linked glycans, such as IgD (Smith et al., 2006). These observations indicate the IgA1 glycosylation defect lies in the upstream regulatory pathway(s) rather than in glycosylation enzymes located in the downstream of IgA synthesis. However, a study by Suzuki et al. (2008) in EBV-immortalized cells from patients with IgAN demonstrating a decrease in C1Gal-T1 activity and an increase in ST6GalNAc2 favours premature sialylation.

Undergalactosylated IgA1 molecules are prone to self-aggregate and to form complexes with IgG antibodies (Tomana et al., 1997, 1999). Epitopes at the hinge region now exposed in the absence of galactose are recognized by IgG and IgA1 with antiglycan specificities (Suzuki et al., 2009). IgA-IC are formed following the binding of glycan-specific IgG from IgAN patients with undergalactosylated IgA1. A possibility of mesangial IgA1-IC formation *in situ* following the initial deposition of IgA1 alone has also been raised.

The binding of plasma polymeric IgA (pIgA) to human mesangial cells is charge dependent. pIgA from IgA nephropathy patients with the highest net anionic charge binds more to human mesangial cells. Pre-incubation with polyanion decreases the binding of pIgA1 to mesangial cells indicating the anionic charge of IgA1 plays an important role in mesangial deposition (Leung et al., 2001). The over-sialylation of the IgA increases the negative charge and also enhances steric hindrance to binding (Leung et al., 1999).

Immunoglobulin A receptors

There are five known IgA receptors: FcαR1 (CD89), asialoglycoprotein receptor (ASGPR), polymeric Ig receptors (pIgR), transferrin

receptor (TfR), and Fcα/μ receptor. The FcαR1 binds both the monomeric and dimeric forms of IgA1 and IgA2 (Monteiro et al., 1992; Morton et al., 1996). Transfection studies in leucocytes showed that the FcαR1 does not bind IgG (Reterink et al., 1997). FcαR1 was originally found to be expressed by neutrophils, monocytes, macrophages, and eosinophils (Monteiro et al., 1990). It was proposed that FcαR1 plays a role in the removal of IgA-antigen complexes from the circulation (Grossetete et al., 1998). Previous works have suggested that mesangial cells possess Fc receptors for IgA (Mostov et al., 1984; Gomez-Guerrero et al., 1993). The pathogenic role of this receptor was suggested by the development of mesangial IgA deposits, glomerular and interstitial macrophage infiltration, haematuria and mild proteinuria in transgenic mice expressing human CD89 (Launay et al., 2000). Moura et al. (2008) proposed that, as a second event, activation of the classic, FcRγ-associated transmembrane FcαRI expressed on circulating myeloid leucocytes takes place. FcαRI/γ2 cross-linking in human FcαRI transgenic animals promotes disease progression by enhancing leucocyte chemotaxis and cytokine production. However, other investigators failed to demonstrate the expression of FcαR1 by human mesangial cells (Diven et al., 1998; Leung et al., 2000), despite the fact that mesangial cells showed Fc-dependent IgA binding that was saturable and dose dependent.

The asialoglycoprotein receptor is a C-type lectin that recognizes galactose and *N*-acetylgalactosamine residues of desialylated glycoproteins and mediates endocytosis of serum glycoproteins (Stockert, 1995). The human ASGPR is an integral transmembrane glycoprotein composed of two units, H1 and H2. Although ASGPR is thought to be exclusively present in liver cells, several investigators have shown that mRNA for rat RHL-1 and RHL2/3 are widely expressed in different tissues and cell lines (Pacifico et al., 1995; Park et al., 1998). The absence of galactose in galactose-deficient IgA1 with its anionic charge reduces the hepatic clearance of IgA1 leading to increased serum IgA1 in IgA nephropathy (Leung et al., 1999). Gomez-Guerrero et al. (1998) first demonstrated that human and rat mesangial cells were able to specifically bind, internalize, and degrade iodine-125-labelled asialo-orosomucoid that was rich in terminal galactose. They also detected RHL-1 and RHL-2 transcripts in RNA extracted from rat mesangial cells. With IgA1 partially inhibiting the binding of asialo-orosomucoid to mesangial cells, they concluded that human mesangial cells expressed ASGPR.

pIgR is an integral membrane secretory component localized on the basolateral surface of secretory epithelial cells. It mediates the transepithelial transport of pIg, particularly, pIgA (Piskurich et al., 1997). pIgR is detected in most human secretory epithelia (Krajci et al., 1989). The pIgR neutralizes extracellular and intracellular pathogens in mucous membranes by epithelial transport of pIgA-pathogen complexes and then excretes them via epithelial transcytosis (Mostov et al., 1984).

Transferrin receptor (TfR) has been suggested as an IgA1 receptor as TfR binds IgA1 but not IgA2, co-localizes with mesangial IgA1 deposits, and is overexpressed in patients with IgA nephropathy (Moura et al., 2001, 2004). However, TfR is expressed ubiquitously in different kidney cells. Fcα/μ receptor was also examined as novel IgA1 receptor on mesangial cells based on *in vitro* culture studies (McDonald et al., 2002). Subsequent in-depth studies failed to detect these five IgA receptors in mesangial cells, podocytes or renal tubular epithelial cells (Leung et al., 2000; Chan et al., 2005; Lai et al., 2008). Hence, the predominant binding of human IgA to human mesangial cells is mediated by other mechanisms yet to be revealed.

Immunoglobulin A immune complexes

Early studies found high titres of IgA-ICs and a low frequency of IgG-ICs in 30–70% of adult and paediatric patients (Coppo et al., 1995). The former is present during acute mucosal infection and in remission, while the latter appears only in relapses. Circulating IgA-fibronectin aggregates are useful as a serologic marker for IgA nephropathy in Caucasian patients, but their values could not be reproduced in Chinese subjects (Lai et al., 1996). More recently, soluble CD89–IgA complexes are identified as a potential new biomarker of risk of progression. In this Swedish study (Vuong et al., 2010), there was no difference in levels of sCD89–pIgA complexes between IgA nephropathy patients, healthy controls, and matched subjects with biopsy-proven glomerulonephritis from non-IgA nephropathy causes. However, within the IgA nephropathy group, there was a significant association between levels of sCD89–pIgA complexes and the likelihood of developing progressive renal disease. Such findings have implications for risk stratification and suggest a role for CD89 in the formation of pathogenic IgA-ICs in IgA nephropathy. Animal data suggest IgA1 interacts with CD89 on mononuclear cells that induces the release of sCD89 and the formation of IgA1–CD89 complexes. These complexes then interact with the transferrin receptor (CD71/TfR1) on mesangial cells and further enhance the expression of TfR1 via transglutaminase-2 (TGase2). TfR1 and TGase2 were both shown to bind sCD89, but also to directly interact with each other, providing an amplification step for IgA1 accumulation and inflammation in the kidney (Berthelot et al., 2012).

T and B cells in circulation

Patients with IgA nephropathy have increased number of IgA-bearing B cells and activated Tα helper cells in circulation. They demonstrate increase of both circulating Th1 and Th2 T lymphocyte subsets (Lai et al., 1994a). The cytokine expression in these cells is characterized by a predominance of interleukin-4, interleukin-5, interleukin-10, and interleukin-13 belonging to Th2 cells (Lai et al., 1994a; Ebihara et al., 2001). The increased interleukin-4 production may explain hyperproduction of IgA while the enhanced interleukin-5 production favours the IgA isotype switch and differentiation (Lai et al., 1994b). The proportion of γδT cells in peripheral blood mononuclear cells is high in IgA nephropathy and correlates well with surface IgA-positive B cells. Both γδ− and CD4-positive T cells produce a large amount of transforming growth factor-β1, which induces IgA class switching on B cells (Lai et al., 1994c; Toyabe et al., 2001).

Plasma IgA levels are determined by the rate of IgA production, uptake by leucocytes, and removal by hepatocytes. In IgA nephropathy, there is increased binding of endogenous IgA to circulating granulocytes and monocytes (Lai et al., 2002). FcαR1 expression on leucocytes is increased, independently of plasma IgA levels, as FcαR1 is not saturated in leucocytes, because of internalization of IgA after uptake. There is binding mechanism other than FcαR1 for pIgA uptake by leucocytes. Migration and/or sequestration of 'activated' leucocytes with predominant λ-IgA in the mononuclear

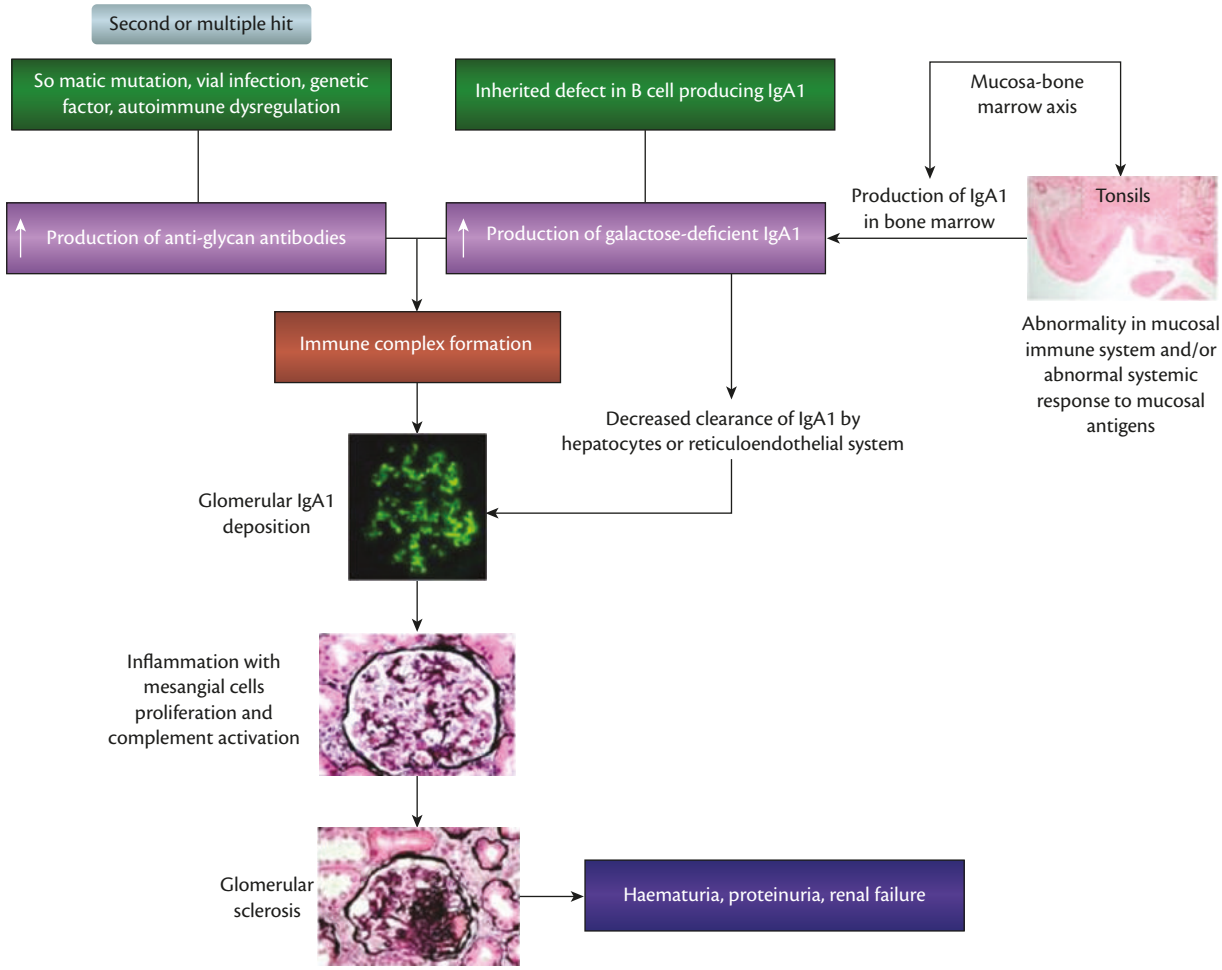

Fig. 69.3 Proposed pathways involved in mesangial IgA deposition in IgAN: multi-hit mechanism. Fundamental to immune complex formation is the enhanced synthesis of aberrant IgA1 with undergalactosylation (hit-1). Genetic factors heavily influence the production of undergalactosylated IgA1 and familial clustering has been well recognized. However, the presence these IgA1 *O*-glycoforms alone is insufficient to cause IgAN. The second hit is the formation of glycan-specific IgG and IgA antibodies that recognize the undergalactosylation IgA molecule (hit-2). These antibodies often with reactivity against antigens from extrinsic microorganisms may arise from recurrent mucosal infection (subsequent hits). Emerging evidence indicates that B cells in the mucosal infections, particularly in tonsillitis, may produce the nephritogenic IgA1. With increased immune complex formation and its decreased clearance, IgA1 (mainly polymeric in nature) binds to glomerular mesangium via yet unidentified receptor. Glomerular IgA1 deposits trigger the local production of cytokines and growth factors, leading to mesangial cell activation and complement activation.

phagocytic system or inflammatory tissues, after the initial binding of λ-pIgA occurs in IgA nephropathy.

Bone marrow–mucosa axis

Most patients with IgA nephropathy have a higher memory repertoire of IgA-bearing B cells in the bone marrow. The displacement of mucosal B cells to systemic lymphoid organs and bone marrow may arise from abnormal trafficking of lymphocytes along the mucosa-bone marrow axis involving changes of chemokines and adhesion molecules (Yu et al., 2010). The connection between the bone marrow compartment and the mucosal immune system acts through the trafficking of antigen presenting cells and/or antigen-specific lymphocytes.

An increased synthesis of both monomeric and pIgA1 occurs in IgA nephropathy with increased number of IgA1-producing plasma cells (van den Wall Bake et al., 1988, 1989). A shift towards IgA1 subclass is present in circulating IgA and mesangial deposits may originate from the bone marrow (Harper et al., 1994a). High serum levels of IgA, IgA-immune complexes, and hyper-responsiveness of lymphocytes to antigens, *in vitro* and *in vivo*, are present in these patients. An abnormal systemic response to tetanus toxoid immunization has been demonstrated in IgA nephropathy (Layward et al., 1992). Animal studies suggest that bone marrow-derived Th1 cells initiate the disease activity and mucosal IgA responses to antigens are altered by Th2-biased background or dysregulation of innate immunity in this disease (Suzuki and Tomino, 2007; Suzuki et al., 2007). These data suggest that the excess marrow-derived IgA may be of 'mucosal' type pIgA, which has abnormal access to the circulation and hence the mesangium.

Increased percentage and number of IgA-positive cells are found in tonsillar tissues of IgA nephropathy patients (Bene et al., 1991). The germinal centres of tonsils in these patients are constituted by follicular dendritic cells with preferential IgA1 localization (Kusakari et al., 1994). A reduced expression of J chain mRNA in duodenal IgA plasma cells is found in IgA nephropathy (Harper

et al., 1994b). Clinical exacerbation of the disease with macrohaematuria is frequently associated with mucosal infection.

Glomerulo-podocyte-tubular crosstalk

The mechanism by which mesangial IgA-triggered inflammation leads to the varied types and rates of glomerular lesion is still not well understood, but some hypotheses are summarized in Fig. 69.2 and reviewed by Lai (2012). Fig. 69.3 summarizes our view on the pathogenesis of IgA nephropathy.

References

Bene, M. C., Hurault De, L. B., Kessler, M., *et al.* (1991). Confirmation of tonsillar anomalies in IgA nephropathy: a multicenter study. *Nephron*, 58, 425–8.

Berthelot, L., Papista, C., Maciel, T.T., *et al.* (2012). Transglutaminase is essential for IgA nephropathy development acting through IgA receptors. *J Exp Med*, 209, 793–806.

Buck, K. S., Smith, A. C., Molyneux, K., *et al.* (2008). B-cell O-galactosyltransferase activity, and expression of O-glycosylation genes in bone marrow in IgA nephropathy. *Kidney Int*, 73, 1128–36.

Chan, L. Y., Leung, J. C., Tsang, A. W., *et al.* (2005). Activation of tubular epithelial cells by mesangial-derived TNF-alpha: glomerulotubular communication in IgA nephropathy. *Kidney Int*, 67, 602–12.

Coppo, R., Amore, A., Gianoglio, B., *et al.* (1995). Macromolecular IgA and abnormal IgA reactivity in sera from children with IgA nephropathy. Italian Collaborative Paediatric IgA Nephropathy Study. *Clin Nephrol*, 43, 1–13.

Diven, S. C., Caflisch, C. R., Hammond, D. K., *et al.* (1998). IgA induced activation of human mesangial cells: independent of FcalphaR1 (CD 89). *Kidney Int*, 54, 837–47.

Ebihara, I., Hirayama, K., Yamamoto, S., *et al.* (2001). Th2 predominance at the single-cell level in patients with IgA nephropathy. *Nephrol Dial Transplant*, 16, 1783–9.

Gomez-Guerrero, C., Duque, N., and Egido, J. (1998). Mesangial cells possess an asialoglycoprotein receptor with affinity for human immunoglobulin A. *J Am Soc Nephrol*, 9, 568–76.

Gomez-Guerrero, C., Gonzalez, E., and Egido, J. (1993). Evidence for a specific IgA receptor in rat and human mesangial cells. *J Immunol*, 151, 7172–81.

Grossetete, B., Launay, P., Lehuen, A., *et al.* (1998). Down-regulation of Fc alpha receptors on blood cells of IgA nephropathy patients: evidence for a negative regulatory role of serum IgA. *Kidney Int*, 53, 1321–35.

Harper, S.J., Allen, A.C., Layward, L., *et al.* (1994a). Increased immunoglobulin A and immunoglobulin A1 cells in bone marrow trephine biopsy specimens in immunoglobulin A nephropathy. *Am J Kidney Dis*, 24, 888–92.

Harper, S. J., Pringle, J. H., Wicks, A. C. *et al.* (1994b). Expression of J chain mRNA in duodenal IgA plasma cells in IgA nephropathy. *Kidney Int*, 45, 836–44.

Hiki, Y., Odani, H., Takahashi, M., *et al.* (2001). Mass spectrometry proves under-O-glycosylation of glomerular IgA1 in IgA nephropathy. *Kidney Int*, 59, 1077–85.

Krajci, P., Solberg, R., Sandberg, M., *et al.* (1989). Molecular cloning of the human transmembrane secretory component (poly-Ig receptor) and its mRNA expression in human tissues. *Biochem Biophys Res Commun*, 158, 783–9.

Kusakari, C., Nose, M., Takasaka, T., *et al.* (1994). Immunopathological features of palatine tonsil characteristic of IgA nephropathy: IgA1 localization in follicular dendritic cells. *Clin Exp Immunol*, 95, 42–8.

Lai, K. N., Chan, L. Y., Tang, S. C., *et al.* (2002). Characteristics of polymeric lambda-IgA binding to leukocytes in IgA nephropathy. *J Am Soc Nephrol*, 13, 2309–19.

Lai, K. N., Ho, R. T., Lai, C. K., *et al.* (1994a). Increase of both circulating Th1 and Th2 T lymphocyte subsets in IgA nephropathy. *Clin Exp Immunol*, 96, 116–21.

Lai, K. N., Ho, R. T., Leung, J. C., *et al.* (1994b). CD4-positive cells from patients with IgA nephropathy demonstrate increased mRNA of cytokines that induce the IgA switch and differentiation. *J Pathol*, 174, 13–22.

Lai, K. N., Ho, R. T., Leung, J. C., *et al.* (1994c). Increased mRNA encoding for transforming factor-beta in CD4+ cells from patients with IgA nephropathy. *Kidney Int*, 46, 862–8.

Lai, K. N., Leung, J. C., Chan, L. Y., *et al.* (2008). Activation of podocytes by mesangial-derived TNF-alpha: glomerulo-podocytic communication in IgA nephropathy. *Am J Physiol Renal Physiol*, 294, F945–F955.

Lai, K. N., To, W. Y., Leung, J. C., *et al.* (1996). Serologic study of immunoglobulin A-fibronectin aggregates in immunoglobulin A nephropathy. *Am J Kidney Dis*, 27, 622–30.

Launay, P., Grossetete, B., Arcos-Fajardo, M., *et al.* (2000). Fcalpha receptor (CD89) mediates the development of immunoglobulin A (IgA) nephropathy (Berger's disease). Evidence for pathogenic soluble receptor-Iga complexes in patients and CD89 transgenic mice. *J Exp Med*, 191, 1999–2009.

Layward, L., Allen, A. C., Harper, S. J., *et al.* (1992). Increased and prolonged production of specific polymeric IgA after systemic immunization with tetanus toxoid in IgA nephropathy. *Clin Exp Immunol*, 88, 394–8.

Leung, J. C., Poon, P. Y., and Lai, K. N. (1999). Increased sialylation of polymeric immunoglobulin A1: mechanism of selective glomerular deposition in immunoglobulin A nephropathy? *J Lab Clin Med*, 133, 152–60.

Leung, J. C., Tang, S. C., Lam, M. F., *et al.* (2001). Charge-dependent binding of polymeric IgA1 to human mesangial cells in IgA nephropathy. *Kidney Int*, 59, 277–85.

Leung, J. C., Tsang, A. W., Chan, D. T., *et al.* (2000). Absence of CD89, polymeric immunoglobulin receptor, and asialoglycoprotein receptor on human mesangial cells. *J Am Soc Nephrol*, 11, 241–9.

Li, G. S., Zhang, H., Lv, J. C., *et al.* (2007). Variants of C1GALT1 gene are associated with the genetic susceptibility to IgA nephropathy. *Kidney Int*, 71, 448–53.

McDonald, K. J., Cameron, A. J., Allen, J. M., *et al.* (2002). Expression of Fc alpha/mu receptor by human mesangial cells: a candidate receptor for immune complex deposition in IgA nephropathy. *Biochem Biophys Res Commun*, 290, 438–42.

Monteiro, R. C., Cooper, M. D., and Kubagawa, H. (1992). Molecular heterogeneity of Fc alpha receptors detected by receptor-specific monoclonal antibodies. *J Immunol*, 148, 1764–70.

Monteiro, R. C., Kubagawa, H., and Cooper, M. D. (1990). Cellular distribution, regulation, and biochemical nature of an Fc alpha receptor in humans. *J Exp Med*, 171, 597–613.

Morton, H. C., van, E. M., and van de Winkel, J. G. (1996). Structure and function of human IgA Fc receptors (Fc alpha R). *Crit Rev Immunol*, 16, 423–40.

Mostov, K. E., Friedlander, M., and Blobel, G. (1984). The receptor for transepithelial transport of IgA and IgM contains multiple immunoglobulin-like domains. *Nature*, 308, 37–43.

Moura, I. C., Benhamou, M., Launay, P., *et al.* (2008). The glomerular response to IgA deposition in IgA nephropathy. *Semin Nephrol*, 28, 88–95.

Moura, I. C., Centelles, M. N., Arcos-Fajardo, M., *et al.* (2001). Identification of the transferrin receptor as a novel immunoglobulin (Ig)A1 receptor and its enhanced expression on mesangial cells in IgA nephropathy. *J Exp Med*, 194, 417–25.

Moura, I. C., Arcos-Fajardo, M., Sadaka, C., *et al.* (2004). Glycosylation and size of IgA1 are essential for interaction with mesangial transferrin receptor in IgA nephropathy. *J Am Soc Nephrol*, 15, 622–34.

Pacifico, F., Laviola, L., Ulianich, L., *et al.* (1995). Differential expression of the asialoglycoprotein receptor in discrete brain areas, in kidney and thyroid. *Biochem Biophys Res Commun*, 210, 138–44.

Park, J. H., Cho, E. W., Shin, S. Y., *et al.* (1998). Detection of the asialoglycoprotein receptor on cell lines of extrahepatic origin. *Biochem Biophys Res Commun*, 244, 304–11.

Pirulli, D., Crovella, S., Ulivi, S., *et al.* (2009). Genetic variant of C1GalT1 contributes to the susceptibility to IgA nephropathy. *J Nephrol*, 22, 152–9.

Piskurich, J. F., Youngman, K. R., Phillips, K. M., *et al.* (1997). Transcriptional regulation of the human polymeric immunoglobulin receptor gene by interferon-gamma. *Mol Immunol*, 34, 75–91.

Raska, M., Moldoveanu, Z., Suzuki, H., *et al.* (2007). Identification and characterization of CMP-NeuAc:GalNAc-IgA1 alpha2,6-sialyltransferase in IgA1-producing cells. *J Mol Biol*, 369, 69–78.

Reterink, T. J., van Zandbergen, G., van Egmond, M., *et al.* (1997). Size-dependent effect of IgA on the IgA Fc receptor (CD89). *Eur J Immunol*, 27, 2219–24.

Smith, A. C., de Wolff, J. F., Molyneux, K., *et al.* (2006). O-glycosylation of serum IgD in IgA nephropathy. *J Am Soc Nephrol*, 17, 1192–9.

Stockert, R. J. (1995). The asialoglycoprotein receptor: relationships between structure, function, and expression. *Physiol Rev*, 75, 591–609.

Suzuki, H., Fan, R., Zhang, Z., *et al.* (2009). Aberrantly glycosylated IgA1 in IgA nephropathy patients is recognized by IgG antibodies with restricted heterogeneity. *J Clin Invest*, 119, 1668–77.

Suzuki, H., Moldoveanu, Z., Hall, S., *et al.* (2008). IgA1-secreting cell lines from patients with IgA nephropathy produce aberrantly glycosylated IgA1. *J Clin Invest*, 118, 629–39.

Suzuki, H., Suzuki, Y., Aizawa, M., *et al.* (2007). Th1 polarization in murine IgA nephropathy directed by bone marrow-derived cells. *Kidney Int*, 72, 319–27.

Suzuki, Y. and Tomino, Y. (2007). The mucosa-bone-marrow axis in IgA nephropathy. *Contrib Nephrol*, 157, 70–9.

Tomana, M., Matousovic, K., Julian, B.A., *et al.* (1997). Galactose-deficient IgA1 in sera of IgA nephropathy patients is present in complexes with IgG. *Kidney Int*, 52, 509–16.

Tomana, M., Novak, J., Julian, B. A., *et al.* (1999). Circulating immune complexes in IgA nephropathy consist of IgA1 with galactose-deficient hinge region and antiglycan antibodies. *J Clin Invest*, 104, 73–81.

Toyabe, S., Harada, W., and Uchiyama, M. (2001). Oligoclonally expanding gammadelta T lymphocytes induce IgA switching in IgA nephropathy. *Clin Exp Immunol*, 124, 110–7.

Van den Wall Bake, A. W., Daha, M. R., Haaijman, J. J., *et al.* (1989). Elevated production of polymeric and monomeric IgA1 by the bone marrow in IgA nephropathy. *Kidney Int*, 35, 1400–4.

Van den Wall Bake, A. W., Daha, M. R., Radl, J., *et al.* (1988). The bone marrow as production site of the IgA deposited in the kidneys of patients with IgA nephropathy. *Clin Exp Immunol*, 72, 321–5.

Vuong, M. T., Hahn-Zoric, M., Lundberg, S., *et al.* (2010). Association of soluble CD89 levels with disease progression but not susceptibility in IgA nephropathy. *Kidney Int*, 78, 1281–7.

Yu, H. H., Chu, K. H., Yang, Y. H., *et al.* (2010). Genetics and immunopathogenesis of IgA nephropathy. *Clin Rev Allergy Immunol*, 41, 198–213.

Zhu, L., Tang, W., Li, G., *et al.* (2009). Interaction between variants of two glycosyltransferase genes in IgA nephropathy. *Kidney Int*, 76, 190–8.

CHAPTER 70

Crescentic (rapidly progressive) glomerulonephritis

Neil Turner

Introduction

Crescentic nephritis was recognized in the nineteenth century (Fig. 70.1). The term rapidly progressive glomerulonephritis (RPGN) was used by Ellis (1942) to describe patients who developed renal failure over weeks or months, most of whom were found to have crescentic nephritis.

Pathological characterization

When immunofluorescence techniques became available, crescentic nephritis was divided into different types depending on fluorescence pattern of staining for immunoglobulins: linear, none, or granular. However, crescent formation can be a consequence of any aggressive nephritis, so it is more important to define the underlying disease than to subclassify crescentic nephritis. Discontinuation of this terminology was aided by the recognition that most patients with the condition previously described as pauci-immune, idiopathic crescentic nephritis have small vessel vasculitis (see Chapter 159). Pauci-immune is the most common histological finding in patients who have the clinical syndrome of RPGN (Table 70.1).

Crescents originate in proliferation of epithelial cells, mostly derived from parietal epithelial cells lining Bowman's capsule (see 'Formation of crescents') (Fig. 70.2). This is termed extracapillary proliferation. In more severe disease, it extends circumferentially around the glomerulus so that in cross-section it has the classic crescentic form (Fig. 70.3). A key additional abnormality is the glomerular basement membrane (GBM) breaks that lead to crescent initiation, seen on silver stains or others that show the structure of the GBM clearly, and on electron microscopy. Other features depend on the initiating disease.

Some diseases may have a stuttering focal nature so that crescents of different ages may be seen in the same biopsy. Most patients with small vessel vasculitis show this to some extent. There may be evidence of inactive focal scarring in affected glomeruli, and fibrotic partial or circumferential crescents. Crescents may also resolve without scarring in some circumstances (see below).

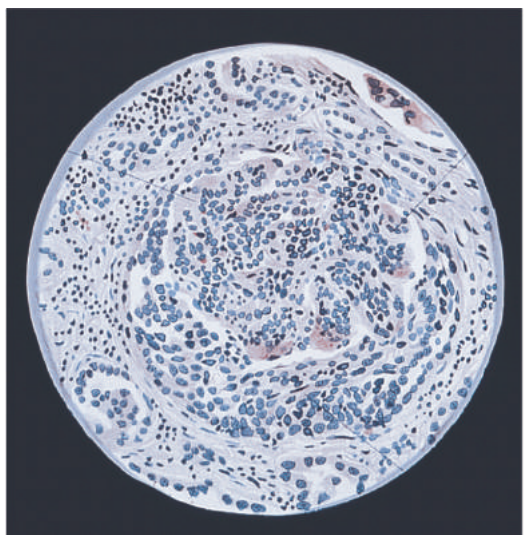

Fig. 70.1 Crescentic nephritis as illustrated by Volhard and Fahr (1914). The glomerular tuft shows proliferative changes, and a cellular crescent occupies the lower segment of Bowman's capsule.

Clinical features of rapidly progressive glomerulonephritis

There are two key characteristics to RPGN:

- Its nature: it is from the 'nephritic' end of the spectrum of glomerulonephritis (see Chapter 45) so is characterized by marked haematuria and a variable amount of proteinuria
- Its tempo: kidney function declines over days or weeks (Fig. 70.4).

In acute and swiftly moving RPGN there may be visibly red urine, or it may be described as smoky or Coca-Cola urine. Blood clots are not a feature of glomerular bleeding. Microscopy shows glomerular-type dysmorphic red cells and usually also red cell casts (see Chapter 6). There may, however, be very little proteinuria if the disease is very acute. Nephrotic-range proteinuria usually implies that there has been an exacerbation of a pre-existing disease.

The patient may complain of loin pain and kidneys may be moderately enlarged on imaging.

Hypertension is not a prominent or early feature in most types of RPGN. Lupus nephritis and post-infectious glomerulonephritis are prominent exceptions. Hypertension is also likely when RPGN is caused by exacerbation of a prior nephritis, most commonly seen in immunoglobulin A (IgA) nephropathy.

Lung haemorrhage with RPGN

This combination encompasses the classic causes of pulmonary renal syndrome, although there is an important differential diagnosis with other causes of simultaneous pulmonary and renal failure (see Table 72.1 and Box 72.1 in Chapter 72). Alveolar and glomerular capillaries appear to share enough antigens and/or properties

for immune attack on both to occur in more than one disease. In anti-GBM disease the target is the basement membrane (see Chapter 72) and in small vessel vasculitis the target appears to be endothelium (see Chapter 158).

Life-threatening disease with aggressive pulmonary and renal disease is most commonly seen in anti-GBM disease and small vessel vasculitis.

Investigations

Although it may be possible to establish the aetiology from associated features, and from serological and other tests, in most cases a renal biopsy is urgently required. This is often the most rapid way to prove the diagnosis with certainty, and may also establish a likely prognosis which may affect decisions about treatment. Useful investigations are shown in Table 70.1.

Causes of crescentic nephritis

Aggressive nephritis

The major causes are shown in Table 70.1. They are inflammatory systemic diseases and types of glomerulonephritis from the 'nephritic' end of the spectrum (see Fig. 45.2, Chapter 45). Children, particularly in series from developing regions, are more likely to have post-infectious disease, but the range of causes is similar to that in adults (Southwest Pediatric Study Group, 1985; Jardim et al., 1992; Srivastava et al., 1992; Dewan et al., 2008; Sinha et al., 2013).

Table 70.1 Distinguishing characteristics of key causes of RPGN. Frequency in different series taken from Heilman (1987), Keller et al. (1989), Andrassy et al. (1991), Angangco et al. (1994), and Levy and Pusey (2005)

Disease	Frequency	Tests	Comments
Small vessel vasculitis	43–68%	MPO and Pr3 antibodies	More specific than ANCA by immunofluorescence. Usually positive in the most frequent primary vasculitides. Note that false positive results occur. Renal biopsy confirms (Chapter 159)
Anti-GBM	6–20%	Anti-GBM antibodies	Always positive in florid disease (Chapter 72). Renal biopsy is the most sensitive and specific test and often also the most rapid.
Lupus nephritis	3–8%	Complement (C3, C4) Anti-ds DNA, ANF	Anti-ds DNA almost always positive (Chapter 162)
Post-infectious glomerulonephritis	0–13%	Complement Throat or other swab	Complement is the most valuable clinical test. Anti-streptococcal titres can be historic (Chapter 77)
Other glomerulonephritis	2–23%		Features of that nephritis

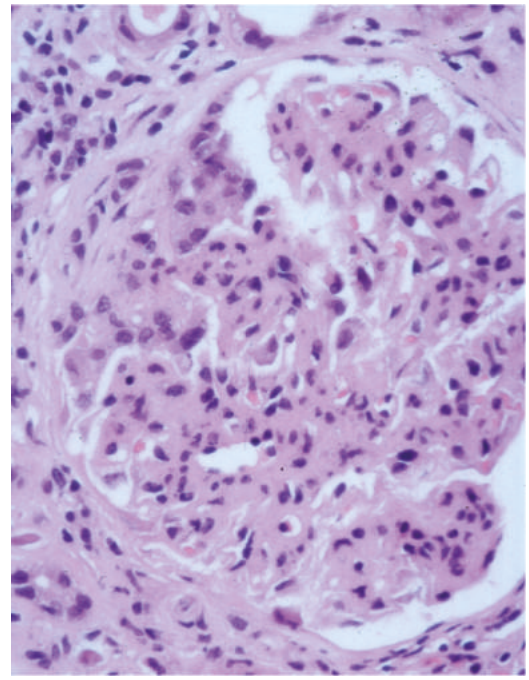

Fig. 70.2 A small segmental crescent in a patient with IgA nephropathy (haematoxylin and eosin).

There are some informative exceptions where crescent formation is encountered in the context of diseases not usually characterized by aggressive inflammation.

Nephrosis transforming into aggressive nephritis

Membranous nephropathy rarely transforms into crescentic nephritis. About half of reported cases have anti-GBM disease (discussed

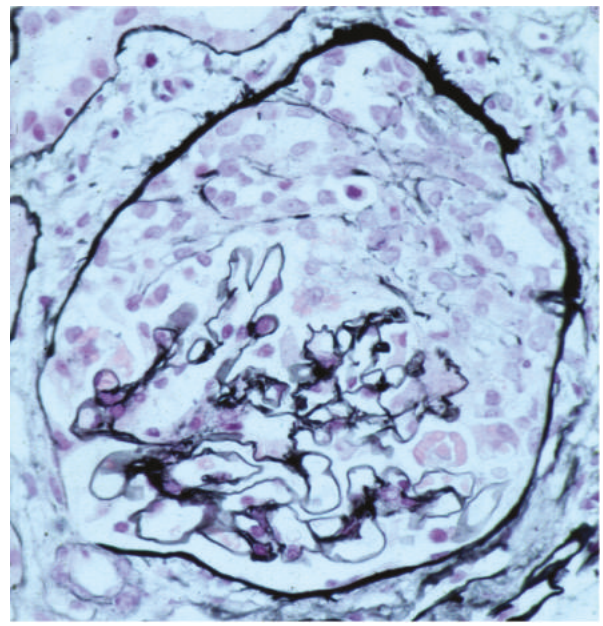

Fig. 70.3 A large cellular crescent surrounds and partially compresses the glomerular tuft in a patient with ANCA-associated vasculitis (methenamine silver–haematoxylin and eosin). GBM breaks can be seen.

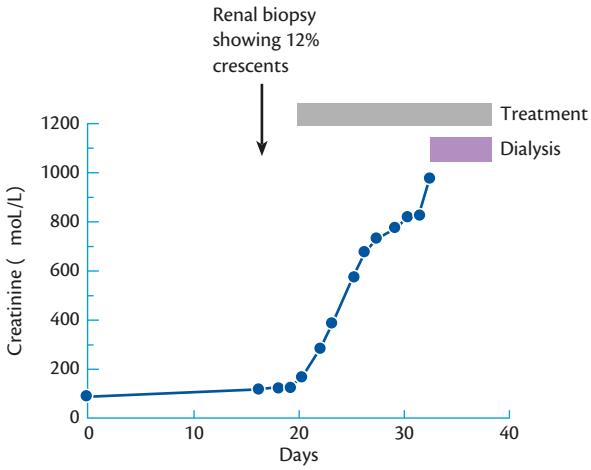

Fig. 70.4 Deterioration of kidney function in crescentic nephritis caused by anti-GBM disease. This is typically the most rapidly progressive RPGN and exacerbation of established disease by infection is well recognized (Rees et al., 1977; Guillen et al., 1995; Erwig et al., 2001).

in Chapters 61 and 74). Diabetes mellitus may also occasionally transform into anti-GBM disease. In both nephropathies a thickened GBM contains increased amounts of the collagen 345 network that includes the Goodpasture antigen. This plus some 'second hit' may enable breaking tolerance to the antigen (see Chapter 74).

Disorders associated with GBM fragility

Several disorders leading to GBM fragility other than through nephritis have been occasionally reported to develop crescentic changes, for example, in amyloidosis (Nagata et al., 2001; Schafernak et al., 2005), in experimental models of Alport syndrome and rarely in humans (Ryu et al., 2012), and in fibrillary nephritis (Sethi et al., 2001).

Formation of crescents

Anti-GBM antibodies define the best-characterised, archetypal crescentic nephritis, but animal experiments clearly define that the characteristic histopathological changes are dependent on cell mediated immunity including macrophages and lymphocytes. Fig. 70.5 illustrates the steps in crescent formation in cartoon form.

Breaks in the GBM

Breaks in the glomerular basement membrane allow plasma proteins to enter Bowman's space. As described above this is usually caused by aggressive immune attack but occasionally crescents are seen when GBM breaks spontaneously because of abnormal composition. Fig. 70.6 shows a scanning electron micrograph of rat capillary basement membranes after induction of nephrotoxic nephritis, a model of anti-GBM disease (Bonsib, 1985, 1988).

Fibrin provokes proliferation of parietal epithelial cells

The formation of fibrin in Bowman's space seems to be critical; crescent formation can be reduced or blocked by defibrination with the snake venom ancrod or by fibrinolysis with urokinase (Naish et al., 1972; Ryu et al., 2012), and is diminished in mice lacking fibrinogen (Drew et al., 2001). Tissue factor is presumed to trigger activation of fibrin formation and has been considered as a therapeutic target, but anticoagulation after a renal biopsy in patients who may have lung haemorrhage would be very hazardous (McCluskey, 1997). Furthermore it is not clear that preventing crescent formation would be enough, if the process causing the underlying glomerular damage continues (Browne et al., 2004). Fig. 70.7 shows an early, fibrin-containing lesion in human disease.

The large cells that participate in the first phase of crescent formation are mostly derived from parietal epithelial cells lining Bowman's capsule, with only occasional cells that seem to have a podocyte origin (Smeets et al., 2009). Indeed this was the origin first suggested by Langerhans (1885). A similar proliferative response of parietal epithelial cells ('the forgotten fourth cell of the glomerulus') is seen in collapsing glomerulopathy and can be induced by injury to parietal epithelial cells themselves (Sicking et al., 2012; Shankland et al., 2014).

Macrophage infiltration and cell mediated immunity

There are many macrophages in more mature crescents (Tipping et al., 1986; Lan et al., 1997) and these are important for mediating injury in many experimental models. It is also clear that crescentic nephritis is dependent on lymphocytes and other effectors of cell-mediated immunity, and that factors that modulate cellular infiltration or inflammatory state (interleukin 1, tumour necrosis factor alpha, and others) will modulate the injury caused by a particular immune insult (Holdsworth et al., 1999; Kitching et al., 2000; Kitching and Holdsworth, 2011; and see Fig. 70.4). Most of these studies have been carried out in animal models of anti-GBM disease.

Breaks in Bowman's capsule

In the final stage of the most destructive disease the damage extends to Bowman's capsule, where breaks seem associated with influx of further cells including fibrocytes. It has been suggested that this determines whether a glomerulus affected by crescentic change recovers or fibroses (Boucher et al., 1987; Lan et al., 1997).

Resolution of crescents

It is clear from reports of outcomes in post-streptococcal glomerulonephritis, from some patients with vasculitis, and rare patients with anti-GBM disease, that recovery can occur despite high proportions of crescents in renal biopsies. Post-streptococcal disease in children probably has the greatest propensity to do this. It has been termed 'pneumonia of the glomerulus', and like pneumococcal pneumonia, can be associated with good recovery and repair despite severe functional impairment and extreme histological hypercellularity and infiltration (Roy et al., 1981).

In anti-GBM disease, crescent score usually predicts the outcome quite accurately (Fig. 70.8). This may be because this is such a destructive diseases that most crescentic glomeruli are irrecoverable, and sclerose. However, one individual in Fig. 70.8 recovered to a serum creatinine of 200 μmol/L at 1 year despite 86% crescents.

Management of crescentic nephritis

The management depends on the cause, but there is some urgency, so it is important to reach a rapid diagnosis. As suggested in

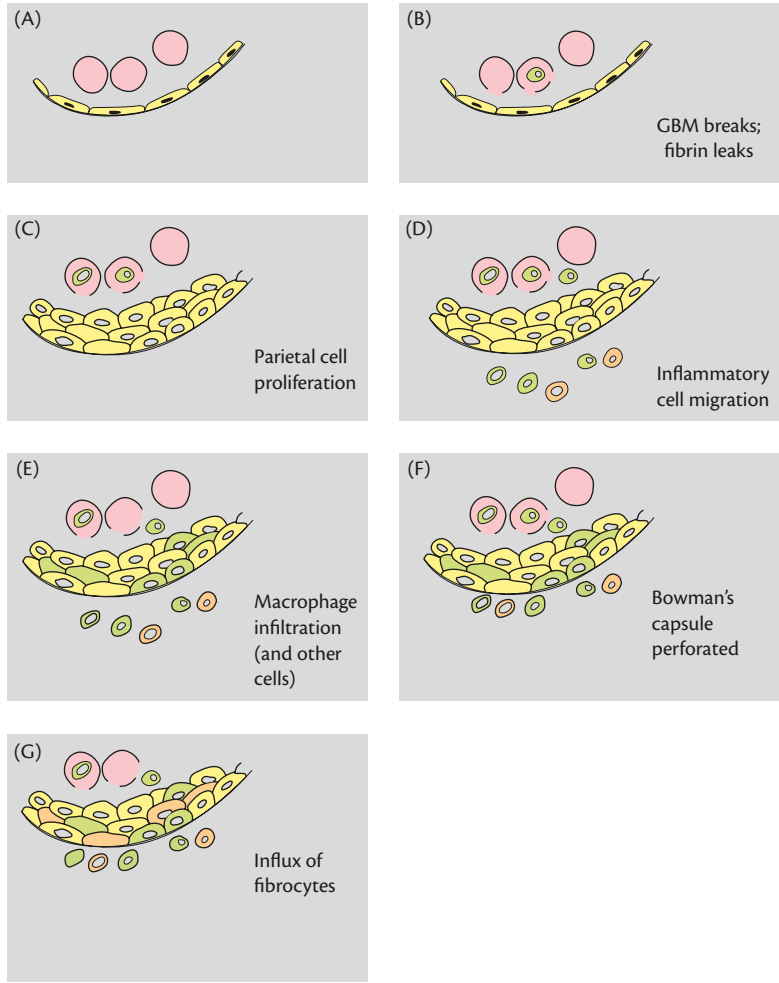

Fig. 70.5 Mechanism of crescent formation.
A. Three schematic capillary loops.
B. Damage to GBM mediated by inflammatory cells, antibodies with complement, etc.
C. Fibrin formation from local tissue factor acting on fibrinogen promotes proliferation of parietal epithelial cells.
D. Macrophages and later lymphocytes, fibrocytes migrate in, leading to further chemokine release.
E. Macrophages found in crescents.
F. Bowman's capsule perforated.
G. Further migration of cells from periglomerular space, including fibrocytes.

Table 70.1, renal biopsy is often the most informative test. It not only determines the cause, but it also gives prognostic information (number of affected glomeruli, age of lesions, degree of architectural damage, and presence or absence of interstitial fibrosis). As many of the therapies for crescentic nephritis involve significant risk, this is important information.

If you can be certain that the patient does not have an infection underlying their disease, and that RPGN is the diagnosis, therapy with cyclophosphamide and prednisolone may be commenced in advance of a firm diagnosis.

Specific treatments

Pulses of methylprednisolone have been used in these circumstances following reports by Bolton and Sturgill (1989). There is little good evidence that this is any more effective than more moderate doses, and as patients are often treated with other immunosuppressive agents the risk of infection is probably significantly elevated by the practice. There is some experimental support for use of high-dose corticosteroids alone, but studies have tended to be short and look more at histological appearances than outcome (Yamamoto-Shuda et al., 1999; Ou et al., 2001). It undoubtedly does 'reduce the appearance of crescents' and reduce leucocyte infiltration. It may improve outcome when used in high dose as sole therapy, but it is associated with risk.

Plasma exchange is proven to add benefit in small vessel vasculitis and is accepted therapy in anti-GBM disease (see Chapters 73 and 160).

Specific diseases

In IgA nephropathy there is little evidence that immunosuppressive agents add additional benefits above those of high-dose steroids (see Chapter 68).

In anti-GBM disease, a significant proportion of patients have no realistic chance of salvaging renal function (Fig. 70.8) (see

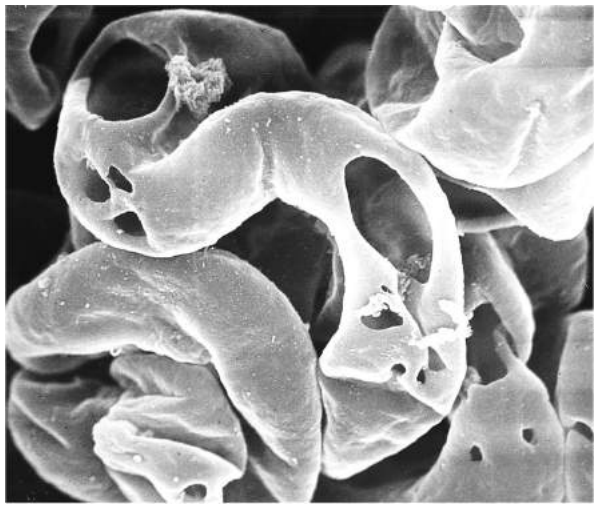

Fig. 70.6 Scanning electron micrograph of glomerular capillary loops of an animal with experimental anti-GBM disease, from Bonsib (1985). The glomerulus has been denuded of cells to show only the fixed GBM. Following anti-GBM antibody binding, fixation of complement and recruitment of cell-mediated effectors has blown holes in the GBM allowing serum proteins and red blood cells to leak into the urinary space. In most human nephritis GBM breaks are more subtle than this.

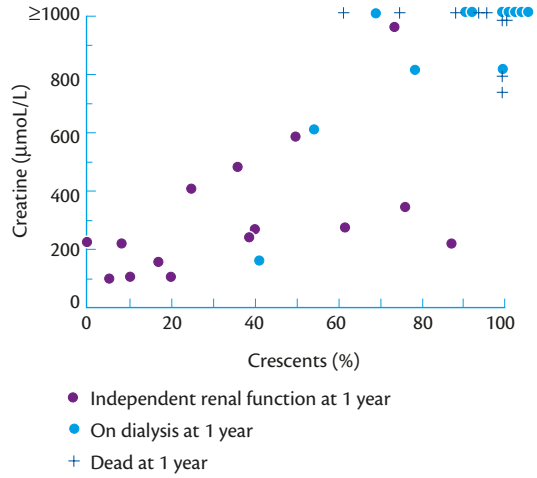

Fig. 70.8 Plasma creatinine concentration at presentation, and the proportion of glomeruli with crescents, in 38 patients treated at Hammersmith Hospital, London. Those who did not receive the combination of plasma exchange, cyclophosphamide, and prednisolone, or who had < 10 glomeruli in their renal biopsies, have been excluded. The following are illustrated: (1) the correlation between the creatinine at presentation and histological evidence of glomerular damage, except in one patient with acute tubular necrosis; (2) the close relationship between the severity of renal damage at presentation and outcome; (3) death from pulmonary haemorrhage occurs predominantly in those with severe renal disease.

Chapter 73). If they do not have lung haemorrhage, a decision not to treat with immunosuppressive agents may be considered.

In systemic vasculitis, the pace of the disease is highly variable, and many patients are elderly and have comorbid conditions which increases the risk of many therapies. Therapeutic strategies need to take this into account (see Chapter 160).

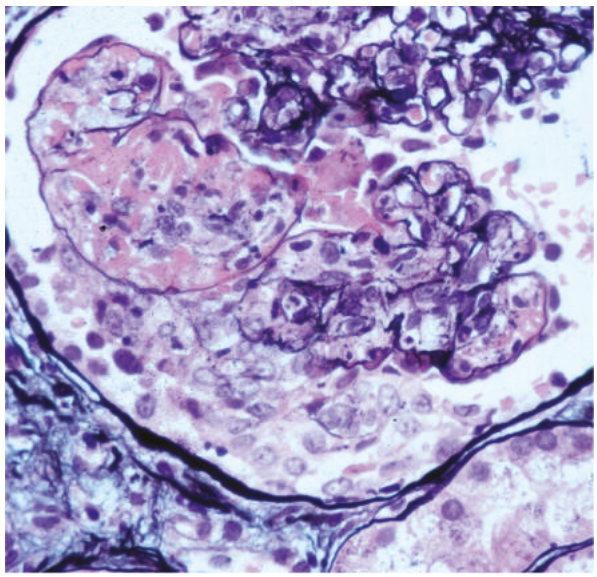

Fig. 70.7 A cellular segmental crescent is seen at 6 o'clock in this patient with Henoch–Schönlein purpura. In the centre of the picture fibrin can be seen leaking into the urinary space from a break in the GBM, stimulating the proliferation of parietal epithelial cells.

References

Andrassy, K., Kuster, S., Waldherr, R., *et al.* (1991). Rapidly progressive glomerulonephritis: analysis of prevalence and clinical course. *Nephron*, 59 (2), 206–12.

Angangco, R., Thiru, S., Esnault, V. L., *et al.* (1994). Does truly 'idiopathic' crescentic glomerulonephritis exist? *Nephrol Dial Transplant*, 9, 630–6.

Bolton, W. K. and Sturgill, B. C. (1989). Methylprednisolone therapy for acute crescentic rapidly progressive glomerulonephritis. *Am J Nephrol*, 9, 368–75.

Bonsib, S. M. (1985). GBM discontinuities. Scanning electron microscopic study of a cellular glomeruli. *Am J Pathol*, 119, 357–60.

Bonsib, S. M. (1988). GBM necrosis and crescent organization. *Kidney Int*, 33, 966–74.

Boucher, A., Droz, D., Adafer, E., *et al.* (1987). Relationship between the integrity of Bowman's capsule and the composition of cellular crescents in human crescentic glomerulonephritis. *Lab Invest*, 56, 526–33.

Browne, G., Brown, P. A., Tomson, C. R., *et al.* (2004). Retransplantation in Alport post-transplant anti-GBM disease. *Kidney Int*, 65, 675–81.

Dewan, D., Gulati, S., Sharma, R. K., *et al.* (2008). Clinical spectrum and outcome of crescentic glomerulonephritis in children in developing countries. *Pediatr Nephrol*, 23, 389–94.

Drew, A. F., Tucker, H. L., Liu, H., *et al.* (2001). Crescentic glomerulonephritis is diminished in fibrinogen-deficient mice. *Am J Physiol Renal Physiol*, 281, F1157–63.

Ellis, A. (1942). Natural history of Bright's disease. Clinical, histological and experimental observations. *Lancet*, i, 34–6.

Erwig, L. P., Kluth, D. C., and Rees, A. J. (2001). Macrophages in renal inflammation. *Curr Opin Nephrol Hypertens*, 10, 341–7.

Guillen, E. L., Ruiz, A. M., Fernandez, M. A., *et al.* (1995). Goodpasture syndrome: re-exacerbations associated with intercurrent infections. *Revista Clinica Española*, 195, 761–4.

Holdsworth, S. R., Kitching, A. R., and Tipping, P. G. (1999). Th1 and Th2 helper cell subsets affect patterns of injury and outcomes in glomerulo-nephritis. *Kidney Int*, 55, 1198–216.

Jardim, H. M., Leake, J., Risdon, R. A., *et al.* (1992). Crescentic glomerulonephritis in children. *Pediatr Nephrol*, 6, 231–5.

Keller, F., Oehlenberg, B., Kunzendorf, U., *et al*. (1989). Long-term treatment and prognosis of rapidly progressive glomerulonephritis. *Clin Nephrol*, 31, 190–7.

Kitching, A. R. and Holdsworth, S. R. (2011). The emergence of TH17 cells as effectors of renal injury. *J Am Soc Nephrol*, 22(2), 235–8.

Kitching, A. R., Holdsworth, S. R., and Tipping, P. G. (2000). Crescentic glomerulonephritis—a manifestation of a nephritogenic Th1 response? *Histol Histopathol*, 15, 993–1003.

Lan, H. Y., Nikolic-Paterson, D. J., Mu, W., *et al*. (1997). Local macrophage proliferation in the pathogenesis of glomerular crescent formation in rat anti-glomerular basement membrane (GBM) glomerulonephritis. *Clin Exp Immunol*, 110, 233–40.

Langerhans, T. (1885). Über die entzundlichen Veranderungen der Glomeruli und die acute Nephritis. *Virchows Archiv*, 99, 193–204.

Levy, J. and Pusey, C. D. (2005). Crescentic glomerulonephritis. In J. S. Cameron, A. M. Davison AM, J. -P. Grunfeld, *et al*. (eds.) *Oxford Textbook of Clinical Nephrology* (3rd ed), pp. 559–78. Oxford: Oxford University Press.

McCluskey, R. T. (1997). Tissue factor in crescentic glomerulonephritis. *Am J Pathol*, 150, 787–92.

Nagata, M., Shimokama, T., Harada, A., *et al*. (2001). Glomerular crescents in renal amyloidosis: an epiphenomenona or distinct pathology? *Pathol Int*, 51, 179–86.

Naish, P., Penn, G. B., Evans, D. J., *et al*. (1972). The effect of defibrination on nephrotoxic serum nephritis in rabbits. *Clin Sci*, 42, 643.

Ou, Z. L., Nakayama, K., Natori, Y., *et al*. (2001). Effective methylprednisolone dose in experimental crescentic glomerulonephritis. *Am J Kidney Dis*, 37, 411–17.

Rees, A. J., Lockwood, C. M., and Peters, D. K. (1977). Enhanced allergic tissue injury in Goodpasture's syndrome by intercurrent bacterial infection. *Br Med J*, 2, 723–6.

Roy, S., Murphy, W. M., and Arant, B. S. (1981). Post-streptococcal glomerulonephritis in children: comparison of quintuple therapy versus supportive care. *Pediatrics*, 98, 403–10.

Ryu, M., Migliorini, A., Miosge, N., *et al*. (2012). Plasma leakage through glomerular basement membrane ruptures triggers the proliferation of parietal epithelial cells and crescent formation in non-inflammatory glomerular injury. *J Pathol*, 228, 482–94.

Schafernak, K. T., Chugh, S. S., and Kanwar, Y. S. (2005). Co-existent crescentic glomerulonephritis and renal amyloidosis: a case report and literature review. *J Nephrol*, 18, 616–22.

Sethi, S., Adeyi, O. A., and Rennke, H. G. (2001). A case of fibrillary glomerulonephritis with linear immunoglobulin G staining of the glomerular capillary walls. *Arch Pathol Lab Med*, 125(4), 534–6.

Shankland, S. J., Smeets, B., Pippin, J. W., *et al*. (2014). The emergence of the glomerular parietal epithelial cell. *Nat Rev Nephrol*, 10, 158–73.

Sicking, E. M., Fuss, A., Uhlig, S., *et al*. (2012). Subtotal ablation of parietal epithelial cells induces crescent formation. *J Am Soc Nephrol*, 23, 629–40.

Sinha, A., Puri, K., Hari, P., *et al*. (2013). Etiology and outcome of crescentic glomerulonephritis. *Indian Pediatr*, 50(3), 283–8.

Smeets, B., Uhlig, S., Fuss, A., *et al*. (2009). Tracing the origin of glomerular extracapillary lesions from parietal epithelial cells. *J Am Soc Nephrol*, 20, 2604–15.

Southwest Pediatric Nephrology Study Group (1985). A clinico- pathologic study of crescentic glomerulonephritis in children. A report of the Southwest Pediatric Nephrology Study Group. *Kidney Int*, 27, 450–8

Srivastava, R. N., Moudgil, A., Bagga, A., *et al*. (1992). Crescentic glomerulonephritis in children: a review of 43 cases. *Am J Nephrol*, 12, 155–61.

Tipping, P. G. and Holdsworth, S. R. (1986). The participation of macrophages, glomerular procoagulant activity, and factor VIII in glomerular fibrin deposition. Studies on anti-GBM antibody-induced glomerulonephritis in rabbits. *Am J Pathol*, 124, 10–17.

Volhard, F. and Fahr, T. (1914). *Die Brightsche Nierenkrankheit*. Berlin: Springer.

Yamamoto-Shuda, Y., Nakayama, K., Saito, T., *et al*. (1999). Therapeutic effect of glucocorticoid on experimental crescen- tic glomerulonephritis. *J Lab Clin Med*, 134, 410–18.

CHAPTER 71

Antiglomerular basement membrane disease: overview

Zhao Cui, Neil Turner, and Ming-hui Zhao

Antiglomerular basement membrane (anti-GBM) disease is characteristically the most rapidly progressive (crescentic) nephritis. It is often accompanied by lung haemorrhage, and occasionally causes lung disease alone (see Chapter 72). Its hallmark is linear deposition of immunoglobulin G (IgG) along the GBM. There are usually few systemic symptoms apart from any related to the lung disease. Urine shows haematuria, often macroscopic in very acute disease.

Diagnosis depends on identification of anti-GBM antibodies in association with tissue damage (Fig. 71.1) (see Chapter 72). Renal biopsy is important for confirming the diagnosis and gives important prognostic information.

Early treatment (see Chapter 73) with cyclophosphamide, plasma exchange, and prednisolone arrests lung haemorrhage and can salvage renal function, but the disease often progresses very rapidly so that renal destruction is advanced by the time the diagnosis is made, and renal recovery partial or absent. The value of alternative therapies is unproven.

Anti-GBM disease tends to be an acute disease not requiring long-term immunosuppression.

Anti-GBM antibody formation and/or disease may be provoked by small vessel vasculitis affecting the kidney, by lithotripsy, and occasionally in other circumstances. There is a strong association with class II human leucocyte antigen (HLA)-DR15 and -DR4 (see Chapter 74).

The disease is caused by autoimmunity to the carboxy-terminal (NC1) domain of one of the tissue-specific basement membrane (type IV) collagen chains, α3(IV)NC1 (see Chapter 74). The antigen is also found in the alveolus, and causes lung haemorrhage in about half of patients with the disease (see Chapter 73). This can be life-threatening and associated with severe renal disease, but it can also occur with minimal renal disease. The antigen in lung is cryptic: additional insults are required to expose it to the immune system leading to lung haemorrhage. Most patients who develop lung haemorrhage in anti-GBM disease are cigarette smokers.

Alport post-transplant anti-GBM disease (see Chapter 75) is a rare post-transplant complication that occurs in a small minority of patients with Alport syndrome. It appears clinically very like spontaneous anti-GBM disease, but causes isolated kidney disease. The target of antibodies is usually the type IV collagen chain that carries the Alport mutation. It is difficult to treat.

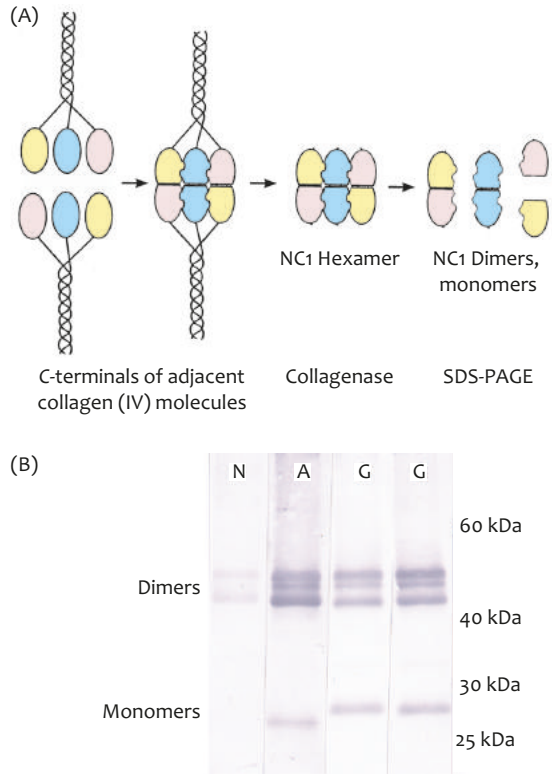

Fig. 71.1 (A) Western blotting of tissue-specific type IV collagen NC1 domains. Collagenase-solubilised basement membrane from bovine testis has been separated by SDS–PAGE in non-reducing conditions and transferred to nitrocellulose. (See also Chapter 74, Fig. 74.2.)
(B) Goodpasture sera (lanes marked 'G') recognize monomers of α3(IV) NC1 domains of approximately 28 kDa and a cluster of dimers. Some Alport post-transplant anti-GBM sera (e.g. the lane marked 'A') contain antibodies to α5(IV)NC1, which can be seen as a monomer of approximately 26 kDa. Normal sera ('N') show no monomer recognition but weak staining of dimer regions after prolonged incubation.

CHAPTER 72

Antiglomerular basement membrane disease: clinical features and diagnosis

Zhao Cui, Neil Turner, and Ming-hui Zhao

Introduction

The name Goodpasture was first applied in 1958 by Stanton and Tange (1958), who described a group of nine patients with similar clinical features. They recognized these as similar to an 18-year-old man presenting with lung haemorrhage and crescentic nephritis at autopsy during an epidemic of influenza, reported by Ernest Goodpasture in 1919 (Goodpasture, 1919). The term 'Goodpasture syndrome' is sometimes used to describe the combination of severe glomerulonephritis and lung haemorrhage, irrespective of cause. Actually, the pulmonary-renal syndrome is more commonly caused by systemic vasculitis, usually associated with antineutrophil cytoplasmic antibodies (ANCAs), but with a wider range of possible causes described below. Patients with pulmonary-renal syndrome and pathogenic antibodies against GBM are best described as anti-GBM disease, but the description 'Goodpasture disease' is still in use.

In 1967, the pathogenicity of autoantibodies against GBM was demonstrated by the antibody transfer experiment of Lerner, Glassock, and Dixon (Lerner et al., 1967). Now, Goodpasture disease is known to be caused by autoimmunity to a target on the non-collagenous domain 1 of the α3 chain of type IV collagen (α3(IV)NC1), present in alveolar and glomerular basement membranes, and now commonly described as the Goodpasture antigen.

Epidemiology

Anti-GBM disease is rare, with an estimated and possibly rising incidence of up to 1 case per million of the population per annum (Savage et al., 1986). It occurs across all racial groups, but not equally affected. Although there is no large study of incidence for Asian populations, cases have been reported from Japan (47 cases from 715 patients with rapidly progressive glomerulonephritis (RPGN)) (Hirayama et al., 2008) and China (221 cases reported, accounting for 16% of crescentic nephritis) (Li et al., 2004; Lin et al., 2010; Cui et al., 2011a). It seems to be particularly rare in black races, although cases in black Americans have been described (Kelly and Haponik, 1994). The disease has been estimated to cause up to 5% of glomerulonephritis (Wilson and Dixon, 1973), 10–20% of crescentic glomerulonephritis (Couser, 1988; Andrassy et al., 1991; Lin et al., 2010), and 2% of end-stage renal disease (Disne, 1986).

All age groups can be affected by the disease. The youngest reported case appears to be 11 months old (Bigler et al., 1997); several patients in their 90s have been described (Cui et al., 2011b). Generally, there are two periods of peak incidence. The first peak is in the second and third decades of life, the second peak is in the sixth and seventh decades (Kluth and Rees, 1999; Pusey, 2003). Early series showed a striking preponderance of young male patients with high frequency of pulmonary haemorrhage. The wide application of immunoassays and immunohistology, and increased awareness of the disease, has led to later series showing a greater proportion of older patients (Savage et al., 1986; Herody et al., 1993; Merkel et al., 1994; Daly et al., 1996; Levy et al., 2001; Fischer and Lager, 2006; Hirayama et al., 2008; Cui et al., 2011b). Glomerulonephritis alone is more common in older patients. At the time of diagnosis, their renal dysfunction is mild or moderate, but the outcome is similar to younger patients. This age and gender distribution is notably different to that of other organ-specific autoimmune disorders.

Lung haemorrhage is more common in men (Fig. 72.1). Glomerulonephritis alone is more common in women. The male:female ratio in 71 cases from the United Kingdom was 1.4, but for those with both pulmonary haemorrhage and glomerulonephritis it was 3.0, and for those with glomerulonephritis alone it was 0.9 (Levy et al., 2001). These differences were probably related to smoking history.

Anti-GBM disease has been associated with a number of other conditions, the most common shown in Table 72.1. Some of these may be causally related, inducing the anti-GBM response. Probably examples of this include small vessel vasculitis (common), and rarely lithotripsy, urinary tract obstruction, and perhaps other examples. An association with lymphocyte depletion (e.g. in alemtuzumab therapy and HIV) may also give clues to the natural control of immunity to this autoantigen (see Chapter 74).

Clinical features

Patients may present with renal disease alone, with renal disease plus lung haemorrhage, or occasionally with lung haemorrhage alone. Although there are hints that the disease may have a long prodrome, presentation is usually acute following an accelerated phase. The prodromal phase can be seen in some patients with low titres of antibodies associated with pulmonary haemorrhage alone,

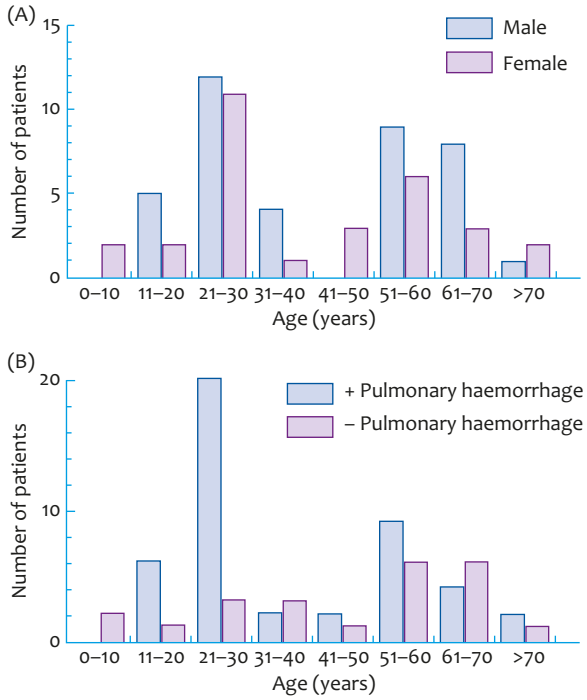

Fig. 72.1 (A) Age and gender of 68 patients with Goodpasture disease treated at Hammersmith Hospital, London.
(B) Pulmonary haemorrhage in 68 patients with Goodpasture disease treated at Hammersmith Hospital, London.

that sometimes progresses, and in historic studies of antibody titres (see Chapter 74).

General manifestations

The clinical features of anti-GBM disease have been well established from the relatively large series of immunologically defined cases published over the last 40 years (Wilson and Dixon, 1973; Beirne et al., 1977; Teague et al., 1978; Briggs et al., 1979; Peters et al., 1982; Johnson et al., 1985; Walker et al., 1985; Savage et al., 1986; Herody et al., 1993; Merkel et al., 1994; Daly et al., 1996; Levy et al., 2001; Shah and Hugghins, 2002; Segelmark et al., 2003; Li et al., 2004; Cui et al., 2005, 2011a; Fischer and Lager, 2006; Hirayama et al., 2008). Systemic symptoms, such as malaise, fever, or weight loss, are less frequently seen and generally mild. Anaemia is common and frequently symptomatic, even in patients who have had little or no haemoptysis. Anti-GBM disease should be considered in patients who present with moderate to severe anaemia together with acute kidney injury. The anaemia is usually microcytic and hypochromic, but sometimes shows evidence of microangiopathy as can be the case with other types of RPGN. The iron deficiency probably reflects subclinical pulmonary haemorrhage, but can on occasion be confused with gastrointestinal disease, especially if uraemia is causing nausea and vomiting.

Table 72.1 Diseases recurrently associated with Goodpasture disease. Alport syndrome following transplantation is bracketed as it has some notable differences (see Chapter 75)

Disease	Number of reports (approx.)
ANCA-associated vasculitis (mostly with anti-myeloperoxidase ANCA)	Hundreds
Membranous nephropathy	< 20
Diabetes mellitus	< 20
Malignancy—lymphoma, bronchial carcinoma	10
Lithotripsy to intrarenal stones	< 10
Lymphocyte depletion (alemtuzumab, HIV)	< 10
(Alport syndrome following renal transplantation)	(Tens)

Renal manifestations

Abnormalities of the urine sediment, usually microscopic haematuria, are the earliest sign of renal damage. Later, the urine contains numerous dysmorphic red blood cells and red cell casts. In severe disease, macroscopic haematuria can occur. Proteinuria is generally mild or moderate (< 3.5 g/24 hours), but some patients may have severe proteinuria and present with nephrotic syndrome. Oliguria is a late feature and a bad prognostic sign. However, the chance of superimposed acute tubular necrosis in hypoxic and severely ill patients is always high. There may be loin pain when inflammation is severe. Hypertension is generally a late feature that accompanies advanced renal failure and fluid retention. Kidney size is usually normal or enlarged due to inflammation.

Disease onset is typically abrupt with oliguria or anuria, haematuria and proteinuria, and end-stage renal disease. Renal function is usually already reduced at presentation and may deteriorate from normal to dialysis requiring levels in a matter of days to weeks.

Several studies found that a subgroup of patients, 3–36%, has normal renal function or only minimal renal dysfunction and mild glomerular lesions (Mathew et al., 1975; Zimmerman et al., 1979; Bailey et al., 1981; Bell et al., 1990; Knoll et al., 1993; Min et al., 1996; Ang et al., 1998; Cui et al., 2007). These patients present mainly with lung haemorrhage, with varying degree of haematuria and proteinuria, but macroscopic haematuria and nephrotic range proteinuria are rare. During follow-up, renal function is preserved in most of this subgroup, although slow progression to renal failure has been seen in some cases, and a typical catastrophic deterioration has occurred after an interval in others.

Renal pathology

An adequate renal biopsy is an essential part of the assessment of patients with the disease, and has prognostic as well as diagnostic importance.

The pathologic finding of linear staining of immunoglobulins along glomerular capillary wall by direct immunofluorescence is indicative of anti-GBM glomerulonephritis (Fig. 72.2). This is predominantly IgG, however, rare patients with IgA-dominant anti-GBM glomerulonephritis have also been reported (Border et al., 1979; Fivush et al., 1986). Most specimens (60–70%) have discontinuous linear or granular capillary wall staining for C3, but a minority has little or no C3 staining. Occasional reports mention IgM alone, IgA alone, or C3 alone (Savage et al., 1986). Linear staining for IgG may also occur along tubular basement membranes in some but not all cases. Fibrin-related antigens are commonly present within the crescents and segmental necrotizing lesions.

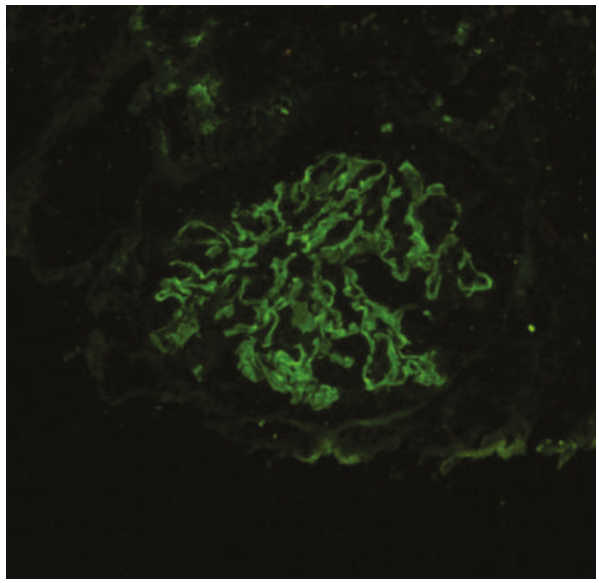

Fig. 72.2 Direct immunofluorescence for IgG in a typical glomerulus from a patient with anti-GBM disease, showing linear fixation of antibody to the GBM (FITC anti-IgG).

Additional granular deposits, associated with subepithelial or sometimes intramembranous or subendothelial deposits ultrastructurally, have been noted in some patients (Rajaraman et al., 1984) and may be associated with resolution. However, there are clear-cut examples of patients with membranous nephropathy developing anti-GBM disease, so this circumstance should be considered in patients with a history of nephrotic syndrome or heavy proteinuria.

The glomeruli may be abnormal even in patients with normal renal function. The earliest and mildest changes consist of segmental mesangial matrix expansion and hypercellularity, progressing to a more generalized, but still focal and segmental, proliferative glomerulonephritis with increased numbers of neutrophils in the glomeruli. Later, glomeruli show a diffuse glomerulonephritis with segmental or total necrosis and extensive crescent formation.

At the time of biopsy, 95% of patients have some degree of crescent formation and 81% have crescents in 50% or more of glomeruli. On average, 77% of glomeruli have crescents. Early crescents are formed by proliferating epithelial cells and infiltrating T lymphocytes, monocytes, and polymorphonuclear leucocytes, whereas older ones are composed predominantly of spindled fibroblast-like cells, with few if any infiltrating leucocytes (see Chapter 70). Glomeruli with crescents typically have fibrinoid necrosis in adjacent glomerular segments. Non-necrotic segments may look entirely normal by light microscopy, or may have slight infiltration by neutrophils or mononuclear leucocytes. This differs from crescentic immune complex glomerulonephritis, which typically has capillary wall thickening and endocapillary hypercellularity in the intact glomeruli. Special stains that outline basement membranes, such as Jones silver methenamine or periodic acid–Schiff stains, often demonstrate focal breaks in glomerular basement membranes in areas of necrosis, and also show focal breaks in Bowman capsule. The most severely injured glomeruli have global glomerular necrosis, circumferential cellular crescents, and extensive disruption of Bowman capsule. There may be a mixture of acute and chronic lesions; however, the glomerular lesions of anti-GBM glomerulonephritis tend to be more in synchrony than those of ANCA-glomerulonephritis, which more often show admixtures of acute and chronic injury.

Tubulointerstitial changes are commensurate with the degree of glomerular injury. Glomeruli with extensive necrosis and disruption of Bowman capsule typically have intense periglomerular inflammation, including occasional multinucleated giant cells (Fig. 72.3). There also is focal tubular epithelial acute simplification or atrophy, focal interstitial oedema and fibrosis, and focal interstitial infiltration of predominantly mononuclear leucocytes.

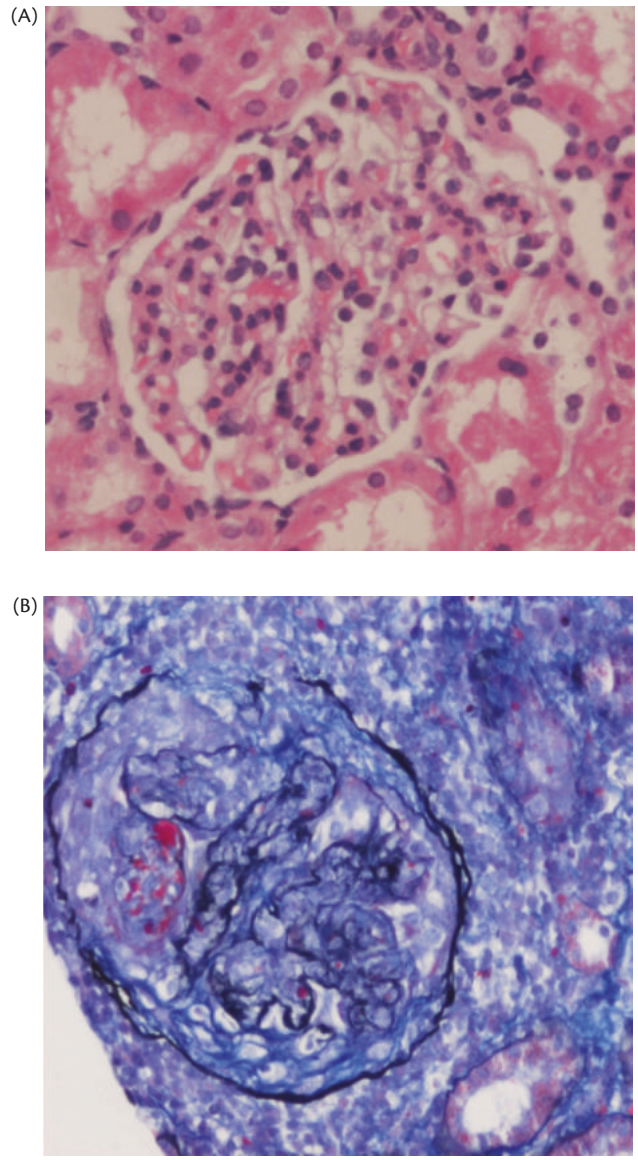

Fig. 72.3 Light microscopy on renal biopsy from patients with anti-GBM disease. (A) A glomerulus showing mild proliferation of mesangial cells, from renal biopsy of a patient with microscopic haematuria and proteinuria, and normal serum creatinine. (B) A typical glomerulus from the renal biopsy of a patient with RPGN and requiring dialysis at presentation, showing global glomerular necrosis, circumferential cellular crescents, and extensive disruption of Bowman capsule (periodic acid–Schiff and silver methenamine stains).

There are no specific changes in arteries or arterioles. If necrotizing inflammation is observed in arteries or arterioles, the possibility of concurrent ANCA should be considered.

The findings by electron microscopy reflect those seen by light microscopy. Ultrastructurally, the GBM usually shows a widespread irregular broadening, often with mottled thickening of the lamina rara interna. Breaks in the GBM are common. Endothelial and epithelial cells are swollen, and epithelial foot processes may be effaced. An important negative observation is the absence of immune complex type electron-dense deposits. These occur only in anti-GBM disease patients who have concurrent immune complex disease.

Pulmonary manifestations

The most frequent extrarenal presentation is pulmonary involvement. Patients present with cough, dyspnoea, shortness of breath, and haemoptysis, although the severity can vary widely—ranging from mild to life-threatening and requiring mechanical ventilation. The prevalence of pulmonary haemorrhage is reported from 50% to 90%, varying on different criteria for diagnosis of pulmonary haemorrhage. Pulmonary haemorrhage presents typically as haemoptysis that may be episodic, varies from the trivial to torrential and is a poor reflection of the actual quantity of pulmonary bleeding. It can also occur without haemoptysis.

In contrast with renal injury, lung disease shows a very poor correlation with antibody titre, even though the autoantigen is present in alveolar as well as glomerular basement membrane. This may reflect the lack of direct contact between circulating antibodies and alveolar basement membrane (Jennings et al., 1981; Downie et al., 1982; Yamamoto and Wilson, 1987). It is consistent with the clear association between pulmonary haemorrhage and cigarette smoking or exposure to other inhaled toxins, notably gasoline or other hydrocarbons (discussed further in Chapter 74). Fluid overload and pulmonary infections have also been shown to provoke lung haemorrhage in anti-GBM disease.

Isolated lung disease is reported regularly, though haematuria is probably always present. It may occur weeks to months before presentation with fulminant renal/ pulmonary disease, and is often associated with false-negative serological tests for anti-GBM antibodies.

Signs

Physical examination can be normal in patients with mild to moderate pulmonary haemorrhage, but the more severely affected are usually tachypnoeic and may be cyanosed. They may expectorate fresh blood and have rather dry-sounding inspiratory crackles on auscultation that are most prominent over the lower lung fields and may be accompanied by areas of bronchial breathing.

Radiology

Most episodes of pulmonary haemorrhage are associated with changes in the chest radiograph (Fig. 72.4). Usually the shadows involve the central lung fields, with peripheral and upper-lobe sparing. The abnormalities are generally symmetrical, but can be markedly asymmetrical. Changes range from ill-defined nodules of size 1–4 mm to confluent consolidation with an air bronchogram. Shadowing is rarely limited or entirely confined by a fissure, and radiographs that show this or those that demonstrate shadowing at the apex strongly suggest infection, either alone or superimposed on pulmonary haemorrhage. Shadows caused by bleeding usually start to clear within 48 hours. Residual minor changes are usually gone within 2 weeks.

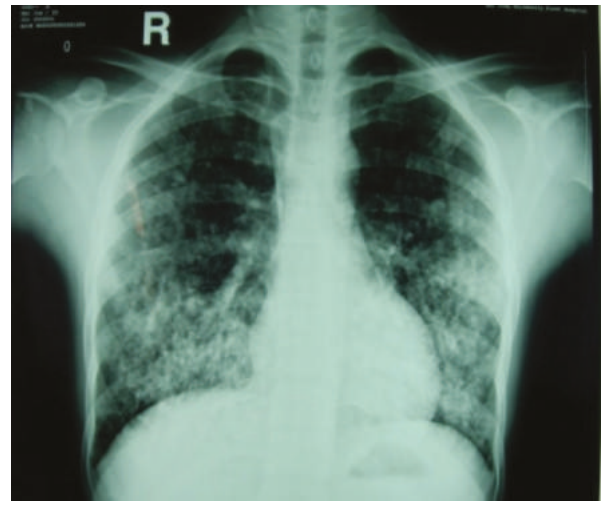

Fig. 72.4 Chest radiograph of a patient with anti-GBM disease who presented with pulmonary haemorrhage and RPGN. Symmetrical parenchymal patchy shadows are shown with some confluent consolidation.

The diagnosis of pulmonary haemorrhage presents few problems in the majority of patients, and difficulties only arise in the minority whose haemoptysis is absent. Other indicators include a sudden otherwise unexplained drop in haemoglobin and new shadows on the chest radiograph.

Pulmonary function

Bleeding into the lung also causes an acute increase in the transfer factor corrected for lung volumes and the patient's haemoglobin, KCO, the diffusion coefficient for carbon monoxide (CO). This is the most sensitive and specific test for fresh pulmonary haemorrhage (Ewan et al., 1976). The cause is the additional free haemoglobin within the alveoli that is able to bind inspired carbon monoxide and so increase values for KCO. These results contrast with the usual situation in renal failure in which the KCO is about 30% lower than predicted, and for most patients with pulmonary oedema who also show reduced values.

The lung lesions resolve almost completely and chronic lung disease is not a described outcome even in those who have survived life-threatening pulmonary haemorrhage (Conlon et al., 1994).

Pulmonary pathology

At autopsy of patients with pulmonary haemorrhage, the lungs are characteristically heavy, showing patchy congestion or haemorrhage. Histologically, intra-alveolar haemorrhage is accompanied by haemosiderin-containing macrophages, deposits of fibrin, and alveolar cell hyperplasia. Electron microscopy shows thickening of the alveolar basement membrane, often with defects. Thickened alveolar walls may show oedema, fibrosis, and modest inflammatory cell infiltration, mainly with polymorphs and lymphocytes. Immunofluorescence investigations are more difficult in the lung, but linear fixation of immunoglobulin can be detected at autopsy in patients with lung haemorrhage. However, the binding is patchy, so

that, although a high success rate in obtaining diagnostic materials by transbronchial biopsy has been reported, the technique is unreliable for diagnostic use (Johnson et al., 1985; Nakajima et al., 1999).

Diagnosis

The specific diagnosis of anti-GBM disease rests on the demonstration of anti-GBM antibodies in the circulation or fixed to the kidney.

Circulating anti-GBM antibodies are predominantly IgG, although there are rare cases in which only anti-GBM IgA could be detected (Border et al., 1979; Savage et al., 1986; Maes et al., 1999; Ho et al., 2008). Indirect immunofluorescence assay using sections of normal kidney is specific but not sensitive and has been replaced by solid phase immunoassays. Radioimmunoassay and enzyme-linked immunosorbent assay (ELISA) for the antibodies are relatively specific and sensitive (Kluth and Rees, 1999; Pusey, 2003). Purified bovine or sheep GBM soluble proteins enriched for α3(IV)NC1 are widely used in commercial available assays with high specificity (> 95%) and sensitivity (> 90%) (Sinico et al., 2006). In some diagnostic centres, recombinant human α1 to α5 (IV)NC1 are used to confirm and validate the presence of anti-GBM antibodies using solid phase immunoassays and Western blotting analysis (Salama et al., 2002; Jia et al., 2012; Mahler et al., 2012).

Circulating anti-GBM antibodies can be detected in > 90% of patients using these solid phase immunoassays, but some assays may still have varying degree of false-negativity or false-positivity (Litwin et al., 1996; Jaskowski et al., 2002; Salama et al., 2002; Jia et al., 2012). False-negative immunoassay results are most likely in patients with isolated pulmonary or low-grade/early renal disease. These patients will have linear antibody binding to the GBM, and the presence of typical but low-titre circulating antibodies can often be detected by more sensitive assays or by Western blotting. Rare non-IgG antibodies are identified by direct immunofluorescence studies of the renal biopsy. False-positive assays may occasionally be seen in states of polyclonal activation including other autoimmune conditions. Renal biopsy will not confirm linear binding of antibody to the GBM.

It is the rapidity of deterioration, and the rapidly changing prognosis for renal recovery, that makes prompt diagnosis and therapy of anti-GBM disease imperative. In the correct clinical context (i.e. raised serum creatinine concentration and active urinary sediment or proteinuria, with or without haemoptysis), positive circulating anti-GBM antibodies can be used as an indication to start treatment in cases where renal biopsy cannot be performed immediately. Renal biopsy should, however, be performed as soon as possible, since in rare cases, circulating anti-GBM antibodies are not detectable despite the presence of antibodies deposited along the GBM in the kidney (Salama et al., 2002; Jia et al., 2012).

From 10% to 38% of patients have both positive anti-GBM antibodies and ANCA usually directed against myeloperoxidase (Rutgers et al., 2005; Cui et al., 2011a). Other serologic tests such as anti-streptolysin O, antinuclear antibodies, serum immunoglobulin and complement levels, rheumatoid factor, cryoglobulins, and circulating immune complex are either negative or normal. Although a strong HLA association is recognised (see Chapter 74), this is not useful diagnostically.

Direct immunofluorescence on the renal biopsy is the most sensitive technique of all, if adequate renal tissue is obtained and glomerular destruction is not severe; this is the main method of diagnosis in many centres. However, the typical linear staining of IgG involving GBM cannot be revealed in all patients, in part because of severe destruction of glomerular capillary walls. In such condition, the immunofluorescence may display segmental linear deposits or absolutely negative. There may also be occasional

Box 72.1 Causes of linear staining on direct immunofluorescence of renal tissue[a]

- Anti-GBM disease
- Diabetes mellitus
- Older patients with hypertensive vascular disease
- Alport syndrome after renal transplantation
- Systemic lupus erythematosus
- Normal autopsy kidneys
- Cadaver kidneys after perfusion
- Transplant biopsies
- Fibrillary nephritis.

(Wilson and Dixon, 1974; Quérin et al., 1986; Alpers et al., 1987; Peten et al., 1991)

[a] In most cases, the appearances are of weak binding than in anti-GBM disease, but confirmation of specificity can only be achieved by elution studies or by testing serum for antibodies to the Goodpasture antigen.

Table 72.2 Differential diagnosis of pulmonary-renal syndrome (Leatherman et al., 1984; Holdsworth et al., 1985; Clutterbuck and Pusey, 1987; Leatherman, 1987; Vats et al., 1999; Masson et al., 1992; Espinosa et al., 2002). Small vessel vasculitis and anti-GBM disease are the key disorders to differentiate when there is true pulmonary haemorrhage with glomerulonephritis

Causing lung haemorrhage and RPGN	Causing simultaneous renal and pulmonary failure by various means
ANCA-associated small vessel vasculitis	Pulmonary oedema secondary to hypervolemia in acute renal failure of any aetiology
Anti-GBM disease	Pulmonary embolism secondary to nephrotic syndrome (most commonly seen in membranous nephropathy)
Systemic lupus erythematosus	Severe pneumonia (including *Legionella* pneumonia) with acute tubular necrosis
Antiphospholipid syndrome	Pulmonary tuberculosis, especially during steroid treatments for glomerulonephritis
Henoch–Schönlein purpura	Hantavirus infections
Behçet disease	Bacterial endocarditis with pulmonary oedema
Mixed essential cryoglobulinaemia	Paraquat poisoning
	Haemolytic uraemic syndrome
Rheumatoid vasculitis	Leptospirosis

false-positive results, as linear fluorescence not attributable to anti-GBM disease has been noted in a number of circumstances, mostly seen in diabetic nephropathy and transplant biopsies (Box 72.1). The clinical data and light microscopic findings should help make this distinction. Serologic confirmation should always be obtained to substantiate the diagnosis of anti-GBM disease. Direct immunofluorescence is not useful for following disease activity as linear antibody fixation may be demonstrable for a year or more after diagnosis, after circulating antibodies have become undetectable, and in the absence of clinical disease (Teague et al., 1978).

It is important to distinguish the syndrome of lung haemorrhage and RPGN from other causes of renal and pulmonary failure (Table 72.2).

References

Alpers, C. E., Rennke, H. G., Hopper, J., Jr., *et al.* (1987). Fibrillary glomerulonephritis: an entity with unusual immunofluorescence features. *Kidney Int*, 31, 781–9.

Andrassy, K., Kuster, S., Waldherr, R., *et al.* (1991). Rapidly progressive glomerulonephritis: analysis of prevalence and clinical course. *Nephron*, 59, 206–12.

Ang, C., Savige, J., Dawborn, J., *et al.* (1998). Anti-glomerular basement membrane (GBM)-antibody-mediated disease with normal renal function. *Nephrol Dial Transplant*, 13, 935–9.

Bailey, R. R., Simpson, I. J., Lynn, K. L., *et al.* (1981). Goodpasture's syndrome with normal renal function. *Clin Nephrol*, 15, 211–15.

Beirne, G. J., Wagnild, J. P., Zimmerman, S. W., *et al.* (1977). Idiopathic crescentic glomerulonephritis. *Medicine (Baltimore)*, 56, 349–81.

Bell, D. D., Moffatt, S. L., Singer, M., *et al.* (1990). Antibasement membrane antibody disease without clinical evidence of renal disease. *Am Rev Respir Dis*, 142, 234–7.

Bigler, S. A., Parry, W. M., Fitzwater, D. S., *et al.* (1997). An 11-month-old with anti-glomerular basement membrane disease. *Am J Kidney Dis*, 30, 710–12.

Border, W. A., Baehler, R. W., Bhathena, D., *et al.* (1979). IgA antibasement membrane nephritis with pulmonary hemorrhage. *Ann Intern Med*, 91, 21–5.

Briggs, W. A., Johnson, J. P., Teichman, S., *et al.* (1979). Antiglomerular basement membrane antibody-mediated glomerulonephritis and Goodpasture's syndrome. *Medicine (Baltimore)*, 58, 348–61.

Clutterbuck, E. J. and Pusey, C. D. (1987). Severe alveolar haemorrhage in Churg-Strauss syndrome. *Eur J Respir Dis*, 71, 158–63.

Conlon, P. J., Jr., Walshe, J. J., Daly, C., *et al.* (1994). Antiglomerular basement membrane disease: the long-term pulmonary outcome. *Am J Kidney Dis*, 23, 794–6.

Couser, W. G. (1974). Goodpasture's syndrome: a response to nitrogen mustard. *Am J Med Sci*, 268, 175–9.

Cui, Z., Zhao, J., Jia, X. Y., *et al.* (2011a). Anti-glomerular basement membrane disease: outcomes of different therapeutic regimens in a large single-center chinese cohort study. *Medicine (Baltimore)*, 90, 303–11.

Cui, Z., Zhao, J., Jia, X. Y., *et al.* (2011b). Clinical features and outcomes of anti-glomerular basement membrane disease in older patients. *Am J Kidney Dis*, 57, 575–82.

Cui, Z., Zhao, M. H., Singh, A. K., *et al.* (2007). Antiglomerular basement membrane disease with normal renal function. *Kidney Int*, 72, 1403–8.

Cui, Z., Zhao, M. H., Xin, G., *et al.* (2005). Characteristics and prognosis of Chinese patients with anti-glomerular basement membrane disease. *Nephron Clin Pract*, 99, c49–55.

Daly, C., Conlon, P. J., Medwar, W., *et al.* (1996). Characteristics and outcome of anti-glomerular basement membrane disease: a single-center experience. *Ren Fail*, 18, 105–12.

Disney, A. P. S. (1986). *Tenth Report of the Australian and New Zealand Combined Dialysis and Transplant Registry (ANZ-DATA)*. Adelaide: Queen Elizabeth Hospital.

Downie, G. H., Roholt, O. A., Jennings, L., *et al.* (1982). Experimental anti-alveolar basement membrane antibody-mediated pneumonitis. II. Role of endothelial damage and repair, induction of autologous phase, and kinetics of antibody deposition in Lewis rats. *J Immunol*, 129, 2647–52.

Espinosa, G., Cervera, R., Font, J., *et al.* (2002). The lung in the antiphospholipid syndrome. *Ann Rheum Dis*, 61, 195–8.

Ewan, P. W., Jones, H. A., Rhodes, C. G., *et al.* (1976). Detection of intrapulmonary hemorrhage with carbon monoxide uptake. Application in goodpasture's syndrome. *N Engl J Med*, 295, 1391–6.

Fischer, E. G. and Lager, D. J. (2006). Anti-glomerular basement membrane glomerulonephritis: a morphologic study of 80 cases. *Am J Clin Pathol*, 125, 445–50.

Fivush, B., Melvin, T., Solez, K., *et al.* (1986). Idiopathic linear glomerular IgA deposition. *Arch Pathol Lab Med*, 110, 1189–91.

Goodpasture, E. W. (1919). The significance of certain pulmonary lesions in relation to the etiology of influenza. *Am J Med Sci*, 158, 863–70.

Herody, M., Bobrie, G., Gouarin, C., *et al.* (1993). Anti-GBM disease: predictive value of clinical, histological and serological data. *Clin Nephrol*, 40, 249–55.

Hirayama, K., Yamagata, K., Kobayashi, M., *et al.* (2008). Anti-glomerular basement membrane antibody disease in Japan: part of the nationwide rapidly progressive glomerulonephritis survey in Japan. *Clin Exp Nephrol*, 12, 339–47.

Ho, J., Gibson, I. W., Zacharias, J., *et al.* (2008). Antigenic heterogeneity of IgA anti-GBM disease: new renal targets of IgA autoantibodies. *Am J Kidney Dis*, 52, 761–5.

Holdsworth, S., Boyce, N., Thomson, N. M., *et al.* (1985). The clinical spectrum of acute glomerulonephritis and lung haemorrhage (Goodpasture's syndrome). *QJM*, 55, 75–86.

Jaskowski, T. D., Martins, T. B., Litwin, C. M., *et al.* (2002). Comparison of four enzyme immunoassays for the detection of immunoglobulin G antibody against glomerular basement membrane. *J Clin Lab Anal*, 16, 143–5.

Jennings, L., Roholt, O. A., Pressman, D., *et al.* (1981). Experimental anti-alveolar basement membrane antibody-mediated pneumonitis. I. The role of increased permeability of the alveolar capillary wall induced by oxygen. *J Immunol*, 127, 129–34.

Jia, X. Y., Qu, Z., Cui, Z., *et al.* (2012). Circulating anti-GBM autoantibodies against alpha3(IV)NC1 undetectable by commercial available enzyme-linked immunosorbent assays. *Nephrology (Carlton)*, 17(2), 160–6.

Johnson, J. P., Moore, J., Jr., Austin, H. A., 3rd., *et al.* (1985). Therapy of anti-glomerular basement membrane antibody disease: analysis of prognostic significance of clinical, pathologic and treatment factors. *Medicine (Baltimore)*, 64, 219–27.

Kelly, P. T. and Haponik, E. F. (1994). Goodpasture syndrome: molecular and clinical advances. *Medicine (Baltimore)*, 73, 171–85.

Kluth, D. C. and Rees, A. J. (1999). Anti-glomerular basement membrane disease. *J Am Soc Nephrol*, 10, 2446–53.

Knoll, G., Rabin, E., and Burns, B. F. (1993). Antiglomerular basement membrane antibody-mediated nephritis with normal pulmonary and renal function. A case report and review of the literature. *Am J Nephrol*, 13, 494–6.

Leatherman, J. W. (1987). Immune alveolar hemorrhage. *Chest*, 91, 891–7.

Leatherman, J. W., Davies, S. F., and Hoidal, J. R. (1984). Alveolar hemorrhage syndromes: diffuse microvascular lung hemorrhage in immune and idiopathic disorders. *Medicine (Baltimore)*, 63, 343–61.

Lerner, R. A., Glassock, R. J., and Dixon, F. J. (1967). The role of anti-glomerular basement membrane antibody in the pathogenesis of human glomerulonephritis. *J Exp Med*, 126, 989–1004.

Levy, J. B., Turner, A. N., and Rees, A. J. (2001). Long-term outcome of anti-glomerular basement membrane antibody disease treated with plasma exchange and immunosuppression. *Ann Intern Med*, 134, 1033–42.

Li, F. K., Tse, K. C., Lam, M. F., *et al.* (2004). Incidence and outcome of antiglomerular basement membrane disease in Chinese. *Nephrology (Carlton)*, 9, 100–4.

Lin, W., Chen, M., Cui, Z., *et al.* (2010). The immunopathological spectrum of crescentic glomerulonephritis: a survey of 106 patients in a single Chinese center. *Nephron Clin Pract*, 116, c65–74.

Litwin, C. M., Mouritsen, C. L., Wilfahrt, P. A., *et al.* (1996). Anti-glomerular basement membrane disease: role of enzyme-linked immunosorbent assays in diagnosis. *Biochem Mol Med*, 59, 52–6.

Maes, B., Vanwalleghem, J., Kuypers, D., *et al.* (1999). IgA antiglomerular basement membrane disease associated with bronchial carcinoma and monoclonal gammopathy. *Am J Kidney Dis*, 33, E3.

Mahler, M., Radice, A., Sinico, R. A., *et al.* (2012). Performance evaluation of a novel chemiluminescence assay for detection of anti-GBM antibodies: an international multicenter study. *Nephrol Dial Transplant*, 27(1), 243–52.

Masson, R. G., Rennke, H. G., and Gottlieb, M. N. (1992). Pulmonary hemorrhage in a patient with fibrillary glomerulonephritis. *N Engl J Med*, 326, 36–9.

Mathew, T. H., Hobbs, J. B., Kalowski, S., *et al.* (1975). Goodpasture's syndrome: normal renal diagnostic findings. *Ann Intern Med*, 82, 215–18.

Merkel, F., Pullig, O., Marx, M., *et al.* (1994). Course and prognosis of anti-basement membrane antibody (anti-BM-Ab)-mediated disease: report of 35 cases. *Nephrol Dial Transplant*, 9, 372–6.

Min, S. A., Rutherford, P., Ward, M. K., *et al.* (1996). Goodpasture's syndrome with normal renal function. *Nephrol Dial Transplant*, 11, 2302–5.

Nakajima, I., Sasaki, M., Ito, T., *et al.* (1999). [Goodpasture's syndrome initially presenting with alveolar hemorrhage]. *Nihon Kokyuki Gakkai Zasshi*, 37, 652–7.

Peten, E., Pirson, Y., Cosyns, J.P., *et al.* (1991). Outcome of 30 patients with Alport's syndrome after renal transplantation. *Transplantation*, 52, 823–6.

Peters, D. K., Rees, A. J., Lockwood, C. M., *et al.* (1982). Treatment and prognosis in antibasement membrane antibody-mediated nephritis. *Transplant Proc*, 14, 513–21.

Pusey, C. D. (2003). Anti-glomerular basement membrane disease. *Kidney Int*, 64, 1535–50.

Quérin, S., Noël, L. H., Grünfeld, J. P., *et al.* (1986). Linear glomerular IgG fixation in renal allografts: incidence and significance in Alport's syndrome. *Clin Nephrol*, 25, 134–40.

Rajaraman, S., Pinto, J. A., and Cavallo, T. (1984). Glomerulonephritis with coexistent immune deposits and antibasement membrane activity. *J Clin Pathol*, 37, 176–81.

Rutgers, A., Slot, M., van Paassen, P., *et al.* (2005). Coexistence of anti-glomerular basement membrane antibodies and myeloperoxidase-ANCAs in crescentic glomerulonephritis. *Am J Kidney Dis*, 46, 253–62.

Salama, A. D., Dougan, T., Levy, J. B., *et al.* (2002). Goodpasture's disease in the absence of circulating anti-glomerular basement membrane antibodies as detected by standard techniques. *Am J Kidney Dis*, 39, 1162–7.

Savage, C. O., Pusey, C. D., Bowman, C., *et al.* (1986). Antiglomerular basement membrane antibody mediated disease in the British Isles 1980-4. *Br Med J (Clin Res Ed)*, 292, 301–4.

Segelmark, M., Hellmark, T., and Wieslander, J. (2003). The prognostic significance in Goodpasture's disease of specificity, titre and affinity of anti-glomerular-basement-membrane antibodies. *Nephron Clin Pract*, 94, c59–68.

Shah, M. K. and Hugghins, S. Y. (2002). Characteristics and outcomes of patients with Goodpasture's syndrome. *South Med J*, 95, 1411–18.

Sinico, R. A., Radice, A., Corace, C., *et al.* (2006). Anti-glomerular basement membrane antibodies in the diagnosis of Goodpasture syndrome: a comparison of different assays. *Nephrol Dial Transplant*, 21, 397–401.

Stanton, M. C. and Tange, J. D. (1958). Goodpasture's syndrome (pulmonary haemorrhage associated with glomerulonephritis). *Australas Ann Med*, 7, 132–44.

Teague, C. A., Doak, P. B., Simpson, I. J., *et al.* (1978). Goodpasture's syndrome: an analysis of 29 cases. *Kidney Int*, 13, 492–504.

Vats, K. R., Vats, A., Kim, Y., *et al.* (1999). Henoch-Schonlein purpura and pulmonary hemorrhage: a report and literature review. *Pediatr Nephrol*, 13, 530–4.

Walker, R. G., Scheinkestel, C., Becker, G. J., *et al.* (1985). Clinical and morphological aspects of the management of crescentic anti-glomerular basement membrane antibody (anti-GBM) nephritis/Goodpasture's syndrome. *QJM*, 54, 75–89.

Wilson, C. B. and Dixon, F. J. (1973). Anti-glomerular basement membrane antibody-induced glomerulonephritis. *Kidney Int*, 3, 74–89.

Yamamoto, T. and Wilson, C. B. (1987). Binding of anti-basement membrane antibody to alveolar basement membrane after intratracheal gasoline instillation in rabbits. *Am J Pathol*, 126, 497–505.

Zimmerman, S. W., Varanasi, U. R., and Hoff, B. (1979). Goodpasture's syndrome with normal renal function. *Am J Med*, 66, 163–71.

CHAPTER 73

Antiglomerular basement membrane disease: treatment and outcome

Zhao Cui, Neil Turner, and Ming-hui Zhao

Treatment

The rarity and fulminant course of antiglomerular basement membrane (GBM) disease prevent the initiation of any large randomized studies to investigate therapeutic benefits. The only reported randomized controlled trial (RCT) tested the need for plasma exchange, but it was small, groups unevenly matched for severity, and the degree of plasmapheresis and doses of cyclophosphamide were lower than those generally used (Johnson et al., 1985). However, it appeared that less severely affected individuals did relatively well without plasma exchange. Retrospective analyses based on a large number of patients provide useful information (Couser, 1988; Cui et al., 2011a). The best summary of this approach is a large retrospective study of anti-GBM disease from Hammersmith Hospital (Levy et al., 2001), including 85 patients seen over 25 years. Seventy-one patients were treated with high-dose prednisone (1 mg/kg/day) tapered over 6–9 months, oral cyclophosphamide for 2–3 months, and daily plasmapheresis for 14 days, or until anti-GBM antibody was no longer detectable. The kidney outcome for this cohort was strongly influenced by kidney function at presentation. Patients who had an initial serum creatinine (SCr) < 5.7 mg/dL (500 μmol/L) had 1-year overall survival of 100% and kidney survival of 95%. If the initial SCr was > 5.7 mg/dL (500 μmol/L) but dialysis was not required immediately, the patient and kidney survivals were 83% and 82% at 1 year, respectively. However, among patients who needed dialysis at presentation, patient and kidney survival were reduced to 65% and 8% at 1 year, respectively. Compared to nearly 100% mortality from pulmonary haemorrhage and kidney failure in historical series, this treatment strategy represent a significant improvement and is widely adopted in multiple centres. See Table 73.1.

Antibody removal

Plasma exchange is ineffective if used alone (Guillen et al., 1995) but hastens the disappearance of circulating antibody when used in combination with immunosuppressive agents (Johnson et al., 1985; Savage et al., 1986). There is a strong clinical impression of its efficacy, and its role as essential component in the treatment of severe anti-GBM disease is accepted. Couser (1988) reviewed 22 published uncontrolled studies involving 186 patients, and reported a favourable effect of plasmapheresis on pulmonary disease in around 90% of patients, and a favourable effect of plasmapheresis on renal disease in about 40% of patients. In a retrospective analysis involving 221 patients Beijing, it was demonstrated that, in comparison with corticosteroids alone and corticosteroids plus cyclophosphamide, the combination of plasma exchange, corticosteroids and cyclophosphamide has an overall beneficial effect on patient and kidney survival. Particular benefit was seen for patients with pulmonary haemorrhage and, in relation to kidney survival, for those with anti-GBM nephritis and initial SCr higher than 6.8 mg/dL (600 μmol/L) (Cui et al., 2011a).

Using immunoadsorption against staphylococcal protein A to adsorb circulating IgG antibodies (Bygren et al., 1985; Laczika et al., 2000) lowers antibody titres more rapidly than plasma exchange and has a number of theoretical advantages. However, it is a more time-consuming and complicated technique, and is not widely available.

Corticosteroids

Prednisolone has been a universal part of treatment regimens. We do not recommend the use of bolus doses of methylprednisolone. This (as methylprednisolone 10 mg/kg intravenously once daily for 1–3 days) has been advocated when there is severe pulmonary haemorrhage or very rapidly declining renal function (Johnson et al., 1985), but may increase the risk of later infection, a major concern because as well as threatening survival directly, it may exacerbate renal and pulmonary injury.

Cytotoxic and immunosuppressive agents

Cyclophosphamide was first used successfully by Couser in 1974 (Couser, 1974), and is a critical element of therapy. Alternative immunosuppressive agents have been tried in only a few cases, including azathioprine, ciclosporin, mycophenolate mofetil, and the anti-B-cell antibody rituximab (Pepys et al., 1982; Quérin et al., 1992; Garcia-Canton et al., 2000). Most of these seem likely to be less effective or only slowly effective.

Outcomes and prognostic factors

Outcomes of the Hammersmith cohort (Levy et al., 2001) are representative of what can be expected with a uniform, aggressive approach to therapy as outlined. In other series, not all necessarily using the same treatment regimens, and encompassing patients

Table 73.1 Therapy of anti-GBM disease

Corticosteroids	1 mg/kg/day ideal body weight (maximum 80 mg), tailed off over 3–6 months (typically 60 mg, 45 mg, 30 mg, 25 mg, 20 mg at 1–2 weekly intervals then more slowly. There is disagreement over possible value for higher dose steroids (e.g. pulsed methylprednisolone) at initiation of treatment; it may increase risk of infection and disease amplification
Cyclophosphamide	2 mg/kg/day orally for 3 months, rounded down to nearest 50 mg, and at lower dose if > 60 years
Plasmapheresis	One 4-L (or plasma-volume) exchange per day with 5% albumin. Add 150–300 mL fresh frozen plasma at the end of each pheresis session if patients have pulmonary haemorrhage, or have had recent surgery, including kidney biopsy. Plasmapheresis should be continued for 14 days or until anti-GBM antibodies are no longer detectable

from the United States, Europe, China, and Japan, patient survival at 6–12 months is approximately 67–94%, and kidney survival is about 15–58% (Johnson et al., 1985; Kelly and Haponik, 1994; Merkel et al., 1994; Daly et al., 1996; Shah and Hugghins, 2002; Li et al., 2004; Hirayama et al., 2008; Cui et al., 2011a). Haemoptysis is no longer the major cause of death. Titres of antibodies against GBM and coexistence of ANCA are independent predictors for death (Cui et al., 2011a). An association between high anti-GBM antibody titres and poor renal outcomes is also reported. There are conflicting findings on the implications of ANCA for renal outcome.

Predictors of kidney survival are serum creatinine at presentation, the need for dialysis at presentation, and the percentage of glomerular crescents (Johnson et al., 1985; Levy et al., 2001; Cui et al., 2011a). Serum creatinine at presentation is an independent predictor for renal failure (Cui et al., 2011a). Patients who required dialysis at presentation may not be able to come off dialysis, despite aggressive treatment (Levy et al., 2004; Li et al., 2004). The most optimistic study observed that no patients with a combination of dialysis at presentation plus 100% crescents on kidney biopsy recovered kidney function sufficiently to come off dialysis (Levy et al., 2001). A survey of several studies shows dialysis dependence at diagnosis in a median of 55% (range 12–83%) of patients, 100% crescents on kidney biopsy in 20.5% (range 7–50%) of patients, and a median initial SCr of 6.8 mg/dL (600 μmol/L) (range 4.9–7.2 mg/dL (430–630 μmol/L)), underscoring the importance of early diagnosis and urgent intervention.

The prognosis of patients with mild renal dysfunction or normal kidney function is good when they are treated aggressively with plasmapheresis and immunosuppressive regimens used for classic anti-GBM disease (Levy et al., 2001; Cui et al., 2007). Indeed, this treatment is indicated in patients who have haemoptysis (Zimmerman et al., 1979; Bell et al., 1990) and in those in whom renal histological abnormalities are seen (Ang et al., 1998). The use of less aggressive strategies or merely supportive treatment has occasionally been associated with spontaneous recovery and persistently normal renal function. However, given the fulminant course and possible severe outcomes of anti-GBM disease, a non-aggressive approach is still not recommended in these patients. Johnson's randomized study (Johnson et al., 1985) suggested that an approach that uses cyclophosphamide without plasma exchange might be reasonable however.

Deciding not to treat

The kidney prognosis of dialysis-dependent patients with 100% crescents in adequate biopsy sample is extremely poor. It has been suggested that, in the absence of pulmonary haemorrhage, aggressive immunosuppression should be withheld, for the risks of therapy outweigh the potential benefits (Flores et al., 1986). However, there are occasional reports of patients who have recovered despite acute kidney injury requiring dialysis (Cohen et al., 1976; Johnson et al., 1978; Schindler et al., 1998; Laczika et al., 2000; Cui et al., 2011a). Their hallmark is that they have either mild glomerular change with superimposed acute tubular necrosis or very new cellular crescents without fibrosis on renal biopsy. These patients emphasize the value of an urgent renal biopsy even when the diagnosis has been established serologically. In those patients, aggressive treatment should continue for at least 4 weeks. If there is no restoration of renal function by 4–8 weeks, and in the absence of pulmonary bleeding, immunosuppression should be discontinued.

Relapse and recurrence

In the early phase, immunological relapse should be distinguished from haemodynamic changes or drug effects, and from exacerbation of injury in association with infection.

In contrast to most other autoimmune diseases, anti-GBM disease is not usually characterized by a recurrent course. Autoantibodies seem to disappear spontaneously after 12–18 months (Wilson and Dixon, 1981; Levy et al., 1996). Recurrences of pulmonary haemorrhage over many years are described in the literature (Dahlberg et al., 1978; Mehler et al., 1987; Klasa et al., 1988), and in these circumstances haematuria may also. Recurrent renal disease is exceptional (Levy et al., 1996). Autoantibody reproduction may occur without signs of tissue damage (Hind et al., 1984). Treatment of recurrences is identical to that of initial disease and probably has a better chance of success.

Kidney transplantation

There is little evidence as to the timing of transplant after anti-GBM disease has caused end-stage renal disease. Most transplant centres require at least 6 months of undetectable anti-GBM antibody levels before kidney transplantation. Recurrent anti-GBM disease in a kidney allograft has been described but is now very unusual (Choy et al., 2006; Joshi et al., 2007).

Alport anti-GBM disease (see Chapter 75) is an alloimmune, rather than autoimmune disease, that occurs in < 5% of patients with Alport syndrome after transplantation. It is caused by the development of antibodies to antigens that are missing or altered in the recipient. Most commonly the missing antigen is α5(IV), rather than α3(IV). Clinically these anti-GBM variants are nearly identical, but Alport anti-GBM disease attacks antigens present in the kidney, so lung haemorrhage is not to be expected (it has been reported in one case). Alloimmune responses like this are hard to

suppress and outcomes of treatment are poor. Alport anti-GBM disease is described further in Chapter 75.

References

Ang, C., Savige, J., Dawborn, J., *et al.* (1998). Anti-glomerular basement membrane (GBM)-antibody-mediated disease with normal renal function. *Nephrol Dial Transplant*, 13, 935–9.

Bell, D. D., Moffatt, S. L., Singer, M., *et al.* (1990). Antibasement membrane antibody disease without clinical evidence of renal disease. *Am Rev Respir Dis*, 142, 234–7.

Bygren, P., Freiburghaus, C., Lindholm, T., *et al.* (1985). Goodpasture's syndrome treated with staphylococcal protein A immunoadsorption. *Lancet*, 2, 1295–6.

Choy, B. Y., Chan, T. M., and Lai, K.,N. (2006). Recurrent glomerulonephritis after kidney transplantation. *Am J Transplant*, 6, 2535–42.

Cohen, L. H., Wilson, C. B., and Freeman, R. M. (1976). Goodpasture syndrome: recovery after severe renal insufficiency. *Arch Intern Med*, 136, 835–7.

Couser, W. G. (1974). Goodpasture's syndrome: a response to nitrogen mustard. *Am J Med Sci*, 268, 175–9.

Couser, W. G. (1988). Rapidly progressive glomerulonephritis: classification, pathogenetic mechanisms, and therapy. *Am J Kidney Dis*, 11, 449–64.

Cui, Z., Zhao, J., Jia, X. Y., *et al.* (2011a). Anti-glomerular basement membrane disease: outcomes of different therapeutic regimens in a large single-center chinese cohort study. *Medicine (Baltimore)*, 90, 303–11.

Cui, Z., Zhao, M. H., Singh, A. K., *et al.* (2007). Antiglomerular basement membrane disease with normal renal function. *Kidney Int*, 72, 1403–8.

Dahlberg, P. J., Kurtz, S. B., Donadio, J. V., *et al.* (1978). Recurrent Goodpasture's syndrome. *Mayo Clin Proc*, 53, 533–7.

Daly, C., Conlon, P. J., Medwar, W., *et al.* (1996). Characteristics and outcome of anti-glomerular basement membrane disease: a single-center experience. *Ren Fail*, 18, 105–12.

Flores, J. C., Taube, D., Savage, C. O., *et al.* (1986). Clinical and immunological evolution of oligoanuric anti-GBM nephritis treated by haemodialysis. *Lancet*, 1, 5–8.

Garcia-Canton, C., Toledo, A., Palomar, R., *et al.* (2000). Goodpasture's syndrome treated with mycophenolate mofetil. *Nephrol Dial Transplant*, 15, 920–2.

Guillen, E. L., Ruiz, A. M., Fernandez, M. A., *et al.* (1995). Goodpasture syndrome: re-exacerbations associated with intercurrent infections, *Revista Clinica Española*, 195, 761–4.

Hind, C. R., Bowman, C., Winearls, C. G., *et al.* (1984). Recurrence of circulating anti-glomerular basement membrane antibody three years after immunosuppressive treatment and plasma exchange. *Clin Nephrol*, 21, 244–6.

Hirayama, K., Yamagata, K., Kobayashi, M., *et al.* (2008). Anti-glomerular basement membrane antibody disease in Japan: part of the nationwide rapidly progressive glomerulonephritis survey in Japan. *Clin Exp Nephrol*, 12, 339–47.

Johnson, J. P., Moore, J., Jr., Austin, H. A., 3rd., *et al.* (1985). Therapy of anti-glomerular basement membrane antibody disease: analysis of prognostic significance of clinical, pathologic and treatment factors. *Medicine (Baltimore)*, 64, 219–27.

Johnson, J. P., Whitman, W., Briggs, W. A., *et al.* (1978). Plasmapheresis and immunosuppressive agents in antibasement membrane antibody-induced Goodpasture's syndrome. *Am J Med*, 64, 354–9.

Joshi, K., Nada, R., Minz, M., *et al.* (2007). Recurrent glomerulopathy in the renal allograft. *Transplant Proc*, 39, 734–6.

Kelly, P. T. and Haponik, E. F. (1994). Goodpasture syndrome: molecular and clinical advances. *Medicine (Baltimore)*, 73, 171–85.

Klasa, R. J., Abboud, R. T., Ballon, H. S., *et al.* (1988). Goodpasture's syndrome: recurrence after a five-year remission. Case report and review of the literature. *Am J Med*, 84, 751–5.

Laczika, K., Knapp, S., Derfler, K., *et al.* (2000). Immunoadsorption in Goodpasture's syndrome. *Am J Kidney Dis*, 36, 392–5.

Levy, J. B., Hammad, T., Coulthart, A., *et al.* (2004). Clinical features and outcome of patients with both ANCA and anti-GBM antibodies. *Kidney Int*, 66, 1535–40.

Levy, J. B., Lachmann, R. H., and Pusey, C. D. (1996). Recurrent Goodpasture's disease. *Am J Kidney Dis*, 27, 573–8.

Levy, J. B., Turner, A. N., and Rees, A. J. (2001). Long-term outcome of anti-glomerular basement membrane antibody disease treated with plasma exchange and immunosuppression. *Ann Intern Med*, 134, 1033–42.

Li, F. K., Tse, K. C., Lam, M. F., *et al.* (2004). Incidence and outcome of antiglomerular basement membrane disease in Chinese. *Nephrology (Carlton)*, 9, 100–4.

Mehler, P. S., Brunvand, M. W., Hutt, M. P., *et al.* (1987). Chronic recurrent Goodpasture's syndrome. *Am J Med*, 82, 833–5.

Merkel, F., Pullig, O., Marx, M., *et al.* (1994). Course and prognosis of anti-basement membrane antibody (anti-BM-Ab)-mediated disease: report of 35 cases. *Nephrol Dial Transplant*, 9, 372–6.

Pepys, E. O., Rees, A. J., and Pepys, M. B. (1982). Enumeration of lymphocyte populations in whole peripheral blood of patients with antibody-mediated nephritis during treatment with cyclosporin A. *Immunol Lett*, 4, 211–14.

Quérin, S., Schurch, W., and Beaulieu, R. (1992). Ciclosporin in Goodpasture's syndrome. *Nephron*, 60, 355–9.

Savage, C. O., Pusey, C. D., Bowman, C., *et al.* (1986). Antiglomerular basement membrane antibody mediated disease in the British Isles 1980-4. *Br Med J (Clin Res Ed)*, 292, 301–4.

Schindler, R., Kahl, A., Lobeck, H., *et al.* (1998). Complete recovery of renal function in a dialysis-dependent patient with Goodpasture syndrome. *Nephrol Dial Transplant*, 13, 462–6.

Shah, M. K. and Hugghins, S. Y. (2002). Characteristics and outcomes of patients with Goodpasture's syndrome. *South Med J*, 95, 1411–18.

Wilson, C. B. and Dixon, F. J. (1981). The renal response to immunological injury. In *The kidney* (ed. B.M. Brenner and F.C. Rector), pp. 1237–350. Philadelphia, P: W.B. Saunders.

Zimmerman, S. W., Varanasi, U. R., and Hoff, B. (1979). Goodpasture's syndrome with normal renal function. *Am J Med*, 66, 163–71.

CHAPTER 74

Antiglomerular basement membrane disease: pathogenesis

Zhao Cui, Neil Turner, and Ming-hui Zhao

Introduction

Elucidating the aetiology of autoimmune disease in man is a formidable task, but anti-GBM disease provides a model in which pathogenesis has been quite well defined. Loss of tolerance with the consequent development of autoimmune disease requires both an underlying genetic susceptibility and exposure to an environmental trigger. There are also many reports of anti-GBM disease occurring in association with other disorders, especially those with damage on the kidneys. One hypothesis suggests that in individuals with susceptible HLA alleles, things that alter antigen presentation in quantity or quality, or that expose epitopes sequestered within the basement membranes, trigger an autoimmune response to α3(IV)NC1 (Nachman et al., 2007). There is no direct evidence to prove this mechanism, only suggestive or circumstantial evidence from animal experiments and case observations.

Predisposing factors

Inherited susceptibility

Genetic predisposition to mount an anti-GBM response is an important requirement for the disease. There is clear evidence of inherited susceptibility from reports of the disease occurring in four sibling pairs (Stanton and Tange, 1958; Gossain et al., 1972) and two sets of identical twins (D'Apice et al., 1978; Simonsen et al., 1982). Further support comes from mouse models of anti-GBM disease in which crescentic glomerulonephritis and lung haemorrhage are restricted to only certain major histocompatibility complex (MHC) haplotypes, despite the ability of mice of all haplotypes to produce anti-α3(IV)NC1 antibodies (Kalluri et al., 1997).

In common with many other human autoimmune disorders, anti-GBM disease has been associated with inheritance of specific class II HLA alleles. A strong association with HLA-DR2 specificity (Rees et al., 1978) has been extended. Meta-analysis of published series confirms that the primary association is with the DRB1 locus (Phelps et al., 2000). It also demonstrates a hierarchy of associations, ranging from a strong positive association with DRB1*1501 (odds ratios (OR) = 8.5), through weaker positive associations with DRB1*0401 and DRB1*0301, to neutral effects and then increasingly strong negative associations with DRB1*0101 and DRB1*0701 (OR = 0.6 and 0.3 respectively). Most patients (63.9–92%) with anti-GBM disease inherit the DRB1*1501 allele (Dunckley et al., 1991; Huey et al., 1993; Fisher et al., 1997; Phelps and Rees, 1999; Kitagawa et al., 2008; Yang et al., 2009). Gene dosage does not affect susceptibility.

The molecular mechanisms that may underlie the strong HLA class II associations with anti-GBM disease has been explored with the precisely defined autoantigen α3(IV)NC1. DRB1*1501 in general bound the α3(IV)NC1-derived peptides with low affinity compared to DRB1*0101 and DRB1*0701. All the major α3(IV)NC1 peptides would bind preferentially to DRB1*01/07 in DRB1*15, 01/07 heterozygote antigen-presenting cells (APC), which present the peptides to T-helper cells (Phelps et al., 2000). Thus, DRB1*0101 and DRB1*0701 could protect by capturing α3(IV)NC1 peptides and preventing their display bound to DRB1*1501.

The immunoglobulin heavy chain Gm locus which encodes the IgG heavy chain constant region was identified as a second genetic influence on anti-GBM disease (Rees et al., 1984).

Anti-GBM antibodies derived from Wistar Kyoto (WKY) rats are only able to transfer crescentic glomerulonephritis to WKY rats but not Lewis rats, suggesting that factors related to the inflammatory response to deposited antibody contribute to disease susceptibility (Reynolds et al., 2006). The genetic basis of this is shown to be owing to a copy number polymorphism of *Fcgr3* (Aitman et al., 2006). In a Chinese cohort susceptibility of the disease was linked to gene polymorphism of *FCGR2B* (232T/I) and the copy number variation of *FCGR3A* (Zhou et al., 2010a, 2010b). These findings need to be tested in different populations.

Lymphocyte depletion and breaking tolerance

Lymphocyte depletion has been associated with autoimmunity in a number of clinical settings and in animal models. Autoimmune phenomena are seen as lymphocytes begin to recover, so presumably with a limited range of affinities and perhaps lacking the usual balance of regulatory and potentially inflammatory cells. In patients with multiple sclerosis the anti-CD52 lymphocyte depleting antibody alemtuzumab (CamPath®) has been associated with a high incidence of autoimmmune thyroiditis and less frequently with other autoimmune conditions, including several instances of anti-GBM disease (Coles et al., 2012; Jones et al., 2013). Multiple sclerosis also has an association with HLA-DR15, so perhaps this is understandable.

Several case reports have also reported an association of anti-GBM disease with HIV infection (Monteiro et al., 2006; Wechsler et al., 2008; reviewed by Hartle et al., 2013). In a cohort of 105 HIV-infected individuals, 18 (17%) sera were identified as containing anti-GBM antibodies, but they were associated with polyclonal activation and had no clinical features (Savige et al., 1994; Szczech et al., 2006).

Environmental triggers

Infectious agents have long been suspected as causative, since an upper respiratory infection precedes disease onset in 20–60% of cases (Appel et al., 2007). Influenza virus has been specifically mentioned in a number of reports, including Goodpasture's original description (1919), but infection with the virus is common and no studies have consistently shown an association with any particular infection (Wilson and Dixon, 1973; Wilson and Smith, 1972). The association with HIV infection may be different (see above paragraph).

For bacterial infections, it was suggest that mimicry of T-cell epitopes by microbial antigens derived from *Clostridium botulinum* is sufficient to induce anti-GBM disease in WKY rats (Arends et al., 2006). However, no relationship has been found between *C. botulinum* (or any other infection) and human anti-GBM disease.

Pulmonary haemorrhage

The link between pulmonary involvement and smoking was established by Donaghy and Rees (1983). They found that pulmonary haemorrhage occurred in nearly all patients with anti-GBM disease who smoke, whereas this complication was rare in non-smokers. Exposure to organic solvents and hydrocarbons has been associated with disease onset (Bombassei and Kaplan, 1992; Stevenson et al., 1995). Around 6% of all patients with anti-GBM disease in the literature have hydrocarbon exposure (Shah, 2002) in retrospective analyses, and a causal relationship has been suggested, but the evidence is weak. It is more likely that it exposes disease in those with a pre-existing immune response. The direct role of smoke inhalation and hydrocarbon exposure is further demonstrated by recurrence of anti-GBM disease. The majority of them are young male patients with recurrent pulmonary haemorrhage, in association with cigarette smoking or hydrocarbon re-exposure after apparent remission (Keller and Nekarda, 1985; Levy et al., 1996).

Similarly, anti-GBM disease has been associated with intranasal or smoked cocaine (crack) (Garcia-Rostan y Perez et al., 1997; Peces et al., 1999), but the same caveats about causation apply.

In experimental models, cigarette smoke and other non-specific irritants (gasoline, oxygen toxicity) act in a similar way of a non-specific irritant or toxic effect on the lungs and precipitates pulmonary haemorrhage in the presence of circulating anti-GBM antibodies (Jennings et al., 1981; Downie et al., 1982; Yamamoto et al., 1987). Similarly, intercurrent infection amplifies the intensity of inflammatory responses and can aggravate disease and so make it clinically apparent (Rees et al., 1978; Daly et al., 1996).

It may be difficult to distinguish agents that exacerbate previously covert disease from true aetiological agents that initiate disease in individual cases, but it is worth noting that in animal models of active immunity the disease takes weeks to months to develop.

Disease associations

Anti-GBM disease has been reported in association with several forms of other diseases, including autoimmune disorders, glomerulonephritis and others.

Dual positivity for anti-GBM and ANCA

Ten to 38% of patients with anti-GBM disease also have ANCA detectable in their sera with specificity mainly for myeloperoxidase (MPO) (Short et al., 1995; Hellmark et al., 1997; Levy et al., 2004; Rutgers et al., 2005; Yang et al., 2007; Zhao et al., 2007; Cui et al., 2011a). These patients are termed as double positive.

Anti-GBM response could occur in genetically predisposed patients following damage to the glomerular or alveolar basement membrane by small vessel vasculitis. However, there are occasional instances where the clinical history suggests that anti-GBM disease antedated the development of vasculitis (O'Donoghue et al., 1989; Peces et al., 2000).

Double-positive patients are mostly older (average age of 55–66 years) and present with higher prevalence of systemic involvement, including muscle pain, arthralgia, skin rash, nasal, ear, eye, throat, pulmonary, gastrointestinal, and nervous system involvement. Pulmonary haemorrhage occurs in about half of the patients, presenting no difference from the patients with anti-GBM antibodies alone or those with ANCA only. Renal involvement behaves more like anti-GBM disease than vasculitis (Table 74.1). Serum creatinine levels and percentages of patients presenting with oliguria or anuria are higher in double-positive and anti-GBM positive patients, compared with MPO-ANCA single-positive patients (Rutgers et al., 2005).

Renal biopsy shows extensive glomerular cellular crescents in most patients and some of them show linear binding of antibodies to GBM by direct immunofluorescence. Nevertheless, granulomatous periglomerular inflammation is found more common in double-positive patients, but not in anti-GBM-positive patients (Levy et al., 2004; Rutgers et al., 2005).

Renal prognosis is worse than that of patients with ANCA-associated vasculitis, but similar to anti-GBM disease. Recovery from severe renal failure is rare. Patient survival is worse than that of anti-GBM disease, but similar to vasculitis (Levy et al., 2004; Rutgers et al., 2005; Cui et al., 2011a). Thus, intensive plasmapheresis and immunosuppressive therapy are crucial in the early stage of treatment, and maintenance therapy may be necessary for patients in remission.

In view of the high frequency of positive ANCA in patients with anti-GBM disease, the coexistence of these two types of autoantibodies cannot be explained by chance. There is no significant correlation observed between the titres of anti-GBM antibodies and ANCA. Irrespective of whether the antibodies are non-pathogenic in healthy individuals or pathogenic in patients with anti-GBM disease, no cross reaction is seen among antibodies against GBM, MPO, or PR3 (Hellmark et al., 1997; Cui et al., 2010). In double-positive patients, the prevalence of antibodies against GBM targeting α1(IV)NC1, α4(IV)NC1 and α5(IV)NC1 is significantly higher, while the prevalence of anti-GBM antibodies targeting α3(IV)NC1, specific epitope E_A and E_B on α3(IV)NC1 are lower, than that in patients with anti-GBM antibodies alone. Thus, the double-positive patients have a broader spectrum of antibodies to type IV collagen than patients with anti-GBM antibodies alone (Yang et al., 2007). This may support the hypothesis that the anti-GBM response is usually secondary to the small vessel vasculitis.

Olson et al. (2011) used historical samples to identify elevated anti-GBM titres in advance of diagnosis of 30 patients with anti-GBM disease who did not in general have 'double positivity' at diagnosis. Four had elevated titres months in advance of the disease, but elevated ANCA, particularly anti-Pr3, were found further in advance in a larger proportion, raising the possibility that subclinical vasculitis is the trigger more often than currently thought.

Table 74.1 Characteristics of patients with MPO-ANCA, anti-GBM antibodies and double positive (Rutgers et al., 2005)

	MPO-ANCA (N = 46)	Double-positive (N = 10)	Anti-GBM (N = 13)
Age (years)	63 ± 12.7	64 ± 8.7	52 ± 20.6
Male sex (%)	67	80	39
Blood pressure (mmHg)	150/83	158/84	145/85
Proteinuria (g/24 hours)	2.0 (0.0–4.1)	1.3 (0.0–2.6)	2.3 (0.6–5.4)
Anuira/oliguria (%)	12	63	50
Serum creatinine (mg/dL)	5.0 ± 2.9	10.3 ± 5.6	9.6 ± 8.1
Cellular crescents (%)	18 (0–75)	27 (0–75)	29 (0–90)
Fibrous crescents (%)	27 (0–100)	31 (0–100)	43 (0–100)
Periglomerular granuloma (%)	11	40	0
Linear deposits of IgG on GBM (%)	0	40	77
No deposit (%)	59	0	0
Scanty, not linear deposits (%)	33	50	15
Renal survival at 1 year (%)	64	10	15
Patient survival at 1 year (%)	75	79	100

Note: to convert serum creatinine in mg/dL to µmol/L, multiply by 88.4.

Membranous nephropathy

An association with idiopathic membranous nephropathy (Fig. 74.1) was first described in 1974 (Klassen et al., 1974), and the literature now includes nearly 30 cases. In some of the reported cases, a sudden deterioration of previously diagnosed membranous nephropathy has been associated with anti-GBM antibodies (Klassen et al., 1974; Kurki et al., 1984). In other examples, both membranous nephropathy and anti-GBM disease were present on initial assessment (Pasternack et al., 1978; Savige et al., 1989; Cui et al., 2006; Basford et al., 2011). Occasionally, there are cases having anti-GBM disease followed by membranous nephropathy (Agodoa et al., 1976; Kielstein et al., 2001; Hecht et al., 2008). It is hypothesized that the GBM may be damaged in membranous nephropathy, or its turnover increased, altering antigen processing so that new (cryptic) epitopes to which there is little immune tolerance are released.

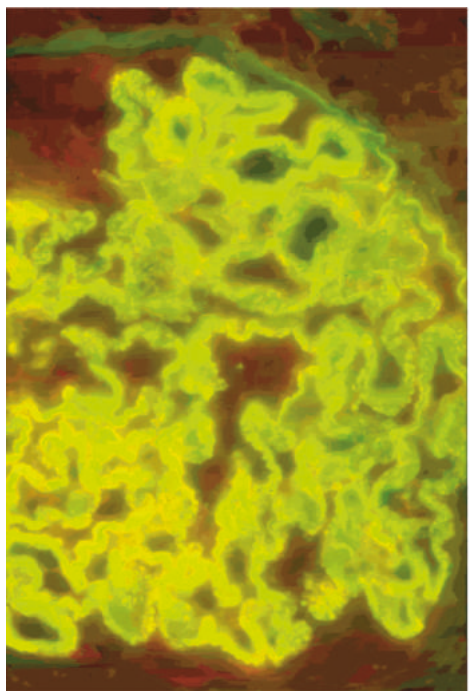

Fig. 74.1 Direct immunofluorescence for IgG on the glomerulus of a 65-year-old man with nephrotic syndrome that deteriorated rapidly to severe renal failure. Linear binding to the endothelial surface of the GBM is accompanied by granular deposits of IgG subepithelially, typical of membranous nephropathy. This was confirmed by electron microscopy. Typical anti-GBM antibodies were detected in serum.

Other conditions

There are numerous but mostly isolated case reports of anti-GBM disease in association with other possible relevant diseases (Table 74.2). Some are likely to be coincidental.

Animal models

Studies in animal models have shown that both antibodies against GBM and antigen-specific T cells are pathogenic. Therefore, humoral and cellular immunity both contribute to glomerular damage in anti-GBM disease.

Two experimental models of anti-GBM disease, mostly in mice or rats, are widely used. In experimental autoimmune glomerulonephritis (EAG), animals from susceptible strains are immunized with homologous or heterologous GBM or α3(IV)NC1 and after some delay develop an autoimmune response that targets their own kidneys. In nephrotoxic nephritis (NTN), animals are injected with heterologous antibodies to GBM, which deposit in kidney and cause transient injury (the heterologous phase). The animal then mounts its own immune response to the foreign immunoglobulin, which acts as a planted antigen on GBM (the autologous phase). This

Table 74.2 Disorders in association with anti-GBM disease (Kalderon et al., 1973; Curtis et al., 1976; Ma et al., 1978; Blake et al., 1980; Kleinknecht et al., 1980; Blake et al., 1980; Wilson and Dixon 1981; Guerin et al., 1990; Tan and Cumming, 1993; Umekawa et al., 1993; Kalluri et al., 1994; Kelly and Haponik, 1994; Wuthrich et al., 1994; Savage et al., 1996; Bindi et al., 1997; Drube et al., 1997; Ahuja et al., 1998; Henderson et al., 1998; Komatsu et al., 1998; Xenocostas et al., 1999; Morello et al., 2001; Curioni et al., 2002; Shaer et al., 2003; Li et al., 2006; Lv et al., 2009; Torok et al., 2010)

Immune (presumed)	Glomerulonephritis	Others	
ANCA associated vasculitis Lymphocyte depletion (alemtuzumab, HIV) Alport post-transplant Penicillamine therapy	Membranous nephropathy Diabetic nephropathy	Lithotripsy	Likely real
Systemic lupus erythematosus Myasthenia gravis Thrombotic thrombocytopenic purpura Dermatomyositis Thyroiditis Primary biliary cirrhosis Multiple sclerosis Cryoglobulinaemia	IgA nephropathy Membranoproliferative glomerulonephritis Focal segmental glomerulosclerosis Henoch–Schönlein purpura	Lymphoma Castleman disease Partial lipodystrophy Coeliac disease Inflammatory bowel disease Vitamin B_{12} deficiency Pneumococcal vaccination Nail-patella syndrome	Probably coincidental

model can be made more severe by pre-immunizing the animal with Ig from the species in which anti-GBM antibodies are raised (the accelerated model of NTN). These animal models permit studies of the role of various inflammatory mediators in the development of disease (Sado and Naito, 1987; Bolton et al., 1993; Sado et al., 1998; Chen et al., 2003, 2006). Conclusions about the pathogenesis of human anti-GBM disease drawn from animal models, however, must be tempered by the realization that findings in animal models might not exactly reflect outcomes in human disease.

Antibody responses

Localization of the Goodpasture antigen

GBM composition is described in Chapter 320. The Goodpasture antigen-bearing type IV collagen chain, α3, is a significant component of the glomerular and alveolar basement membranes (Saus et al., 1988; Kalluri et al., 1995; Pedchenko et al., 2010), and has been found also in testis, choroid plexus, Bruch's membrane in the eye, cochlea and neuromuscular junction. However, clinical manifestations outside the kidney and lung are uncommon. Autoantibodies from patients with anti-GBM disease bind exclusively to the C-terminal globular NC1 domain, a3(IV)NC1 (Leinonen et al., 1999).

Epitope mapping has defined major and minor conformational epitopes of α3(IV)NC1, designated as E_A, which encompasses residues 17–31, and E_B, which encompasses residues 127–141 (Netzer et al., 1999; David et al., 2001). The epitopes appear to be structurally sequestered (hidden) to some extent by adjacent α5(IV)NC1 and α4(IV)NC1 in α3.α4.α5(IV) hexamer (Fig. 74.2) (Borza et al., 2002). Perturbation of the quaternary structure of α3.α4.α5(IV) hexamer by denaturation or possibly in conditions of inflammation increases the accessibility of epitopes (Kalluri et al., 2000). This is the reason that Goodpasture epitopes are described as 'cryptic'.

In addition to reactivity to α3(IV)NC1, which is detectable in all patients, other antibodies recognizing other α chains of collagen IV may be present, such as α1, α2, α4 and α5(IV)NC1 (Johansson et al., 1993; Hellmark et al., 1994; Kalluri et al., 1995, 1996; Dehan et al., 1996; Ghohestani et al., 2000, 2003; Yang et al., 2007; Zhao et al., 2009b; Pedchenko et al., 2010). A few reports suggest that relatively minor disease can occasionally be associated with such antibodies in the absence of reactivity for α3(IV)NC1 (Ghohestani et al., 2000; Chen et al., 2003; Zhao et al., 2009b; Pedchenko et al., 2010). In the presence of linear binding on immunofluorescence and active disease, low α3 titres are a likely alternative explanation for most negative immunoassay results. Western blotting or individual immunoassays at a specialist centre would be required for certainty.

Alport anti-GBM disease is a special example which is considered in Chapter 75.

Antibodies against GBM

There is good evidence that antibodies against GBM have a directly causal role. In the classic adoptive transfer experiments by Lerner et al. (1967), antibodies eluted from the kidneys of patients with anti-GBM disease and injected into squirrel monkeys could fix to

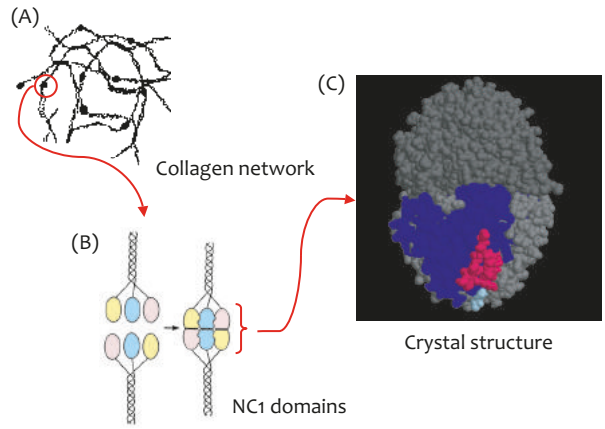

Fig. 74.2 (A) Cartoon of the network of the type IV collagen network within GBM. (B) An NC1 hexamer, as produced by digestion of GBM with collagenase. (C) The crystal structure of a type IV collagen NC1 domain hexamer. A model of the α1/α2 hexamer crystal structure. The NC1 domain of a single type IV collagen chain is shown in blue, with its N terminus (where it joins the collagenous part of the molecule) in light blue. The equivalent location of the major B cell epitope on α3(IV)NC1 is shown in red. Fig. 71.1 in Chapter 71 shows the Western blotting pattern of these domains.
Modified from Than et al. (2002) by Dr R. Phelps.

the GBM of squirrel monkeys *in vivo* and cause pathological glomerular changes and pulmonary haemorrhage. In patients with anti-GBM disease, antibody concentrations are correlated with the severity of kidney damage at presentation and the raised titres are independently prognostic for kidney outcome and patient survival (Hellmark et al., 1994, 1999; Ang et al., 1998; Cui et al., 2005, 2011a; Yang et al., 2009). The removal of circulating antibodies with plasmapheresis is associated with clinical recovery after lung haemorrhage and renal dysfunction (Lockwood et al., 1975; Jindal, 1999; Levy et al., 2001). Furthermore, anti-GBM disease recurs immediately in renal allografts if circulating anti-GBM antibodies remain positive in recipients (Wilson and Dixon, 1973).

The α3(IV)NC1-specific human anti-GBM antibodies can be of any IgG subclass but are typically IgG1 (high affinity for Fcγ receptors, probably Th1 associated) or IgG4 (low affinity for Fcγ receptors, probably Th2 associated), accounting for less than 1% of circulating IgG (Bowman et al., 1987; Segelmark et al., 1990; Zhao et al., 2009a). Clinical reports, together with passive transfers of monoclonal anti-GBM antibodies of different IgG subclasses in WKY rats suggests that high affinity for Fcγ receptors is important as an IgG1 (in rodents as IgG2a), but not an IgG4 (rodent IgG1) skewed humoral immune response is pathogenic (Kohda et al., 2004). Anti-GBM antibodies have high affinity, with relatively high on (binding) rates and slow off (dissociation) rates (Rutgers et al., 2000). The specificities of circulating and tissue bound antibodies are identical (Pedchenko et al., 2010), with predominant target always α3(IV)NC1, and conformational epitopes, E_A and E_B, as described above.

In healthy individuals, natural antibodies against GBM can be purified from IgG fractions in sera or plasma. They recognize human α3(IV)NC1 and are specific for E_A and E_B, as are antibodies against GBM in patients, but affinity is lower and antibodies are restricted to IgG2 and IgG4 subclasses (Cui et al., 2010; 181, 182). Their significance is not entirely clear.

Studies investigating disease development focus on the differences in immunologic characteristics between natural antibodies and disease-associated ones (Table 74.3) (Cui and Zhao, 2005; Cui et al., 2006, 2007; Zhao et al., 2009a, 2009b). In patients with anti-GBM disease and normal kidney function, anti-GBM antibodies in circulation generally present lower titre, lower affinity, less of IgG1 subclass, and limited reaction to α3(IV)NC1 and α5(IV)NC1. In patients with mild and moderate renal dysfunction, the circulating antibodies reveal higher titre, higher affinity, more of IgG1 subclass and broader spectrum of target antigens for the five α chains of type IV collagen. In patients with dialysis-dependent acute renal failure, the antibodies have the highest titre, highest affinity, of IgG1 predominance (Sado et al., 1998; Rutgers et al., 2000; Radeke et al., 2002; Kohda et al., 2004; Bolton et al., 2005; Chen et al., 2006). Table 74.3 shows some of the characteristics of antibodies in different circumstances.

Cell-mediated immunity

Although anti-GBM disease is seen as a prototypic autoantibody-mediated disease, there is strong experimental evidence that the full expression of disease is dependent on cell-mediated autoimmunity, in particular autoreactive T cells. T-cell involvement can be implied from the strong HLA associations in human anti-GBM disease. Help from T cells is required for affinity maturation, antigen specificity, subclass switching and epitope spreading of antibody response. Effector T cells also contribute directly to tissue injury of anti-GBM disease with the finding of CD4+ and CD8+ T cells in affected glomeruli (Nolasco et al., 1987). In rodent models, one T-cell epitope, pCol(28–40), derived from α3(IV)NC1, induces severe glomerulonephritis in WKY rats and triggers a diversified anti-GBM antibody response through B-cell spreading of epitopes (Wu et al., 2001). In the absence of antibodies against GBM, α3(IV)NC1-specific CD4+ T cells alone is sufficient to initiate glomerular injury and reveals a direct pathogenic role (Wu et al., 2001, 2003, 2004; Robertson et al., 2005; Arends et al., 2006). The disease can be inhibited by anti-T-cell therapies, including CD28–B7 or CD154–CD40 co-stimulatory blockade (Reynolds et al., 2000, 2004), and anti-CD4 and anti-CD8

Table 74.3 Different characteristics of anti-GBM antibodies in healthy versus diseased individuals

Patient group	Percentage of IgG	Titre	Affinity (aK)	IgG subtype	Target antigens
Healthy individuals with natural anti-GBM antibodies	0.5%	1:60	$9.09 \times 10^6\ M^{-1}$	IgG2 (100%) IgG4 (100%)	α3(IV)NC1 (100%) α4(IV)NC1 (100%)
Patients with pathogenic anti-GBM antibodies and normal renal function	Unknown	1:200	$4.0 \times 10^7\ M^{-1}$	IgG1 (8%) IgG2 (62%) IgG4 (39%)	α3(IV)NC1 (100%) α4(IV)NC1 (14%) α5(IV)NC1 (86%)
Patients with pathogenic anti-GBM antibodies and moderate renal dysfunction	Unknown	1:400	$2.4 \times 10^8\ M^{-1}$	IgG1 (69%) IgG2 (59%) IgG3 (35%) IgG4 (66%)	α3(IV)NC1 (100%) α1(IV)NC1 (40%) α2(IV)NC1 (35%) α4(IV) NC1 (45%) α5(IV)NC1 (70%)
Patients with pathogenic anti-GBM antibodies and severe renal injury and dependent on dialysis	1%	1:800	$3.26 \times 10^8\ M^{-1}$	IgG1 (94%) IgG2 (87%) IgG3 (32%) IgG4 (61%)	α3(IV)NC1 (100%) α1(IV)NC1 (50%) α2(IV)NC1 (43%) α4(IV) NC1 (60%) α5(IV)NC1 (93%)

aK = affinity constant of antibodies against GBM, measured as the reciprocal value of molar concentration of α(IV)NC1 needed for 50% inhibition of the binding capacity; GBM = glomerular basement membrane.

monoclonal antibodies (Reynolds et al., 2002). Furthermore, T-cell tolerance can be induced via oral or nasal administration of Goodpasture antigen prior to induction of disease (Reynolds et al., 2005).

In EAG models, Th1 responses are found pathogenetic, while Th2 responses are protective (Holdsworth et al., 1999; Phoon et al., 2008). A polarity shift from Th2 to Th1 response seems feasible during disease process. Besides that, Th17 cell subset, which is maintained by IL-23, plays a dominant part in the development of disease (Holdsworth et al., 1999; Hopfer et al., 2003; Phoon et al., 2008; Ooi et al., 2009; Summers et al., 2009). Mice deficient in IL-23 immunized with α3(IV)NC1 develop anti-GBM disease, although autoantibody titres are lower, cellular reactivity is reduced, and renal injury is less severe than in mice with IL-23 (Ooi et al., 2009). A move from an antibody-associated phase to a more aggressive phase associated with Th1 and Th17 CD4+ T cells was described in DBA/1 mice (Hopfer et al. 2012).

α3 and α4 and α5 chains of type IV collagen are all expressed in normal human thymus (Wong et al., 2001). It is not known what proportion of T cells that recognize the respective α chains are deleted in the thymus, but some α3(IV)NC1-specific CD4+ T cells escape thymic deletion and exist in the periphery circulation in healthy individuals (Zou et al., 2008). Peripheral blood T cells from healthy individuals have similar specificities to the α3(IV)NC1-reactive T cells found in patients with anti-GBM disease. However, T cells that proliferate in response to α3(IV)NC1 are much less abundant in healthy individuals, in the absence of stimulation. Therefore, the naïve autoimmunity to α3(IV)NC1 can be classified as quiescent. In patients with active anti-GBM disease, concentrations of autoreactive T cells at onset are higher than that in healthy controls, decline in remission and reach to similar levels of healthy controls after several years (Salama et al., 2001).

Unlike most autoimmune diseases, anti-GBM disease does not follow a relapsing and remitting course. Treatment reduces the time to disappearance of antibody, but even without treatment most patients no longer have detectable circulating antibody after 12–18 months (Levy et al., 1996). Re-establishment of autoimmune tolerance coincides with the alteration and persistence of α3(IV) NC1 reactive T cells from T-helper cells to T-regulatory cells during the evolution of disease (Cairns et al., 2003; Salama et al., 2003). These could be responsible for terminating the anti-GBM response. Regulatory CD4+CD25+ T cell depletion in peripheral blood mononuclear cells from convalescent patients markedly increases the number of Goodpasture antigen-specific IFN-γ producing cells (by ELISPOT) (Salama et al., 2003). In EAG models, transfer of regulatory CD4+CD25+ T cells prior to disease induction significantly attenuates glomerular injury (Wolf et al., 2005).

T-cell epitope

T cells recognize antigen only when presented in the form of peptides bound to MHC class II molecules on the surface of APCs. Thus, T-cell epitopes are critically dependent on the peptides generated by digestion within APCs (antigen processing) and the ability of these peptides to bind MHC class II molecules (antigen presentation). α3(IV)NC1-derived peptides have been characterized that are naturally processed and presented by cells bearing HLA-DRB1*1501, which consists of nested sets centred on core MHC-binding motifs and overlaps the major autoantibody epitope. The linear epitopes of α3(IV)NC1 recognized by autoreactive T cells have proven hard to define (Hellmark et al., 1996). A mapping study of human T-cell epitopes has defined the specificity of α3(IV)NC1 reactive T cells as being highly focused on two peptides: $\alpha3_{71-90}$ and $\alpha3_{131-150}$ (Cairns et al., 2003). The epitopes are highly susceptible to early endopeptidases, such as cathepsins D and E, and might be destroyed by APCs before presentation in the thymus. The $\alpha3_{131-150}$ epitope overlaps with E_B region and binds with high affinity to the disease-associated allele HLA-DRB1*1501. The key residues have been mapped to residues $\alpha3_{134-148}$, which stimulate T cells from patients with anti-GBM disease to proliferate and secrete IFN-γ (Zou et al., 2007). The same peptide was identified by a different method of mapping and modelling in murine models by Ooi et al. (2013).

References

Abrahamson, D. R., Hudson, B. G., Stroganova, L., *et al.* (2009). Cellular origins of type IV collagen networks in developing glomeruli. *J Am Soc Nephrol*, 20, 1471–9.

Agodoa, L. C., Striker, G. E., George, C. R., *et al.* (1976). The appearance of nonlinear deposits of immunoglobulins in Goodpasture's syndrome. *Am J Med*, 61, 407–13.

Ahuja, T. S., Velasco, A., Deiss, W., Jr., *et al.* (1998). Diabetic nephropathy with anti-GBM nephritis. *Am J Kidney Dis*, 31, 127–30.

Aitman, T. J., Dong, R., Vyse, T. J., *et al.* (2006). Copy number polymorphism in Fcgr3 predisposes to glomerulonephritis in rats and humans. *Nature*, 439, 851–5.

Ang, C., Savige, J., Dawborn, J., *et al.* (1998). Anti-glomerular basement membrane (GBM)-antibody-mediated disease with normal renal function. *Nephrol Dial Transplant*, 13, 935–9.

Appel, G. B., Radhakrishnan, J., and D'Agati, V. (2007). Secondary glomerular disease. In B. M. Brenner (ed.) *Brenner and Rector's The Kidney*, pp. 1067–126. Philadelphia, PA: Saunders.

Arends, J., Wu, J., Borillo, J., *et al.* (2006). T cell epitope mimicry in antiglomerular basement membrane disease. *J Immunol*, 176, 1252–8.

Basford, A. W., Lewis, J., Dwyer, J. P., *et al.* (2011). Membranous nephropathy with crescents. *J Am Soc Nephrol*, 22, 1804–8.

Bindi, P., Gilson, B., Aymard, B., *et al.* (1997). Antiglomerular basement membrane glomerulonephritis following D-penicillamine-associated nephrotic syndrome. *Nephrol Dial Transplant*, 12, 325–7.

Blake, D. R., Rashid, H., McHugh, M., *et al.* (1980). A possible association of partial lipodystrophy with anti-GBM nephritis (Goodpasture's syndrome). *Postgrad Med J*, 56, 137–9.

Bolton, W. K., Chen, L., Hellmark, T., *et al.* (2005). Epitope spreading and autoimmune glomerulonephritis in rats induced by a T cell epitope of Goodpasture's antigen. *J Am Soc Nephrol*, 16, 2657–66.

Bolton, W. K., May, W. J., and Sturgill, B. C. (1993). Proliferative autoimmune glomerulonephritis in rats: a model for autoimmune glomerulonephritis in humans. *Kidney Int*, 44, 294–306.

Bombassei, G. J. and Kaplan, A. A. (1992). The association between hydrocarbon exposure and anti-glomerular basement membrane antibody-mediated disease (Goodpasture's syndrome). *Am J Ind Med*, 21, 141–53.

Borza, D. B., Bondar, O., Colon, S., *et al.* (2005). Goodpasture autoantibodies unmask cryptic epitopes by selectively dissociating autoantigen complexes lacking structural reinforcement: novel mechanisms for immune privilege and autoimmune pathogenesis. *J Biol Chem*, 280, 27147–54.

Borza, D. B., Bondar, O., Todd, P., *et al.* (2002). Quaternary organization of the goodpasture autoantigen, the alpha 3(IV) collagen chain. Sequestration of two cryptic autoepitopes by intrapromoter interactions with the alpha4 and alpha5 NC1 domains. *J Biol Chem*, 277, 40075–83.

Bowman, C., Ambrus, K., and Lockwood, C. M. (1987). Restriction of human IgG subclass expression in the population of auto-antibodies to glomerular basement membrane. *Clin Exp Immunol*, 69, 341–9.

Cairns, L. S., Phelps, R. G., Bowie, L., *et al.* (2003). The fine specificity and cytokine profile of T-helper cells responsive to the alpha3 chain of type IV collagen in Goodpasture's disease. *J Am Soc Nephrol*, 14, 2801–12.

Chen, L., Hellmark, T., Pedchenko, V., *et al.* (2006). A nephritogenic peptide induces intermolecular epitope spreading on collagen IV in experimental autoimmune glomerulonephritis. *J Am Soc Nephrol*, 17, 3076–81.

Chen, L., Hellmark, T., Wieslander, J. *et al.* (2003). Immunodominant epitopes of alpha3(IV)NC1 induce autoimmune glomerulonephritis in rats. *Kidney Int*, 64, 2108–20.

Coles, A. J., Fox, E., Vladic, A., *et al.* (2012). Alemtuzumab more effective than interferon β-1a at 5-year follow-up of CAMMS223 clinical trial. *Neurology*, 78(14), 1069–78.

Cui, Z. and Zhao, M. H. (2005). Avidity of anti-glomerular basement membrane autoantibodies was associated with disease severity. *Clin Immunol*, 116, 77–82.

Cui, Z., Wang, H. Y., and Zhao, M. H. (2006). Natural autoantibodies against glomerular basement membrane exist in normal human sera. *Kidney Int*, 69, 894–9.

Cui, Z., Zhao, J., Jia, X. Y., *et al.* (2011). Anti-glomerular basement membrane disease: outcomes of different therapeutic regimens in a large single-center chinese cohort study. *Medicine (Baltimore)*, 90, 303–11.

Cui, Z., Zhao, J., Jia, X. Y., *et al.* (2011b). Clinical features and outcomes of anti-glomerular basement membrane disease in older patients. *Am J Kidney Dis*, 57, 575–82.

Cui, Z., Zhao, M. H., Segelmark, M., *et al.* (2010). Natural autoantibodies to myeloperoxidase, proteinase 3, and the glomerular basement membrane are present in normal individuals. *Kidney Int*, 78, 590–7.

Cui, Z., Zhao, M. H., Singh, A. K., *et al.* (2007). Antiglomerular basement membrane disease with normal renal function. *Kidney Int*, 72, 1403–8.

Cui, Z., Zhao, M. H., Wang, S. X., *et al.* (2006). Concurrent antiglomerular basement membrane disease and immune complex glomerulonephritis. *Ren Fail*, 28, 7–14.

Cui, Z., Zhao, M. H., Xin, G., *et al.* (2005). Characteristics and prognosis of Chinese patients with anti-glomerular basement membrane disease. *Nephron Clin Pract*, 99, c49–55.

Curioni, S., Ferrario, F., Rastaldi, M. P., *et al.* (2002). Anti-GBM nephritis complicating diabetic nephropathy. *J Nephrol*, 15, 83–7.

Curtis, J. J., Bhathena, D., Leach, R. P., *et al.* (1976). Goodpasture's syndrome in a patient with the Nail-Patella syndrome. *Am J Med*, 61, 401–6.

D'Apice, A. J., Kincaid-Smith, P., Becker, G. H., *et al.* (1978). Goodpasture's syndrome in identical twins. *Ann Intern Med*, 88, 61–2.

Daly, C., Conlon, P. J., Medwar, W., *et al.* (1996). Characteristics and outcome of anti-glomerular basement membrane disease: a single-center experience. *Ren Fail*, 18, 105–12.

David, M., Borza, D. B., Leinonen, A., *et al.* (2001). Hydrophobic amino acid residues are critical for the immunodominant epitope of the Goodpasture autoantigen. A molecular basis for the cryptic nature of the epitope. *J Biol Chem*, 276, 6370–7.

Dehan, P., Weber, M., Zhang, X., *et al.* (1996). Sera from patients with anti-GBM nephritis including goodpasture syndrome show heterogenous reactivity to recombinant NC1 domain of type IV collagen alpha chains. *Nephrol Dial Transplant*, 11, 2215–22.

Donaghy, M. and Rees, A. J. (1983). Cigarette smoking and lung haemorrhage in glomerulonephritis caused by autoantibodies to glomerular basement membrane. *Lancet*, 2, 1390–3.

Downie, G. H., Roholt, O. A., Jennings, L., *et al.* (1982). Experimental anti-alveolar basement membrane antibody-mediated pneumonitis. II. Role of endothelial damage and repair, induction of autologous phase, and kinetics of antibody deposition in Lewis rats. *J Immunol*, 129, 2647–52.

Drube, S., Maurin, N., and Sieberth, H. G. (1997). Coincidence of myasthenia gravis and antiglomerular basement membrane glomerulonephritis: a combination of two antibody-mediated autoimmune diseases on day 15. *Nephrol Dial Transplant*, 12, 1478–80.

Duncan, D. A., Drummond, K. N., Michael, A. F. *et al.* (1965). Pulmonary hemorrhage and glomerulonephritis. report on six cases and study of the renal lesion by the fluorescent antibody technique and electron microscopy. *Ann Intern Med*, 62, 920–38.

Dunckley, H., Chapman, J. R., Burke, J., *et al.* HLA-DR and -DQ genotyping in anti-GBM disease. *Dis Markers*, 9, 249–56.

Fisher, M., Pusey, C. D., Vaughan, R. W., *et al.* (1997). Susceptibility to anti-glomerular basement membrane disease is strongly associated with HLA-DRB1 genes. *Kidney Int*, 51, 222–9.

Garcia-Rostan y Perez, G. M., Garcia Bragado, F., and Puras Gil, A.,M. (1997). Pulmonary hemorrhage and antiglomerular basement membrane antibody-mediated glomerulonephritis after exposure to smoked cocaine (crack): a case report and review of the literature. *Pathol Int*, 47, 692–7.

Ghohestani, R. F., Hudson, B. G., Claudy, A., *et al.* (2000). The alpha 5 chain of type IV collagen is the target of IgG autoantibodies in a novel autoimmune disease with subepidermal blisters and renal insufficiency. *J Biol Chem*, 275, 16002–6.

Ghohestani, R. F., Rotunda, S. L., Hudson, B., *et al.* (2003). Crescentic glomerulonephritis and subepidermal blisters with autoantibodies to alpha5 and alpha6 chains of type IV collagen. *Lab Invest* 83, 605–11.

Goodpasture, E. W. (1919). The significance of certain pulmonary lesions in relation to the etiology of influenza. *Am J Med Sci*, 158, 863–70.

Gossain, V. V., Gerstein, A. R., and Janes, A. W. (1972). Goodpasture's syndrome: a familial occurrence. *Am Rev Respir Dis*, 105, 621–4.

Guerin, V., Rabian, C., Noel, L. H., *et al.* (1990). Anti-glomerular-basement-membrane disease after lithotripsy. *Lancet*, 335, 856–7.

Hartle, P. M., Carlo, M. E., Dwyer, J. P., *et al.* (2013) AKI in an HIV patient. *J Am Soc Nephrol*, 24, 1204–8.

Hecht, N., Omoloja, A., Witte, D., *et al.* (2008). Evolution of antiglomerular basement membrane glomerulonephritis into membranous glomerulonephritis. *Pediatr Nephrol*, 23, 477–80.

Hellmark, T., Brunmark, C., Trojnar, J., *et al.* (1996). Epitope mapping of anti-glomerular basement membrane (GBM) antibodies with synthetic peptides. *Clin Exp Immunol*, 105, 504–10.

Hellmark, T., Johansson, C. and Wieslander, J. (1994). Characterization of anti-GBM antibodies involved in Goodpasture's syndrome. *Kidney Int*, 46, 823–9.

Hellmark, T., Niles, J. L., Collins, A. B., *et al.* (1997). Comparison of anti-GBM antibodies in sera with or without ANCA. *J Am Soc Nephrol*, 8, 376–85.

Hellmark, T., Segelmark, M., Unger, C., *et al.* (1999). Identification of a clinically relevant immunodominant region of collagen IV in Goodpasture disease. *Kidney Int*, 55, 936–44.

Henderson, R. D., Saltissi, D., and Pender, M. P. (1998). Goodpasture's syndrome associated with multiple sclerosis. *Acta Neurol Scand*, 98, 134–5.

Holdsworth, S. R., Kitching, A. R., and Tipping, P. G. (1999). Th1 and Th2 T helper cell subsets affect patterns of injury and outcomes in glomerulonephritis. *Kidney Int*, 55, 1198–216.

Hopfer, H., Holzer, J., Hunemorder, S., *et al.* (2012). Characterization of the renal CD4+ T-cell response in experimental autoimmune glomerulonephritis. *Kidney Int*, 82, 60–71.

Hopfer, H., Maron, R., Butzmann, U., *et al.* (2003). The importance of cell-mediated immunity in the course and severity of autoimmune anti-glomerular basement membrane disease in mice. *FASEB J*, 17, 860–8.

Hudson, B. G., Tryggvason, K., Sundaramoorthy, M., *et al.* (2003). Alport's syndrome, Goodpasture's syndrome, and type IV collagen. *N Engl J Med*, 348, 2543–56.

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*, 44, 307–12.

Jennings, L., Roholt, O. A., Pressman, D., *et al.* (1981). Experimental anti-alveolar basement membrane antibody-mediated pneumonitis. I. The role of increased permeability of the alveolar capillary wall induced by oxygen. *J Immunol*, 127, 129–34.

Jindal, K. K. (1999). Management of idiopathic crescentic and diffuse proliferative glomerulonephritis: evidence-based recommendations. *Kidney Int*, Suppl 70, S33–40.

Johansson, C., Butkowski, R., Swedenborg, P., *et al.* (1993). Characterization of a non-Goodpasture autoantibody to type IV collagen. *Nephrol Dial Transplant*, 8, 1205–10.

Jones, J. L., Thompson, S. A., Loh, P., *et al.* (2013). Human autoimmunity after lymphocyte depletion is caused by homeostatic T-cell proliferation. *Proc Natl Acad Sci U S A*, 110(50), 20200–5.

Kalderon, A. E., Bogaars, H. A., and Diamond, I. (1973). Ultrastructural alterations of the follicular basement membrane in Hashimoto's thyroiditis. Report of eight cases with basement deposits. *Am J Med*, 55, 485–91.

Kalluri, R., Cantley, L. G., Kerjaschki, D., *et al.* (2000). Reactive oxygen species expose cryptic epitopes associated with autoimmune goodpasture syndrome. *J Biol Chem*, 275, 20027–32.

Kalluri, R., Danoff, T.M., Okada, H., *et al.* (1997). Susceptibility to anti-glomerular basement membrane disease and Goodpasture syndrome is linked to MHC class II genes and the emergence of T cell-mediated immunity in mice. *J Clin Invest*, 100, 2263–75.

Kalluri, R., Petrides, S., Wilson, C. B., *et al.* (1996). Anti-alpha1(IV) collagen autoantibodies associated with lung adenocarcinoma presenting as the Goodpasture syndrome. *Ann Intern Med*, 124, 651–3.

Kalluri, R., Weber, M., Netzer, K. O., *et al.* (1994). COL4A5 gene deletion and production of post-transplant anti-alpha 3(IV) collagen alloantibodies in Alport syndrome. *Kidney Int*, 45, 721–6.

Kalluri, R., Wilson, C. B., Weber, M., *et al.* (1995). Identification of the alpha 3 chain of type IV collagen as the common autoantigen in antibasement membrane disease and Goodpasture syndrome. *J Am Soc Nephrol*, 6, 1178–85.

Keller, F. and Nekarda, H. (1985). Fatal relapse in Goodpasture's syndrome 3 years after plasma exchange. *Respiration*, 48, 62–6.

Kelly, P. T. and Haponik, E. F. (1994). Goodpasture syndrome: molecular and clinical advances. *Medicine (Baltimore)*, 73, 171–85.

Kielstein, J. T., Helmchen, U., Netzer, K. O., *et al.* (2001). Conversion of Goodpasture's syndrome into membranous glomerulonephritis. *Nephrol Dial Transplant*, 16, 2082–5.

Kitagawa, W., Imai, H., Komatsuda, A., *et al.* (2008). The HLA-DRB1*1501 allele is prevalent among Japanese patients with anti-glomerular basement membrane antibody-mediated disease. *Nephrol Dial Transplant*, 23, 3126–9.

Klassen, J., Elwood, C., Grossberg, A. L., *et al.* (1974). Evolution of membranous nephropathy into anti-glomerular-basement-membrane glomerulonephritis. *N Engl J Med*, 290, 1340–4.

Kleinknecht, D., Morel-Maroger, L., Callard, P., *et al.* (1980). Antiglomerular basement membrane nephritis after solvent exposure. *Arch Intern Med*, 140, 230–2.

Kohda, T., Okada, S., Hayashi, A., *et al.* (2004). High nephritogenicity of monoclonal antibodies belonging to IgG2a and IgG2b subclasses in rat anti-GBM nephritis. *Kidney Int*, 66, 177–86.

Komatsu, T., Utsunomiya, K., and Oyaizu, T. (1998). Goodpasture's syndrome associated with primary biliary cirrhosis. *Intern Med*, 37, 611–13.

Kurki, P., Helve, T., von Bonsdorff, M., *et al.* (1984). Transformation of membranous glomerulonephritis into crescentic glomerulonephritis with glomerular basement membrane antibodies. Serial determinations of anti-GBM before the transformation. *Nephron*, 38, 134–7.

Leinonen, A., Netzer, K. O., Boutaud, A., *et al.* (1999). Goodpasture antigen: expression of the full-length alpha3(IV) chain of collagen IV and localization of epitopes exclusively to the noncollagenous domain. *Kidney Int*, 55, 926–35.

Lerner, R. A., Glassock, R. J., and Dixon, F. J. (1967). The role of anti-glomerular basement membrane antibody in the pathogenesis of human glomerulonephritis. *J Exp Med*, 126, 989–1004.

Levy, J. B., Hammad, T., Coulthart, A., *et al.* (2004). Clinical features and outcome of patients with both ANCA and anti-GBM antibodies. *Kidney Int*, 66, 1535–40.

Levy, J. B., Lachmann, R. H., and Pusey, C. D. (1996). Recurrent Goodpasture's disease. *Am J Kidney Dis*, 27, 573–8.

Levy, J. B., Turner, A. N., and Rees, A. J. (2001). Long-term outcome of anti-glomerular basement membrane antibody disease treated with plasma exchange and immunosuppression. *Ann Intern Med*, 134, 1033–42.

Li, C. H., Li, Y. C., Xu, P. S., *et al.* (2006). Clinical significance of anti-glomerular basement membrane antibodies in a cohort of Chinese patients with lupus nephritis. *Scand J Rheumatol*, 35, 201–8.

Lionaki, S., Jennette, J. C. and Falk, R. J. (2007). Anti-neutrophil cytoplasmic (ANCA) and anti-glomerular basement membrane (GBM) autoantibodies in necrotizing and crescentic glomerulonephritis. *Semin Immunopathol*, 29, 459–74.

Lockwood, C. M., Boulton-Jones, J. M., Lowenthal, R. M., *et al.* (1975). Recovery from Goodpasture's syndrome after immunosuppressive treatment and plasmapheresis. *Br Med J*, 2, 252–54.

Lockwood, C. M., Rees, A. J., Pearson, T. A., *et al.* (1976). Immunosuppression and plasma-exchange in the treatment of Goodpasture's syndrome. *Lancet*, 1, 711–15.

Lv, J., Zhang, H., Zhou, F., *et al.* (2009). Antiglomerular basement membrane disease associated with Castleman disease. *Am J Med Sci*, 337, 206–9.

Ma, K. W., Golbus, S. M., Kaufman, R., *et al.* (1978). Glomerulonephritis with Hodgkin's disease and herpes zoster. *Arch Pathol Lab Med*, 102, 527–9.

McCoy, R. C., Johnson, H. K., Stone, W. J., *et al.* (1982). Absence of nephritogenic GBM antigen(s) in some patients with hereditary nephritis. *Kidney Int*, 21(4), 642–52.

Monteiro, E. J., Caron, D., Balda, C. A., *et al.* (2006). Anti-glomerular basement membrane glomerulonephritis in an HIV positive patient: case report. *Braz J Infect Dis*, 10, 55–8.

Morello, R., Zhou, G., Dreyer, S. D., *et al.* (2001). Regulation of glomerular basement membrane collagen expression by LMX1B contributes to renal disease in nail patella syndrome. *Nat Genet*, 27, 205–8.

Nachman, P. H., Jennette, J. C., and Falk, R. J. (2007). Primary glomerular disease. in B.M. Brenner (ed.) *Brenner and Rector's The Kidney*, pp. 987–1279. Philadelphia, PA: Saunders.

Nagashima T, Ubara Y, Tagami T, *et al.* (2002). Anti-glomerular basement membrane antibody disease: a case report and a review of Japanese patients with and without alveolar hemorrhage. *Clin Exp Nephrol*, 6, 49–57.

Netzer, K. O., Leinonen, A., Boutaud, A., *et al.* (1999). The goodpasture autoantigen. Mapping the major conformational epitope(s) of alpha3(IV) collagen to residues 17-31 and 127-141 of the NC1 domain. *J Biol Chem*, 274, 11267–74.

Nolasco, F. E., Cameron, J. S., Hartley, B., *et al.* (1987). Intraglomerular T cells and monocytes in nephritis: study with monoclonal antibodies. *Kidney Int*, 31, 1160–66.

O'Donoghue, D. J., Short, C. D., Brenchley, P. E., *et al.* (1989). Sequential development of systemic vasculitis with anti-neutrophil cytoplasmic antibodies complicating anti-glomerular basement membrane disease. *Clin Nephrol*, 32, 251–5.

Olson, S. W., Arbogast, C. V., Baker, T. P., *et al.* (2011). Asymptomatic autoantibodies associate with future anti-glomerular basement membrane disease. *J Am Soc Nephrol*, 22, 1946–52.

Ooi, J. D., Chang, J., O'Sullivan, K. M., *et al.* (2013). The HLA-DRB1*15:01-restricted Goodpasture's T cell epitope induces GN. *J Am Soc Nephrol*, 24, 419–31.

Ooi, J. D., Phoon, R. K., Holdsworth, S. R., *et al.* (2009). IL-23, not IL-12, directs autoimmunity to the Goodpasture antigen. *J Am Soc Nephrol*, 20, 980–9.

Pasternack, A., Tornroth, T., and Linder, E. (1978). Evidence of both anti-GBM and immune complex mediated pathogenesis in the initial phase of Goodpasture's syndrome. *Clin Nephrol*, 9, 77–85.

Peces, R., Navascues, R. A., Baltar, J., *et al.* (1999). Antiglomerular basement membrane antibody-mediated glomerulonephritis after intranasal cocaine use. *Nephron*, 81, 434–8.

Peces, R., Rodriguez, M., Pobes, A., *et al.* (2000). Sequential development of pulmonary hemorrhage with MPO-ANCA complicating anti-glomerular basement membrane antibody-mediated glomerulonephritis. *Am J Kidney Dis*, 35, 954–7.

Pedchenko, V., Bondar, O., Fogo, A. B., *et al.* (2010). Molecular architecture of the Goodpasture autoantigen in anti-GBM nephritis. *N Engl J Med*, 363, 343–54.

Phelps, R. G. and Rees, A. J. (1999). The HLA complex in Goodpasture's disease: a model for analyzing susceptibility to autoimmunity. *Kidney Int*, 56, 1638–53.

Phelps, R. G., Jones, V., Turner, A. N., *et al.* (2000). Properties of HLA class II molecules divergently associated with Goodpasture's disease. *Int Immunol*, 12, 1135–43.

Phoon, R. K., Kitching, A. R., Odobasic, D., *et al.* (2008). T-bet deficiency attenuates renal injury in experimental crescentic glomerulonephritis. *J Am Soc Nephrol*, 19, 477–85.

Radeke, H. H., Janssen-Graalfs, I., Sowa, E. N., *et al.* (2002). 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*, 277, 27535–44.

Rees, A. J., Demaine, A. G., and Welsh, K. I. (1984). Association of immunoglobulin Gm allotypes with antiglomerular basement membrane antibodies and their titer. *Hum Immunol*, 10, 213–20.

Rees, A. J., Peters, D. K., Compston, D. A., *et al.* (1978). Strong association between HLA-DRW2 and antibody-mediated Goodpasture's syndrome. *Lancet*, 1, 966–8.

Reynolds, J., Albouainain, A., Duda, M. A., *et al.* (2006). Strain susceptibility to active induction and passive transfer of experimental autoimmune glomerulonephritis in the rat. *Nephrol Dial Transplant*, 21, 3398–408.

Reynolds, J., Khan, S. B., Allen, A. R., *et al.* (2004). Blockade of the CD154-CD40 costimulatory pathway prevents the development of experimental autoimmune glomerulonephritis. *Kidney Int*, 66, 1444–52.

Reynolds, J., Norgan, V. A., Bhambra, U., *et al.* (2002). Anti-CD8 monoclonal antibody therapy is effective in the prevention and treatment of experimental autoimmune glomerulonephritis. *J Am Soc Nephrol*, 13, 359–69.

Reynolds, J., Prodromidi, E. I., Juggapah, J. K., *et al.* (2005). Nasal administration of recombinant rat alpha3(IV)NC1 prevents the development of experimental autoimmune glomerulonephritis in the WKY rat. *J Am Soc Nephrol*, 16, 1350–9.

Reynolds, J., Tam, F. W., Chandraker, A., *et al.* (2000). CD28-B7 blockade prevents the development of experimental autoimmune glomerulonephritis. *J Clin Invest*, 105, 643–51.

Robertson, J., Wu, J., Arends, J., *et al.* (2005). Activation of glomerular basement membrane-specific B cells in the renal draining lymph node after T cell-mediated glomerular injury. *J Am Soc Nephrol*, 16, 3256–63.

Rutgers, A., Meyers, K. E., Canziani, G., *et al.* (2000). High affinity of anti-GBM antibodies from Goodpasture and transplanted Alport patients to alpha3(IV)NC1 collagen. *Kidney Int*, 58, 115–22.

Rutgers, A., Slot, M., van Paassen, P., *et al.* (2005). Coexistence of anti-glomerular basement membrane antibodies and myeloperoxidase-ANCAs in crescentic glomerulonephritis. *Am J Kidney Dis*, 46, 253–62.

Sado, Y. and Naito, I. (1987). Experimental autoimmune glomerulonephritis in rats by soluble isologous or homologous antigens from glomerular and tubular basement membranes. *Br J Exp Pathol*, 68, 695–704.

Sado, Y., Boutaud, A., Kagawa, M., *et al.* (1998). Induction of anti-GBM nephritis in rats by recombinant alpha 3(IV)NC1 and alpha 4(IV)NC1 of type IV collagen. *Kidney Int*, 53, 664–71.

Salama, A. D., Chaudhry, A. N., Holthaus, K. A., *et al.* (2003). Regulation by CD25+ lymphocytes of autoantigen-specific T-cell responses in Goodpasture's (anti-GBM) disease. *Kidney Int*, 64, 1685–94.

Salama, A. D., Chaudhry, A. N., Ryan, J. J., *et al.* (2001). In Goodpasture's disease, CD4(+) T cells escape thymic deletion and are reactive with the autoantigen alpha3(IV)NC1. *J Am Soc Nephrol*, 12, 1908–15.

Saus, J., Wieslander, J., Langeveld, J. P., *et al.* (1988). Identification of the Goodpasture antigen as the alpha 3(IV) chain of collagen IV. *J Biol Chem*, 263, 13374–80.

Savage, C. O., Pusey, C. D., Bowman, C., *et al.* (1986). Antiglomerular basement membrane antibody mediated disease in the British Isles 1980-4. *Br Med J (Clin Res Ed)*, 292, 301–4.

Savige, J. A., Chang, L., Horn, S., *et al.* (1994). Anti-nuclear, anti-neutrophil cytoplasmic and anti-glomerular basement membrane antibodies in HIV-infected individuals. *Autoimmunity*, 18, 205–11.

Savige, J. A., Dowling, J., and Kincaid-Smith, P. (1989). Superimposed glomerular immune complexes in anti-glomerular basement membrane disease. *Am J Kidney Dis*, 14, 145–53.

Segelmark, M., Butkowski, R., and Wieslander, J. (1990). Antigen restriction and IgG subclasses among anti-GBM autoantibodies. *Nephrol Dial Transplant*, 5, 991–6.

Shaer, A. J., Stewart, L. R., Cheek, D. E., *et al.* (2003). 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*, 41, 1097–109.

Shah, M. K. (2002). Outcomes in patients with Goodpasture's syndrome and hydrocarbon exposure. *Ren Fail*, 24, 545–55.

Short, A. K., Esnault, V. L., and Lockwood, C. M. (1995). 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*, 26, 439–45.

Simonsen, H., Brun, C., Thomsen, O. F., *et al.* (1982). Goodpasture's syndrome in twins. *Acta Med Scand*, 212, 425–8.

Stanton, M. C. and Tange, J. D. (1958). Goodpasture's syndrome (pulmonary haemorrhage associated with glomerulonephritis). *Australas Ann Med*, 7, 132–44.

Stevenson, A., Yaqoob, M., Mason, H., *et al.* (1995). Biochemical markers of basement membrane disturbances and occupational exposure to hydrocarbons and mixed solvents. *QJM*, 88, 23–8.

Summers, S. A., Steinmetz, O. M., Li, M., *et al.* (2009). Th1 and Th17 cells induce proliferative glomerulonephritis. *J Am Soc Nephrol*, 20, 2518–24.

Szczech, L. A., Anderson, A., Ramers, C., *et al.* (2006). The uncertain significance of anti-glomerular basement membrane antibody among HIV-infected persons with kidney disease. *Am J Kidney Dis*, 48, e55–9.

Tan, S. Y. and Cumming, A. D. (1993). Vaccine related glomerulonephritis. *BMJ*, 306, 248.

Than, M. E., Henrich, S., Huber, R., *et al.* (2002). The 1.9-A crystal structure of the noncollagenous (NC1) domain of human placenta collagen IV shows stabilization via a novel type of covalent Met-Lys cross-link. *Proc Natl Acad Sc U S A*, 99, 6607–12.

Torok, N., Niazi, M., Al Ahwel, Y., *et al.* (2010).Thrombotic thrombocytopenic purpura associated with anti-glomerular basement membrane disease. *Nephrol Dial Transplant*, 25, 3446–9.

Umekawa, T., Kohri, K., Iguchi, M., *et al.* (1993). Glomerular-basement-membrane antibody and extracorporeal shock wave lithotripsy. *Lancet*, 341, 556.

Vanacore, R. M., Ham, A. J., Cartailler, J. P., *et al.* (2008). A role for collagen IV cross-links in conferring immune privilege to the Goodpasture autoantigen: structural basis for the crypticity of B cell epitopes. *J Biol Chem*, 283, 22737–48.

Vanacore, R., Ham, A. J., Voehler, M., *et al.* (2009). A sulfilimine bond identified in collagen IV. *Science*, 325, 1230–4.

Wang, X. P., Fogo, A. B., Colon, S., *et al.* (2005). Distinct epitopes for anti-glomerular basement membrane alport alloantibodies and goodpasture autoantibodies within the noncollagenous domain of alpha3(IV) collagen: a janus-faced antigen. *J Am Soc Nephrol*, 16, 3563–71.

Wechsler, E., Yang, T., Jordan, S. C., *et al.* (2008). Anti-glomerular basement membrane disease in an HIV-infected patient. *Nat Clin Pract Nephrol*, 4, 167–71,

Wilson, C. B. and Dixon, F. J. (1973). Anti-glomerular basement membrane antibody-induced glomerulonephritis. *Kidney Int*, 3, 74–89.

Wilson, C. B. and Smith, R. C. (1972). Goodpasture's syndrome associated with influenza A2 virus infection. *Ann Intern Med*, 76, 91–4.

Wolf, D., Hochegger, K., Wolf, A. M., *et al.* (2005). CD4+CD25+ regulatory T cells inhibit experimental anti-glomerular basement membrane glomerulonephritis in mice. *J Am Soc Nephrol*, 16, 1360–70.

Wong, D., Phelps, R. G. and Turner, A. N. (2001). The Goodpasture antigen is expressed in the human thymus. *Kidney Int*, 60, 1777–83.

Wu, J., Arends, J., Borillo, J., *et al.* (2004). A self T cell epitope induces autoantibody response: mechanism for production of antibodies to diverse glomerular basement membrane antigens. *J Immunol*, 172, 4567–74.

Wu, J., Borillo, J., Glass, W. F., *et al.* (2003). T-cell epitope of alpha3 chain of type IV collagen induces severe glomerulonephritis. *Kidney Int*, 64, 1292–301.

Wu, J., Hicks, J., Ou, C., *et al.* (2001). Glomerulonephritis induced by recombinant collagen IV alpha 3 chain noncollagen domain 1 is not associated with glomerular basement membrane antibody: a potential T cell-mediated mechanism. *J Immunol*, 167, 2388–95.

Wuthrich, R. P. (1994). Pernicious anemia, autoimmune hypothyroidism and rapidly progressive anti-GBM glomerulonephritis. *Clin Nephrol*, 42, 404.

Xenocostas, A., Jothy, S., Collins, B., *et al.* (1999). Anti-glomerular basement membrane glomerulonephritis after extracorporeal shock wave lithotripsy. *Am J Kidney Dis*, 33, 128–32.

Yamamoto, T. and Wilson, C. B. (1987). Binding of anti-basement membrane antibody to alveolar basement membrane after intratracheal gasoline instillation in rabbits. *Am J Pathol*, 126, 497–505.

Yang, R., Cui, Z., Hellmark, T., *et al.* (2007). Natural anti-GBM antibodies from normal human sera recognize alpha3(IV)NC1 restrictively and recognize the same epitopes as anti-GBM antibodies from patients with anti-GBM disease. *Clin Immunol*, 124, 207–12.

Yang, R., Cui, Z., Zhao, J., *et al.* (2009). The role of HLA-DRB1 alleles on susceptibility of Chinese patients with anti-GBM disease. *Clin Immunol*, 133, 245–50.

Yang, R., Hellmark, T., Zhao, J., *et al.* (2007). Antigen and epitope specificity of anti-glomerular basement membrane antibodies in patients with goodpasture disease with or without anti-neutrophil cytoplasmic antibodies. *J Am Soc Nephrol*, 18, 1338–43.

Yang, R., Hellmark, T., Zhao, J., *et al.* (2009). Levels of epitope-specific autoantibodies correlate with renal damage in anti-GBM disease. *Nephrol Dial Transplant*, 24, 1838–44.

Zhao, J., Cui, Z., Yang, R., *et al.* (2009b). Anti-glomerular basement membrane autoantibodies against different target antigens are associated with disease severity. *Kidney Int*, 76, 1108–15.

Zhao, J., Yan, Y., Cui, Z., *et al.* (2009a). The immunoglobulin G subclass distribution of anti-GBM autoantibodies against rHalpha3(IV)NC1 is associated with disease severity. Hum Immunol, 70, 425–9.

Zhao, J., Yang, R., Cui, Z., *et al.* (2007). Characteristics and outcome of Chinese patients with both antineutrophil cytoplasmic antibody and antiglomerular basement membrane antibodies. *Nephron Clin Pract*, 107, c56–62.

Zhou, X. J., Lv, J. C., Bu, D. F., *et al.* (2010a). Copy number variation of FCGR3A rather than FCGR3B and FCGR2B is associated with susceptibility to anti-GBM disease. *Int Immunol*, 22, 45–51.

Zhou, X. J., Lv, J. C., Yu, L., *et al.* (2010a). FCGR2B gene polymorphism rather than FCGR2A, FCGR3A and FCGR3B is associated with anti-GBM disease in Chinese. *Nephrol Dial Transplant*, 25, 97–101.

Zou, J., Hannier, S., Cairns, L. S., *et al.* (2008). Healthy individuals have Goodpasture autoantigen-reactive T cells. *J Am Soc Nephrol*, 19, 396–404.

Zou, J., Henderson, L., Thomas, V., *et al.* (2007). Presentation of the Goodpasture autoantigen requires proteolytic unlocking steps that destroy prominent T cell epitopes. *J Am Soc Nephrol*, 18, 771–9.

CHAPTER 75

Alport post-transplant antiglomerular basement membrane disease

Zhao Cui, Neil Turner, and Ming-hui Zhao

Introduction

When studied by indirect immunofluorescence, the Goodpasture antigen is absent or greatly diminished in most patients with Alport syndrome (see Chapter 321). Transplant of a normal kidney may allow the development of anti-GBM antibodies to foreign antigen(s) (alloantigens) in the donor kidney. Severe disease is an unusual occurrence though. Overall the outcome of transplantation in Alport syndrome is better than average (Byrne et al., 2002; Temme et al., 2012).

Clinical features

Linear IgG fixation to the GBM occurs in about 15% of Alport recipients (Quérin et al., 1986; Byrne et al., 2002) but only a minority of these (possibly 10–20%, 2–3% in total) progress to crescentic nephritis or other glomerular abnormalities characteristic of anti-GBM disease. As the immune response is to allo-antigens in the allograft, lung haemorrhage does not usually occur. The histological appearances in the kidney and clinical progression are indistinguishable from those of spontaneous Goodpasture disease (see Chapter 72). Many examples of the phenomenon have now been described, and the graft has been lost in the majority (summarized in Browne et al. 2004).

The typical sequence of events is that a first allograft is biopsied months to years after the transplant with a suspicion of chronic rejection, and unexpectedly crescentic changes are seen in glomeruli. A second allograft is lost more rapidly, in weeks to months. The response to a third allograft is brisker still and disease may be apparent in days. However, there are examples of slower tempo disease in the literature, and also of patients in whom a second graft has not triggered an aggressive immune response.

Diagnosis

Immunoassays for anti-GBM antibodies may be falsely negative as Alport anti-GBM antibodies are usually directed towards the molecule in which the genetic defect lies. The NC1 domain is targeted as in spontaneous anti-GBM disease, but in the most common X-linked variety of the disease the target is usually the NC1 domain of the α5 chain of type IV collagen, encoded by COL4A5 (Brainwood et al., 1998). Therefore it is important to maintain a high index of suspicion, undertake early renal biopsy, and if there is evidence of glomerular disease to look for linear binding of IgG to the GBM. Linear binding to GBM without glomerular damage is common and most do not progress to overt disease (Quérin et al., 1986; Peten et al., 1991; Byrne et al., 2002). Reliable techniques for distinguishing anti-α3 from anti-α5 antibodies are not routine, though anti-α5 antibodies are more likely to bind strongly to Bowman's capsule. Other techniques for distinguishing the molecular target do not affect management and are not routinely available, but Figs 75.1 and 71.1 in Chapter 71 illustrate some characteristics.

Differential diagnosis

It is not uncommon for this diagnosis to be made in patients who had not previously been diagnosed with Alport syndrome—often labelled as aetiology unknown, or some type of glomerulonephritis. Important features include other signs of Alport syndrome, notably sensorineural deafness, and a family history of renal disease.

The major differential diagnosis is with spontaneous anti-GBM disease. However, recurrence of anti-GBM disease while taking immunosuppressive drugs is very unusual (see Chapter 74).

Treatment and outcome

Treatment is as for spontaneous anti-GBM disease (see Chapter 73), and although it has not been successful in most reported cases, success or partial success has been achieved in some. Diagnosis and treatment have often been late, and it is possible that the most severe examples are over-represented in published cases. In addition to treatment of the type used in anti-GBM disease, patients with this complication have been treated with anti-rejection therapies of almost every type, sometimes before the true diagnosis has been reached. Overall though, none of the therapies used in published reports have been very successful, even in retransplantation when the diagnosis must have been expected. Browne et al. (2004) reviewed 16 cases of retransplantation. Anti-GBM nephritis was seen in 15 out of the 16 cases, with 12 of those grafts damaged irrevocably. It is not yet possible to suggest a better treatment strategy. Foreign antigen immune responses like this are notoriously hard to suppress.

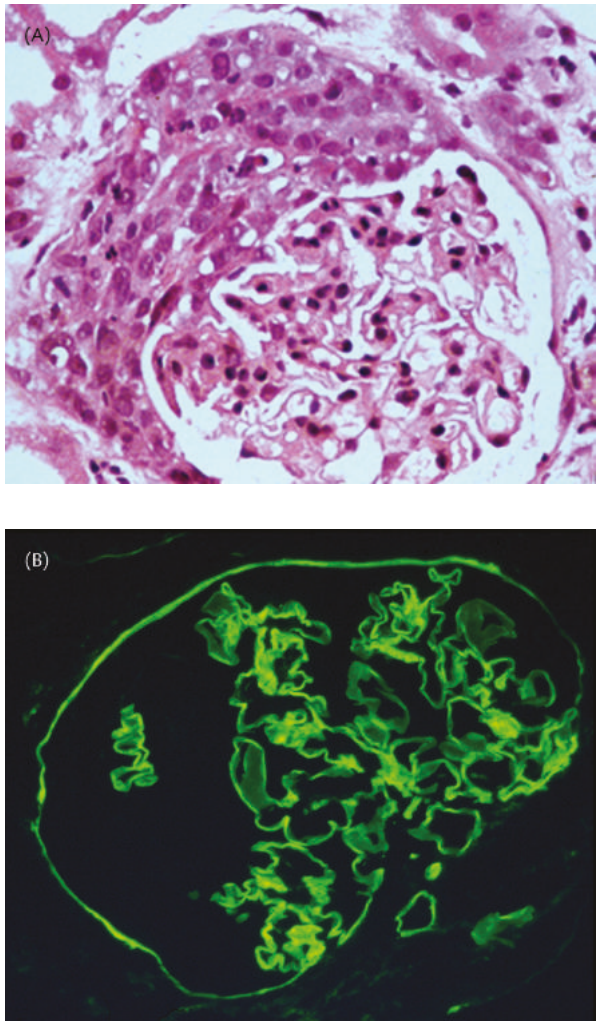

Fig. 75.1 Moderately severe anti-a5(IV) collagen-mediated Alport post-transplant anti-GBM disease in the third renal transplant of a patient with a large COL4A5 deletion. Biopsy at day 44 showed cellular crescents (A) with focal necrotizing lesions in three out of five glomeruli. By direct immunofluorescence, linear staining for IgG and C3 was seen on the GBM, and strongly on Bowman's capsule and in distal tubules (B).
From Browne et al. (2004).

Diagnosis in a second transplant is likely to be more prompt as suspicions will be high. Our current recommendation is that patients who lost a second allograft from this complication should not usually be put forward for further attempts at transplantation unless there are strong reasons to believe that a new approach is likely to be more successful.

Pathogenesis

The molecular defect in Alport syndrome involves a gene encoding one of the tissue-specific type IV collagen chains. The disease is usually X-linked, involving *COL4A5*, but mutations in one chain may destabilize the 345 supramolecular network involving α3, α4, and α5 chains (further described in Chapter 320), producing the degenerative disorder of certain specialized basement membranes that we recognize as Alport syndrome.

Patients with the (most common) X-linked form of Alport syndrome who develop this complication are more likely to have a substantial α5 chain gene deletion, perhaps because this prevents any α5 chain production, so that the antigen in the allograft is truly foreign to the recipient's immune system (Ding et al., 1994). In a European study (Jais et al., 2000), only 3 of the 118 transplanted male patients with identified *COL4A5* mutation developed post-transplant anti-GBM disease. All three had a large deletion. The risk for these patients of developing this complication was 15%, which represents a sixfold increase compared to the total Alport syndrome population. However, 16 other patients with large *COL4A5* rearrangements and 32 with mutations expected to produce a truncated protein lacking the NC1 domain, did not develop anti-GBM glomerulonephritis in the graft, showing that other factors must contribute to alloimmunization.

Other genetic factors may also be important, as may the immunosuppression given to prevent rejection of the allograft. Detailed studies show that the target of anti-GBM antibodies in these circumstances is most likely to be the NC1 domain of the α5 chain if the genetic abnormality is in the gene (*COL4A5*) encoding it (Brainwood et al., 1998), that is, different from the classic Goodpasture antigen. In patients with autosomal disease the target may be α3(IV)NC1, the Goodpasture antigen.

It seems clear that the disease is, like spontaneous anti-GBM disease (see Chapter 73), mediated by both antibody and cellular (T lymphocyte, macrophage) components. Possibly the disease may be triggered by increased presentation of the inciting antigen in an inflammatory context, but at present the reasons for the unpredictability of development of this complication are unknown.

References

Brainwood, D., Kashtan, C., Gubler, M. C., *et al.* (1998). Target of alloantibodies in Alport anti-glomerular basement membrane disease after renal transplantation. *Kidney Int*, 53, 762–6.

Browne, G., Brown, P. A., Tomson, C. R., *et al.* (2004). Retransplantation in Alport post-transplant anti-GBM disease. *Kidney Int*, 65, 675–81.

Bygren, P., Freiburghaus, C., Lindholm, T., *et al.* (1985). Goodpasture's syndrome treated with staphylococcal protein A immunoadsorption. *Lancet*, 2, 1295–6.

Byrne, M. C., Budisavljevic, M. N., Fan, Z., *et al.* (2002). Renal transplant in patients with Alport's syndrome. *Am J Kidney Dis*, 39, 769–75.

Ding, J., Zhou, J., Tryggvasson, K., *et al.* (1994). COL4A5 deletions in three patients with Alport syndrome and posttransplant antiglomerular basement membrane nephritis. *J Am Soc Nephrol*, 5, 161–8.

Jais, J. P., Knebelmann, B., Giatras, I., *et al.* (2000). X-linked Alport syndrome: natural history in 195 families and genotype- phenotype correlations in males. *J Am Soc Nephrol*, 11, 649–57.

Peten, E., Pirson, Y., Cosyns, J. P., *et al.* (1991). Outcome of thirty patients with Alport's syndrome after renal transplantation. *Transplantation*, 52, 823–6.

Quérin, S., Noël, L. H., Grünfeld, J. P., *et al.* (1986). Linear glomerular IgG fixation in renal allografts: incidence and significance in Alport's syndrome. *Clin Nephrol*, 25, 134–40.

Temme, J., Gross, O., Jager, K. J., *et al.* (2012). Outcomes of male patients with Alport syndrome undergoing renal replacement therapy. *Clin J Am Soc Nephrol*, 7, 1969–76.

CHAPTER 76

Post-infectious glomerulonephritis: overview

Bernardo Rodriguez-Iturbe and Mark Haas

Post-infectious glomerulonephritis (GN) defines an inflammatory lesion involving exclusively or predominantly the glomeruli that is a consequence of an infectious disease. There are numerous bacterial, viral, and fungal infections associated with GN (Table 76.1). In the following chapters, we will discuss only post-streptococcal GN (Chapter 77), immunoglobulin A (IgA)-dominant GN usually associated with staphylococcal infections (Chapter 78), and GN associated with bacterial endocarditis, with infected ventriculo-atrial shunts ('shunt nephritis'), and with deep-seated infections (osteomyelitis, visceral abscesses, pleural suppuration, pneumonia) (Chapter 79).

Almost all of these lesions result from antigen–antibody reactivity that causes local activation of the complement system and of the coagulation cascade. Specific types of post-infectious GN, such as those caused by staphylococcal infections associated with IgA deposition may involve superantigens causing intense T-cell activation that engage cytokine-mediated polyclonal B-cell responses.

In disadvantaged populations with poor hygienic conditions and limited access to early medical care, post-infectious GNs, particularly post-streptococcal GN, are frequent. In more affluent societies, post-infectious glomerulonephritis is less common, and tends to occur in older patients or those with significant comorbidity already receiving medical care and/or with implanted catheters or devices.

Recent studies indicate that bacteria causing GN are more frequently *Staphylococcus* (46%), *Streptococcus* (16%), and Gram-negative organisms (Nasr et al., 2011) and the most common sites of infection are the upper respiratory tract (23%), skin (17%), lung (17%), and heart valves (11.6%). Chronic GN develops in about 25% of these patients (Nasr et al., 2008).

The clinical presentation and the pathological characteristics of post-infectious GN are not uniform. Even in a specific disease these characteristics may have considerable variability. For instance, post-streptococcal GN may be clinically asymptomatic, may present with acute nephritic or nephrotic syndrome, or have a rapidly progressive course, and the typical endocapillary GN may be associated with crescent formation followed by sclerosis. This lack of uniformity results from the variability of size, load, and charge of the antigen, the intensity of the antibody response, the site of the immune reactivity, and the efficiency of immune-complex clearance. In post-infectious GN, the immune complexes may be predominantly located in the mesangium, in and on the glomerular basement membrane (GBM), or both. Prominent deposition of immune complexes in and on the GBM is usually associated with heavy proteinuria while mesangial deposits alone are usually

Table 76.1 Infectious agents associated with endocapillary glomerulonephritis

Associated with infectious syndromes
Skin and throat (*Streptococcus* group A)
IgA-dominant glomerulonephritis (methicillin-resistant *Staphylococcus*)
Bacterial endocarditis (*Staphylococcus aureus, Streptococcus viridans*)
Pneumonia (*Diplococcus pneumoniae, Mycoplasma*)
Meningitis (*Meningococcus pneumoniae, Mycoplasma*)
Visceral abscesses and osteomyelitis (*Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Proteus mirabilis, Klebsiella, Clostridium perfringens*)
'Shunt' nephritis (*Staphylococcus aureus, Staph. albus, Streptococcus viridans*)
Infected vascular prosthesis (*Staphylococcus aureus*)
Guillain–Barré syndrome (G-B infectious agent?).
Associated with specific bacterial diseases
Typhoid fever (*Salmonella typhi*)
Leprosy
Yersiniosis
Brucellosis
Leptospirosis.
Associated with viral infections
Hepatitis A, B, and C
Epstein–Barr virus
Parvovirus B19
Cytomegalovirus
Measles
Mumps
Varicella
Coxsackie virus.
Associated with parasitic infestation
Malaria (*Plasmodium falciparum, P. malariae*)
Schistosomiasis (*Schistosoma haematobium, S. mansoni*)
Toxoplasmosis
Trichinosis (*Trichinella spiralis*)
Filiarasis (*Onchocerca volvulus, Loa Loa*).
Associated with other infectious organisms
Rickettsiae (*Coxiella*)
Fungi (*Candida albicans, Coccidioides immitis, Histoplasma capsulatum*).

associated with only mild-to-moderate proteinuria and microscopic haematuria. As demonstrated in serum sickness experimental models, circulating immune complexes are usually deposited in subendothelial and mesangial regions and, if the condition is self-limited, induce a transient glomerulonephritis. *In situ* immune complex formation, a consequence of the penetration of free antigen through the GBM, tends to form subepithelial immune complexes. This mechanism is favoured when there is antigen excess that facilitates dissociation of circulating immune complexes and when the antigen is cationic and therefore attracted by the negatively charged GBM.

References

Nasr, S. H., Fidler, M. E., Valeri, A. M., *et al.* (2011). Postinfectious glomerulonephritis in the elderly. *J Am Soc Nephrol*, 22, 187–95.

Nasr, S. H., Markowitz, G. S., Stokes, M. B., *et al.* (2008). Acute postinfectious glomerulonephritis in the modern era: experience with 86 adults and review of the literatura. *Medicine*, 87, 21–32.

CHAPTER 77

Post-streptococcal glomerulonephritis

Bernardo Rodríguez-Iturbe and Mark Haas

Introduction

In autopsy studies during the nineteenth century, the renal lesion most frequently found in patients who developed oliguria during scarlet fever was, surprisingly, interstitial nephritis (Councilman, 1898) but the observation that 'dark and scanty urine' was a serious complication of the convalescent dates back to descriptions of epidemics in the fourteenth century (Becker and Murphy, 1968). The disease was very common in central Europe in the pre-antibiotic era and acute post-streptococcal glomerulonephritis (PSGN) (Fig. 77.1) was probably the cause of the untimely death of Wolfgang Amadeus Mozart in 1791 (Zegers et al., 2009).

In 1910, Clemens von Pirquet, in a landmark paper that opened the field of immune diseases (von Pirquet 1910), postulated that the post-scarlatinal nephritis was caused by the development of harmful antibodies (as opposed to the beneficial antibodies in vaccination) and coined the term 'allergy' (altered reactivity) to define this pathogenic modality. The streptococcal aetiology of scarlet fever (Dochez and Sherman, 1924) prompted the recognition of PSGN as the first and most extensively studied glomerulonephritis (GN) associated with bacterial infections.

Acute rheumatic fever and GN are both non-infectious complications of streptococcal infection, but have epidemiological and biological differences and only rarely if ever occur in the same patient. Therefore, Seegal and Earle (1941) postulated the existence of distinct rheumatogenic and nephritogenic strains of the bacterium. Since recurrence of PSGN is a very rare event it is likely that putative antigen(s) shared among nephritogenic strains confer long-lasting immunity.

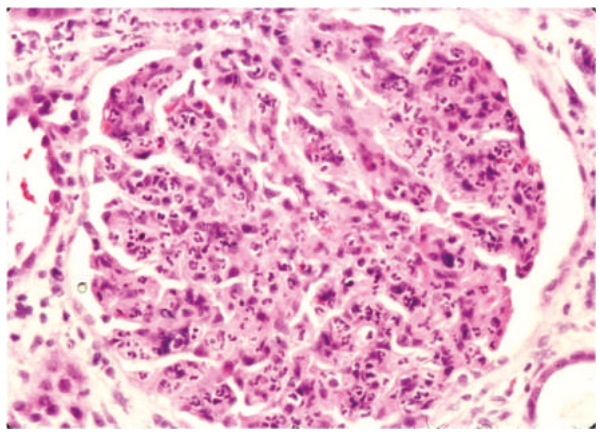

Fig. 77.1 Acute post-streptococcal glomerulonephritis: proliferative and exudative glomerulonephritis. The glomerulus shows marked global hypercellularity with many neutrophils, and is representative of all of the glomeruli on this biopsy. (Haematoxylin and eosin stain, original magnification 400×.)

Epidemiology

The incidence of PSGN has decreased in recent decades in industrialized countries (Ahn and Ingulli, 2008). At present it is usually associated with diabetes, alcoholism, intravenous drug addiction, and debilitating conditions. The reduction of the incidence in the United States, United Kingdom, and Western continental Europe is probably the result of easier and earlier access to appropriate medical care for streptococcal infections and perhaps widespread fluorination of the water since virulence factors in *Streptococcus pyogenes* are reduced with fluoride exposure (Thongboonkerd et al., 2002). The rarity of PSGN in affluent societies has been cited as a factor for delayed diagnosis in patients who do not have gross haematuria (Pais et al., 2008).

The incidence of PSGN is also decreasing in developing countries (Rodríguez-Iturbe and Mezzano, 2005) but it is not uncommon. Two independent studies (Carapetis et al., 2005, Rodriguez-Iturbe and Musser, 2008) have estimated that the incidence of PSGN in developing countries is at least 9.3–9.8 cases per 100,000 population per year and may be in fact as much as three times these values (Carapetis et al., 2005). PSGN is particularly frequent in populations with deficient hygienic conditions and substandard medical services (Sarkissian et al., 1997; Orta and Moriyón, 2001) and particularly in aboriginal communities (Currie and Brewster, 2001; Marshall et al., 2011). The importance of educational programmes in the control of the disease is underlined by the finding that programmes designed to reduce the incidence of rheumatic fever have led also to the decline in the incidence of GN in French Caribbean islands (Bach et al., 1966).

Pharyngitis and tonsillitis are the usual sites of antecedent infection in the winter and the spring in temperate climates and impetigo is more frequent in the tropics in the summer months. PSGN may occur in sporadic cases, in clusters of cases and in epidemic outbreaks. Clusters of cases are more frequently reported in poor communities in industrialized countries with adequate health systems that allow detection and documentation of the disease, while epidemics of > 100 cases are usually reported from countries in the middle range of Human Development Index and a mean annual

health expenditure per capita of about US$550 (Rodríguez-Iturbe and Musser, 2008).

Epidemics have been reported in periodic outbreaks in specific regions in Minnesota (Anthony et al., 1969), Trinidad (Poon-King et al., 1967), Venezuela (Rodríguez-Iturbe, 1984), and in the Northern Territory of Australia (Marshall et al., 2011). The risk of nephritis in epidemics of streptococcal infections varies significantly, ranging from 5% in throat infections, to as high as 25% in pyoderma caused by M type 49 streptococci.

Clinical observations suggest a genetic predisposition to PSGN and prospective studies have found that that 38% of the siblings of index cases have evidence of symptomatic or subclinical nephritis, an incidence that is higher than the attack rate in the general population in epidemic conditions that ranges between 5% and 28% (Rodríguez-Iturbe, 1984). Associations of PSGN with human leucocyte antigen (HLA)-DR4 and DR-1 have been reported but definite genetic associations have not been detected.

Clinical features

The infection preceding nephritis

The usual sites of infection with a nephritogenic *Streptococcus* are the skin or the throat, but other foci are possible. Unusual sites of infection include spider bites (Lung and Mallory, 2000) and infected circumcision wounds (Tasic and Polenakovic, 2000). Streptococcal pharyngitis may cause only sore throat or be accompanied by tonsillar exudate, fever, and cervical lymphadenopathy. Scarlatinal rash is due to an erythrogenic toxin that is produced by the bacteria. Vomiting may be a prominent initial symptom in scarlet fever. Streptococcal impetigo is characterized by clusters of small vesicles that appear in exposed skin areas. They break rapidly and leave lesions covered with a thick yellow crust. Regional lymphadenopathy is usually present in patients with active skin infection. Impetigo is frequently associated with scabies and a history of intense itching, particularly if it is also present in other family members, is a diagnostic clue. Outbreaks of ulcerated ecthyma skin lesions in soldiers have also been associated with acute PSGN (Wasserzug et al., 2009).

The latent period after infection is shorter after throat infections (about 2 weeks) than after pyodermitis (several weeks). When throat and skin infections are present at the same time, the throat infection is usually due to a contamination from the skin (Anthony et al., 1969).

Clinical features of acute nephritis

The clinical features of PSGN are different in adults and in children (Table 77.1). In children the typical presentation of acute PSGN is the acute nephritic syndrome; however, the disease may be subclinical, or it may be manifested by nephrotic syndrome or rapidly progressive GN. *Asymptomatic disease* is recognized by microscopic haematuria and reduced complement levels with or without hypertension. Patients with subclinical PSGN are 1.5 times more frequent than patients with clinical disease in epidemics. In non-epidemic situations, prospective family studies of index cases indicate that the ratio of subclinical/clinical disease is 4.0 (Rodríguez-Iturbe et al., 1981b) to 5.3 (Dodge et al., 1967). The *acute nephritic syndrome* is the classic clinical presentation of acute PSGN. The typical patient is a boy (male:female ratio 2:1) 4–14 years of age who develops haematuria, oedema, hypertension, and oliguria. At least two of these manifestations are present in almost all patients and the full clinical picture in about 40% of the patients. The nephrotic syndrome may occur at the onset in 2% of children and 20% of adults (Table 77.1).

Table 77.1 Clinical manifestations of acute post-streptococcal glomerulonephritis in children and elderly adults[a]

	Children (%)	Elderly patients (%)
Haematuria	100	100
Proteinuria	80	92
Oedema	90	75
Hypertension[b]	60–80	80–86
Oliguria	10–50	58
Dyspnoea/heart failure	< 5	43
Nephrotic proteinuria	< 4	20
Azotaemia	25–40	70–83
Early mortality	< 0.1	25

[a] Data in elderly patients taken from the review of Melby et al. (1987). Data in children taken from several studies reviewed in the text.

[b] At discharge, hypertension persists in 43% of the elderly patients and exceptionally, if ever, in children.

Rapidly progressive renal failure, resulting from GN with extensive crescent formation, occurs in < 1% of patients. Impaired renal function occurs in 25–40% of children and in up to 83% of adults (Rodríguez-Iturbe, 1984).

In acute PSGN, *haematuria* is a constant finding. Gross haematuria is present in one-third of the patients and microscopic haematuria is found in the rest. Microscopic haematuria usually persists for many months after the acute attack and disappears usually within 1 year and always within 4 years in children (Kasahara et al., 2001). *Oedema* is the chief complaint in most patients. It is usually confined to the face and legs in adolescents, while generalized oedema is more common in younger children. Ascites is unusual unless the nephrotic syndrome is present. Traditionally the reduced glomerular filtration rate is thought to be responsible for sodium retention as it diminishes the filtered sodium. This is clearly an oversimplification because a reduced glomerular filtration rate is seen in a variety of other conditions where fluid retention does not occur. Furthermore, profound sodium retention can occur in patients with a mildly reduced glomerular filtration rate and spontaneous diuresis can occur before glomerular filtration rate has improved. At any rate, the fractional sodium excretion is usually < 1% and the urine:plasma creatinine concentration ratio, > 40. *Hypertension* is present in > 80% of patients, but only 50% require drug treatment. Haemodynamic measurements indicate that increased plasma volume, increased cardiac output, and elevated peripheral vascular resistance contribute to the hypertension in the acute nephritic syndrome. Plasma renin and aldosterone levels are suppressed, consistent with the volume-dependent nature of arterial hypertension in the acute nephritic syndrome. *Oliguria* is noted as a symptom on admission by less than half of the children or their parents/guardians. Non-specific symptoms, such as dull

lumbar pain, malaise, weakness, and nausea frequently accompany the cardinal manifestations of the acute nephritic syndrome.

In a typical case of post-streptococcal nephritis, improvement is observed after 2–7 days when the urine volume increases, followed rapidly by resolution of oedema and return of the blood pressure to normal levels.

Diagnosis of post-streptococcal glomerulonephritis

The differential diagnosis of a patient presenting with acute nephritic syndrome includes distinguishing a primary renal disease from the initial manifestation of an otherwise silent systemic disease. Systemic lupus erythematosus, essential cryoglobulinaemia, subacute and acute bacterial endocarditis, 'shunt' nephritis, visceral abscess, antineutrophil cytoplasmic antibody (ANCA)-associated vasculitis and GN, and anti-glomerular basement membrane (anti-GBM) antibody disease may all potentially present as an isolated nephritis. Measurement of serum complement activity has been suggested as a first-line test in evaluation of acute GN because low serum complement is a feature of 90% or more of the cases of acute PSGN and the finding of a normal serum complement would make this diagnosis unlikely. Low levels of C3 and normal or mildly depressed values of C4 are typical of diseases in which there is preferential activation of the alternative pathway, such as dense deposit disease and PSGN. Serum complement returns to normal usually within 1 month in patients with uncomplicated PSGN. Serum immunoglobulin G (IgG) and IgM are elevated in 80% of the cases, and in contrast with another post-streptococcal disease, rheumatic fever, IgA serum level is normal. Cryoglobulins, elevated rheumatoid factor, and anti-C1q antibodies are present in up to one-third of patients, and rarely patients may have low titters of anti-DNA and ANCA (Rodríguez-Iturbe, 1984).

While the diagnosis of post-streptococcal aetiology may be suspected on clinical grounds its confirmation requires positive bacterial culture or serological evidence of recent streptococcal infection. Positive cultures maybe obtained in as many as 70% of the cases in epidemics but at best in 25% of sporadic cases. Increasing or high titres of serum anti-streptolysin O are found in 60–80% of the patients with streptococcal throat infection and high anti-DNAse B titres in about 70% of the cases with pyodermitis. The streptozyme test, which includes four antigens (DNAse B, Streptolysin O, hyaluronidase and streptokinase) may be found elevated in nearly 80% of the cases. Antibodies against a nephritis-associated plasmin receptor (Yamakami et al., 2000) and antibodies against the zymogen precursor of streptococcal pyrogenic exotoxin B (Parra et al., 1998), both of which are assumed to be nephritogenic antigens (see below), are more sensitive and specific for nephritogenic strains than the rest of anti-streptococcal antibodies but they are not clinically available.

In children with uncomplicated PSGN, renal biopsy is only indicated when there are unusual features that make the diagnosis doubtful. Among these features are proteinuria in the nephrotic range, progressive azotaemia suggesting crescentic GN, and serum complement levels that are not depressed in the acute phase or remain reduced for > 1 month. In adult patients, kidney biopsy is the norm because in adults PSGN carries a much poorer immediate and long-term prognosis.

Aetiology and pathogenesis

Nephritogenic antigens

Nephritogenic strains of group A streptococcus pyogenes include impetigo-associated M types 47, 49 and throat infection-associated M types 1, 2, 4 and 12. One recent large epidemic (Balter et al., 2000) and several clusters of cases (Francis et al 1993; Nicholson el al., 2000) have been related to the ingestion of unpasteurized milk contaminated with group C streptococcus (*Streptococcus zooepidemicus*).

Throughout the years, many putative streptococcal nephritogenic antigens have been suggested without confirmation of a causal relationship with GN (Rodríguez-Iturbe and Batsford, 2007). At the present time two antigens are associated with PSGN: the so-called nephritis-associated plasmin receptor (NAPLr) identified as glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (Yoshizawa et al., 2004) and the cationic (pK 8.0) streptococcal proteinase exotoxin B (SPEB) and its more immunogenic precursor zymogen (Vogt et al., 1983; Poon-King et al., 1993). Both antigens injected intravenously have affinity for the glomeruli. Antibody titres to these two antigens are present in the serum at the time of convalescence. GAPDH has been localized in areas of the glomeruli with plasmin-like activity, suggesting a direct mechanism for glomerular inflammatory damage but is not co-localized with complement or immunoglobulin (Oda et al., 2005, 2010). Patients with acute PSGN have increased urinary plasmin activity resistant to alpha(2)-AP, likely due to urinary excretion of NAPlr (Oda et al., 2008). SPEB is co-localized with both complement and IgG in the glomeruli suggesting a participation in immune-mediated renal damage and is the only putative antigen demonstrated in the subepithelial electron dense deposits ('humps') that are the typical ultrastructural lesion of PSGN (Batsford et al., 2005). Vogt et al. (1990) have emphasized that cationic antigens, such as SPEB and its zymogen precursor are attracted by the negatively charged GBM, and penetrate towards subepithelial locations where they react *in situ* with the antibody. SPEB/zymogen induce leucocyte infiltration in the glomerulus, possibly as a result of the induction of chemotactic and migration inhibition factor activities (Romero et al., 1999) and increased angiotensin II production in mesangial cells (Viera et al., 2009). *In situ* immune complex formation would easily explain the formation of subepithelial electron dense deposits (humps) which are extremely difficult to produce by the injection of preformed immune complexes and, in contrast, are the rule with the injection of cationic antigens. Heavy, confluent immunoglobulin and complement deposition in and on the GBM (garland-type deposits) are associated with the existence of a large number of humps in biopsies, heavy proteinuria and worse prognosis (see later), therefore the *in situ* mechanism may have significant clinical relevance. Nevertheless, SPEB cannot be the single nephritogenic antigen of *Streptococcus*, since the SPEB gene was not present in the *Streptococcus zooepidemicus* responsible for a recent major epidemic (Beres et al., 2008).

Other cationic components that could have pathogenetic relevance are the streptococcal histones that may readily accumulate in the glomeruli (Choi et al., 1995). After streptococcal lysis, histones can enter the blood and bind to the anionic proteoglycans to trigger an *in situ* immune complex formation or directly induce the production of proinflammatory cytokines (Zhang et al., 1999).

Complement activation in PSGN

In PSGN, serum C3 levels are more frequently and more intensively depressed than C4. Activation of the alternate complement pathway may be mediated by transient expression of C3 NeF autoantibody (Frémeaux-Bacchi et al., 1994). Berge et al. (1997) have shown that protein H, a surface protein of *Streptococcus pyogenes*, in association with IgG is capable of activating the classic complement pathway. These findings may explain why 15–30% of the patients with PSGN show evidence of reduction of the C1 and C4 complement components. The lectin pathway of complement may also be activated in PSGN, likely as a result of the recognition of the starter molecule mannose-binding lectin by the N-acetyl glucosamine residues of the bacterial wall (Oshawa et al., 1999) but individuals genetically unable to activate this pathway may still develop PSGN (Skattum et al., 2006).

The complement system recruits inflammatory cells but, in addition, individual components have a direct nephritogenicity. C3a and C5a cause histamine release and increased capillary permeability, and the terminal C5b-C9 membrane attack complex has a direct effect on the glomerular capillary membrane. Plasma levels of C5b-C9 complement complexes are consistently elevated at the onset of acute PSGN (Matsell et al., 1994). Non-lytic effects of the membrane attack complex may result from platelet activation; reduction of platelet counts and survival time and increased platelet-activating factor are present in acute PSGN (Mezzano et al., 1993).

Cell-mediated immunity

Early studies demonstrated the infiltration of CD4 T lymphocytes (Parra et al., 1984) and the overexpression of intercellular adhesion molecules in renal biopsies of patients with acute PSGN (Parra et al., 1994). Subsequent studies showed associations between interleukin (IL)-6, IL-8, transforming growth factor beta (TGFβ), and tumour necrosis factor alpha (TNFα) with nephritogenic antigens and proliferative changes in PSGN (Mezzano et al., 1997; Pedreanez et al., 2006).

Autoimmunity

Autoimmune reactivity is present in many cases of acute PSGN. Elevated titres of IgG rheumatoid factor were found in over one-third of the patients in the first week of the disease and anti-IgG deposition has been demonstrated in the kidney (Rodríguez-Iturbe, 1984). Anti-IgG reactivity may result from the loss of sialic acid from autologous IgG due to streptococcal neuraminidase (sialidase). Neuraminidase activity and free sialic acid have been found in the plasma of patients with acute PSGN (Rodríguez-Iturbe et al., 1981a; Asami et al., 1985). Neuraminidase may have the additional effect of facilitating the infiltration of desialized leucocytes in the glomeruli (Marín et al., 1997). Duvic et al. (2000) reported the simultaneous presentation of acute PSGN and thrombotic microangiopathy and suggested a role for neuraminidase in the combined clinical picture.

Another possible mechanism for the production of anti-Ig is the binding of the Fc fragment of IgG to type II receptors on the surface of group A streptococcus. This binding induces intense anti-IgG reactivity and GN with anti-IgG deposits. Burova et al. (1998, 2012) have postulated that PSGN might be triggered by this mechanism. Additional autoimmune phenomena have been found in acute PSGN patients. Anti-DNA antibodies, anti-C1q antibodies (Kozyro et al., 2006), and ANCAs are present in some patients. Interestingly, the latter have been found in two-thirds of the patients with azotaemia and 70% of the patients with crescentic acute PSGN and a rapidly progressive course (Ardiles et al., 1997). Despite these findings the clinical relevance of autoimmune reactivity in PSGN remains undefined.

Pathology

Acute post-streptococcal glomerulonephritis

Light microscopic findings

The majority of cases, including 72% in a recently published series of adult patients (Nasr et al., 2008), show diffuse proliferative and exudative GN (Fig. 77.1), with the most of the remainder showing focal proliferative and exudative GN or predominantly mesangial proliferative GN (Fig. 77.2) (Rosenberg et al., 1985; Montseny et al., 1995; D'Agati et al., 2005; Nasr et al., 2008). A membranoproliferative GN (MPGN) pattern is rarely seen (Montseny et al., 1995; Nasr et al., 2008). With the Masson's trichrome stain, fuschinophilic, red-orange subepithelial and mesangial deposits may be evident (Fig. 77.3). Crescents, primarily segmental cellular crescents, are present in up to half of cases, and may be accompanied by segmental fibrinoid necrosis with disruption of the GBM evident on the silver methenamine stain (Montseny et al., 1995; Nasr et al., 2008).

Interstitial inflammation, typically comprised of a mixture of lymphocytes, monocytes, plasma cells, and neutrophils, is present in most cases. Focal intratubular neutrophils are not infrequent, with these cells coming from the inflamed glomeruli. In the recent study of adult patients by Nasr et al. (2008), histologic evidence of acute tubular injury, characterized by flattening of proximal tubular epithelium with loss of brush borders and nuclear enlargement, was seen in 60% of cases. Mild to moderate arteriosclerosis was also seen in the majority of these cases; cases with underlying diabetic nephropathy tended to have more frequent and more severe arteriosclerosis, as well as arteriolar hyalinization and thickening (Nasr et al., 2008).

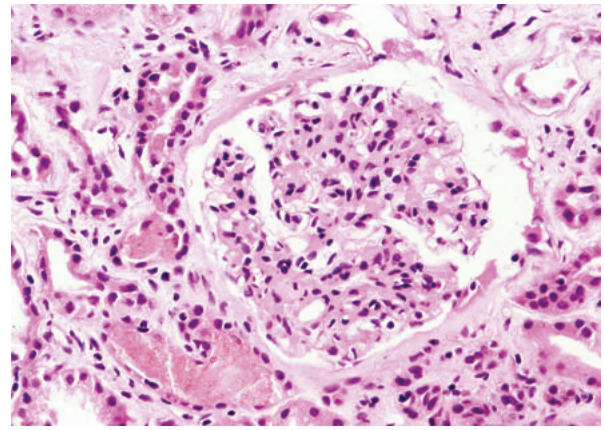

Fig. 77.2 Post-streptococcal glomerulonephritis: mesangial proliferative glomerulonephritis. This pattern is most often seen in lesions undergoing resolution. (Haematoxylin and eosin stain, original magnification 400×.)

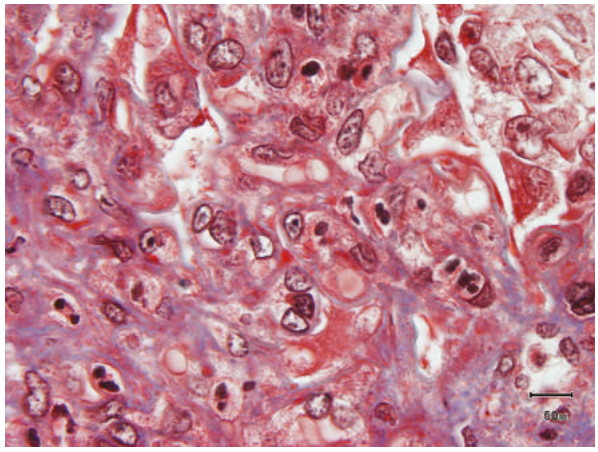

Fig. 77.3 Acute post-streptococcal glomerulonephritis: Masson's trichrome stain. Distinct immune complex deposits are noted that stain a red-orange colour; note especially the subepithelial, 'hump-like' deposit near the centre of the field. (Masson's trichrome stain, original magnification 1000×, scale bar 50 microns.)

Immunofluorescence microscopy

Immunofluorescence findings in evolving stages of PSGN were elegantly defined by Sorger et al. (1982). These investigators defined three patterns of glomerular staining, and correlated these with time after disease onset, relative intensity of IgG and C3 staining, and location and frequency of immune complex deposits by electron microscopy (see below). In the early phase of the disease (initial two or three weeks), the glomeruli show finely granular deposits of C3 and usually IgG in the capillary walls and mesangial areas, in what has been termed a 'starry sky' pattern (Fig. 77.4). Later in the disease, with resorption of many of the subepithelial and subendothelial deposits, there is a predominantly mesangial pattern of staining (Fig. 77.5) with a predominance of C3. In truth, not all of the deposits contributing to the mesangial pattern are actually within the mesangium; as discussed below many are subepithelial deposits within the mesangial 'waist' that are resorbed more slowly than deposits in peripheral portions of the glomerular capillaries. A third pattern of staining, characterized by coarse granular to confluent staining along the glomerular capillary walls (Fig. 77.6), termed the 'garland' pattern, is most often seen early in the disease may but may be seen later as well. It is this pattern that best shows the individual subepithelial 'humps' that are the characteristic ultrastructural feature of this disease.

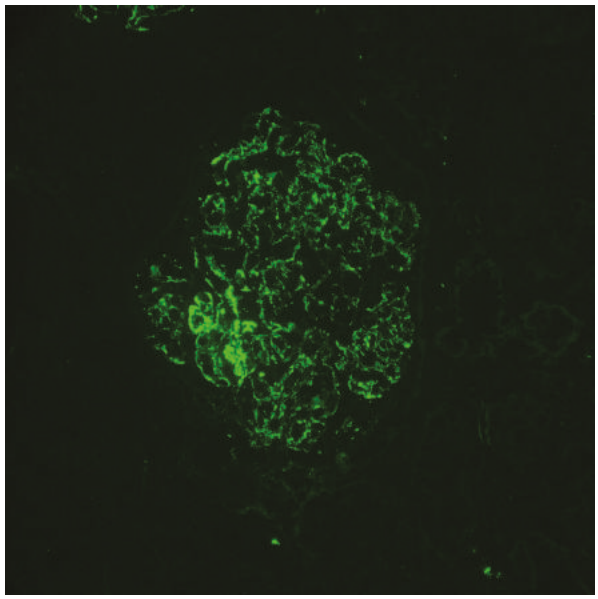

Fig. 77.4 Acute post-streptococcal glomerulonephritis: immunofluorescence microscopy for C3. Numerous granular deposits typical of immune complexes are present in the glomerular capillary walls and mesangial areas, giving a 'starry sky' pattern. (Fluorescein isothiocynate (FITC) conjugated anti-human C3, original magnification 400×.)

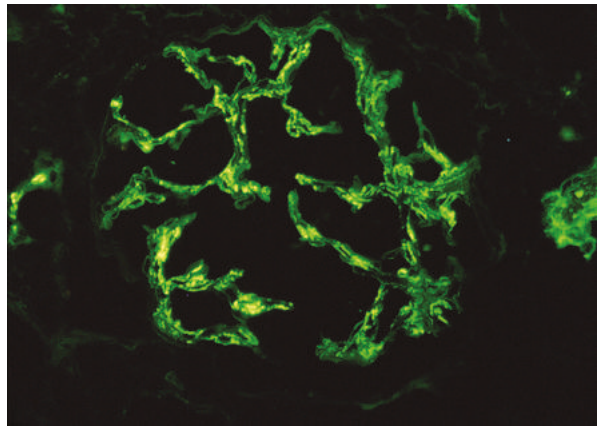

Fig. 77.5 Resolving post-streptococcal glomerulonephritis: immunofluorescence microscopy for C3. Light microscopy from this same biopsy shown in Fig. 77.2. Deposits of C3 appear to be limited to mesangial areas, although electron microscopy also showed subepithelial deposits localized to mesangial 'waist' regions; such deposits cannot be distinguished from mesangial deposits by immunofluorescence and contribute to the mesangial pattern. (FITC conjugated anti-human C3, original magnification 400×.)

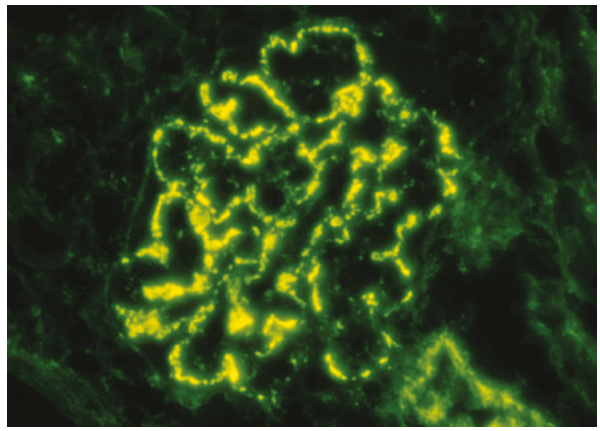

Fig. 77.6 Acute post-streptococcal glomerulonephritis: immunofluorescence microscopy for IgG. There is global, coarse to confluent granular staining mainly along the glomerular capillary walls, corresponding to subepithelial deposits (including large subepithelial 'humps') and likely small subendothelial deposits as well. This 'garland' pattern is most often seen early in the disease, particularly when there is IgG as well as C3 deposition, although C3 deposits with this pattern may also be seen later in the disease course. (FITC conjugated anti-human IgG, original magnification 400×.)

In acute (and subacute) PSGN, deposits of C3 are invariably present, accompanied by IgG in most cases, and IgM in approximately 50%, although the latter staining tends to be of low intensity (D'Agati et al., 2005; Nasr et al., 2008). IgA staining is uncommon and of low intensity when present, although IgA is often the dominant immunoglobulin present in post-staphylococcal lesions, as discussed below. Nasr et al. (2008) found C1q staining, typically of low intensity, in approximately one-third of their cases. Staining for kappa and lambda light chains mirrors that for IgG with respect to pattern with similar staining intensity for both light chains. Focal and segmental, blotchy to amorphous staining for fibrinogen, most typically at the periphery of glomerular tufts, is frequently noted within cellular crescents when these are present.

Electron microscopy

The characteristic ultrastructural finding of acute PSGN is the presence of large subepithelial electron-dense deposits with a 'hump-like' appearance (Fig. 77.7). The number of these deposits varies considerably between different cases; they can be quite segmental or rather numerous, although not so much so as to suggest a membranous nephropathy. The size of the subepithelial 'humps' may also vary considerably within any given glomerulus. In early post-infectious lesions, these deposits are distributed at various points along glomerular capillaries, although even at this stage there is some tendency for the greatest number of deposits to be concentrated at or near the glomerular basement membrane reflection over mesangial areas (the mesangial 'waist' or 'notch'; Fig. 77.7), a finding first noted by Heptinstall (1974). In subacute and resolving cases, the fraction of subepithelial deposits localized to mesangial 'waist' areas increases as more peripheral deposits are resorbed (Sorger et al., 1982; Haas, 2003; Nasr et al., 2008). Mesangial deposits are present in the great majority of cases of acute PSGN and may be abundant, and most cases also show subendothelial deposits, although these tend to be small and segmental (D'Agati et al., 2005; Nasr et al., 2008) (Fig. 77.7). Extraglomerular deposits are not a feature of this disease.

Resolving post-streptococcal glomerulonephritis

The resolution of PSGN proceeds through various stages that are best identified by correlating light, immunofluorescence, and electron microscopic (EM) findings. After the first 1–2 weeks of the disease, in which the glomeruli appear enlarged and markedly hypercellular with prominent numbers of neutrophils (Fig. 77.1), there is a progressive decline in cellularity, initially with the loss of the neutrophils resulting in a combined mesangial and endocapillary proliferative GN (Fig. 77.8). At this stage, the histologic appearance of the glomeruli may resemble that of an early MPGN (without the numerous double contours of the GBM) or even a highly active IgA nephropathy, although subepithelial 'humps' are still quite evident by EM, often in early stages of resorption (Fig. 77.9). Over the ensuing weeks, endocapillary hypercellularity is lost, together with 'humps' in peripheral portions of the GBM. The result is a predominantly mesangial proliferative GN by light microscopy (Fig. 77.2), with an accompanying mesangial pattern of C3 deposits by immunofluorescence (Fig. 77.5) and subepithelial deposits limited largely to mesangial 'waist' regions by EM (Fig. 77.10), the latter often showing evidence of partial resorption. Notably, weak, granular staining for C3 (most typically in a mesangial pattern) and partially or largely resorbed subepithelial deposits (often partially or even completely incorporated into the GBM) remain evident months to years after the resolution of haematuria, renal insufficiency, and light microscopic findings of proliferative GN (Tornroth, 1976; Sorger et al., 1982; Rosenberg et al., 1985; Haas, 2003). These largely resorbed deposits are variably electron-lucent, containing granular, vesicular, or membrane-like structures (Tornroth, 1976; Haas, 2003) (Fig. 77.10). These findings are not infrequently associated with sub-nephrotic proteinuria, and may occasionally represent the only pathologic findings on a renal biopsy done for such proteinuria (Baldwin et al., 1974; Haas, 2003).

It has been our experience that incidental, ultrastructural findings of old, largely healed post-infectious GN, namely partially or largely resorbed subepithelial deposits with at least some localized to mesangial 'waist' areas, are evident in approximately 10%

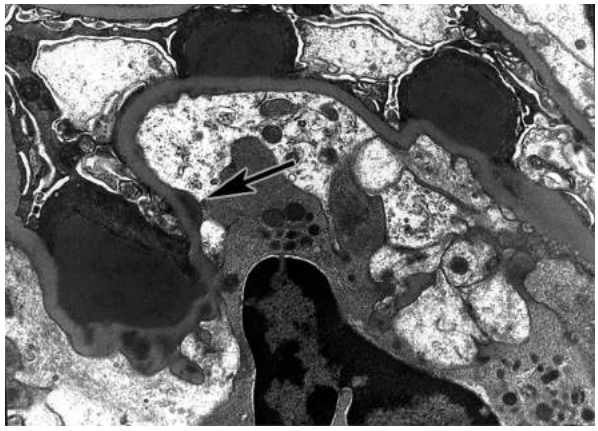

Fig. 77.7 Acute post-streptococcal glomerulonephritis: electron microscopy. There are three large subepithelial 'humps'; the one at left is in a mesangial 'waist' region. There are also some small mesangial deposits underlying this latter deposit, and a small subendothelial deposit (arrow). (Uranyl acetate and lead citrate stain, original magnification 6300×.)

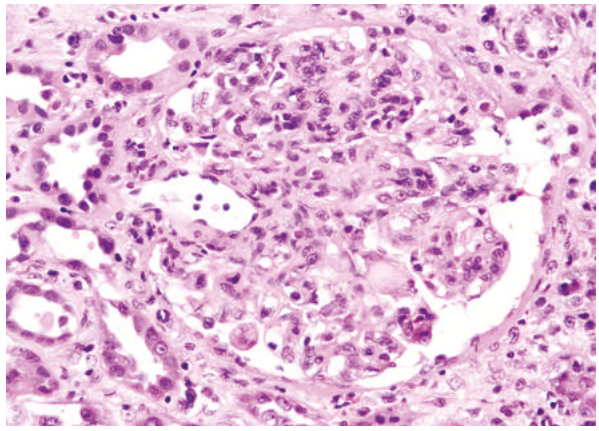

Fig. 77.8 Subacute post-streptococcal glomerulonephritis. The glomerulus, representative all of glomeruli on this biopsy, still shows mesangial and endocapillary hypercellularity. However, the degree of cellularity less than that in Fig. 77.1, and neutrophils are no longer prominently seen. (Haematoxylin and eosin stain, original magnification 400×.)

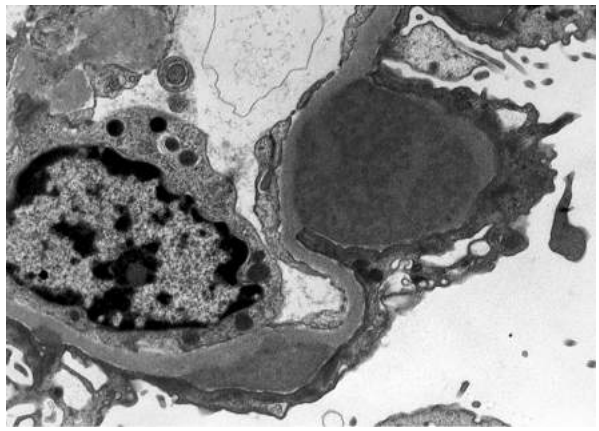

Fig. 77.9 Subacute post-streptococcal glomerulonephritis: electron microscopy. The subepithelial deposits, including the large 'hump', have a somewhat variegated appearance consistent with early resorption, and there is relative electron-lucency at the periphery of the large deposit. (Uranyl acetate and lead citrate stain, original magnification 8000×.)

of native renal biopsy specimens (Haas, 2003). In the vast majority of these cases there is no documented clinical evidence of an acute episode of post-infectious GN. Findings of incidental, healed post-infectious GN are particularly common in biopsies from patients with diabetic nephropathy, being evident in 30–40% of such biopsies (Haas, 2003), although whether these lesions have any clinical significance remains unknown.

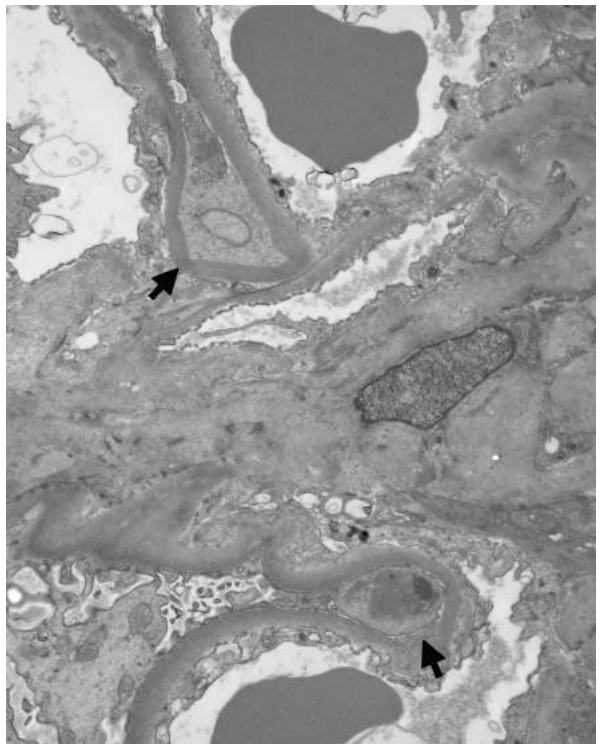

Fig. 77.10 Resolving post-streptococcal glomerulonephritis: electron microscopy. There are two large, partially resorbed subepithelial deposits in mesangial 'waist' regions (arrows). The lower deposit is more electron-dense at its centre and less so at its periphery; the upper deposit contains a membrane-like structure. (Uranyl acetate and lead citrate stain, original magnification 7200×.)

Subepithelial humps, while characteristic of post-infectious GN, are by no means specific for this entity and may be present in other forms of GN, most notably C3 glomerulonephritis (C3GN) and dense deposit disease, both of the latter characterized by abnormal regulation of the alternative pathway of complement (Pickering et al., 2013). Interestingly, Sethi et al. (2012) likewise found evidence of abnormalities in the alternative pathway in 10 of 11 cases of atypical (persistent) post-infectious GN, including mutations in complement factor H (CFH) and CFH-related proteins, and C3 Nef autoantibody.

Treatment

Antibiotic treatment

The diagnosis of impetigo is usually straightforward. In contrast, the clinical judgement may incorrectly diagnose a sore throat as being streptococcal in 20–40% of the cases (Cebul and Poses, 1986). Clinical scores have been proposed to increase the accuracy of this diagnosis (McIsaac et al., 1998). Newly developed rapid high sensitivity tests require culture confirmation if the results are negative (American Academy of Pediatrics, 2000) but the decision to withhold treatment based on this rapid diagnostic test of streptococcal sore throat does not carry increased risk of post-streptococcal complications (Webb et al., 2000).

We recommend antibiotic treatment in all patients with acute PSGN at the time of diagnosis whether the streptococcal infection is obvious or not, to ensure the elimination of the responsible antigen. Penicillin or cephalosporins (Casey and Pichichero, 2004) are adequate treatment. Erythromycin is the treatment of choice in patients allergic to penicillin.

Prophylactic antibiotic treatment should be prescribed in household members of index cases, particularly in high-risk communities since most of them have evidence of recent streptococcal infection and about one-third develop GN (Rodríguez-Iturbe et al., 1981b). This strategy has resulted in a reduction of cases in aboriginal communities (Johnston et al., 1999). As discussed earlier, educational programmes have resulted in a reduction of the incidence of PSGN (Bach et al., 1966).

Treatment of acute PSGN

Children with subclinical disease may be followed in the clinic but patients with the acute nephritic syndrome usually require hospitalization. Strict bed rest is of doubtful value. Restriction of water and particularly sodium intake should be prescribed to all patients with the acute nephritic syndrome.

Early studies (Powell et al., 1980) showed that loop diuretics increase urine output severalfold in most patients and entail a 50% reduction in the time required for normalization of blood pressure and disappearance of oedema, including pulmonary oedema.

Antihypertensive medication may be needed in approximately half the children with PSGN and nearly 80% of adults. A rare patient with convulsions will require sedation and intubation. Pulmonary oedema may complicate the clinical course. Dialysis may be required if there is severe azotaemia or hyperkalaemia. Posterior reversible leucoencephalopathy has been reported in acute PSGN (Ahn and Ingulli, 2008), probably as a manifestation of severe hypertension, and is manifested by drowsiness progressing to stupor, visual hallucinations, headache, and convulsions. Firm diagnosis requires magnetic resonance studies. Immune-mediated

pneumonitis has been also reported in an adult patient with PSGN (Wiles et al., 2011).

Patients with crescentic PSGN are sometimes given intravenous pulses of methylprednisolone, but it has not been demonstrated that intravenous steroids, immunosuppression, or anticoagulation improve the outcome of crescentic PSGN (Zaffanello et al., 2010).

Prognosis

The early mortality of acute endocapillary GN is very low in children but significant in adults (Melby et al., 1987) (Table 77.1). Cardiovascular complications are the main cause of death in acute PSGN. Irreversible renal failure may follow acute GN if widespread extracapillary (crescentic) proliferation develops, but crescentic PSGN has a more favourable prognosis than other types of rapidly progressive GN.

The medium-term prognosis of PSGN has been studied extensively. Initial reports in 1930 and 1940 indicated an excellent prognosis but follow-up periods were relatively short. Subsequent studies have produced variable results. The incidence of abnormal laboratory findings during the follow-up varies from 3.5% (Potter et al., 1982) to 60% (Baldwin et al., 1974). Discrepancies may result, in part, from the different prognosis of PSGN in adults and in children, which is not always taken into account in the reported series. The worse prognosis in adults may result in part from age-related tendency to fibrosis (see Chapter 140), or other changes such as impairment of the Fc-receptor function of the mononuclear phagocyte system (Mezzano et al., 1991).

Several risk factors have a definite influence on the long-term prognosis of acute PSGN. A subgroup of adult patients that had massive proteinuria as the initial manifestation had an incidence of chronic renal failure as high as 77% (Vogl et al., 1986). In an outbreak of PSGN in adults resulting from consumption of cheese contaminated with *Streptococcus zooepidemicus*, there was an alarming incidence of chronic renal disease: impaired renal function was found in 30% of the patients after 2 years of follow-up (10% of them in chronic dialysis therapy) (Balter et al., 2000; Pinto et al., 2001). Recent studies suggest that deficiency of complement factor H-related protein 5 may predispose to the development of chronic renal disease (Vernon et al., 2012).

Studies after 1970 reporting the findings in children, 10–20 years after the acute episode, found that approximately 20% of the patients have an abnormal urinalysis or creatinine clearance, but < 1% develop end-stage kidney disease. However even 20 years leaves many more years at risk for individuals not yet in their middle years. Proteinuria and hypertension occur in 8–13% of the patients in most studies (range 1.4–46%). The incidence of glomerular sclerosis and fibrosis is nearly 50% (Gallo et al., 1980), but the clinical relevance of these histological characteristics is uncertain. Our own data, that include 110 children with epidemic and sporadic PSGN followed prospectively over 15–18 years after the acute episode, indicate an incidence of 7.2% of proteinuria, 5.4% of microhaematuria, 3.0% of arterial hypertension, and 0.9% of azotaemia. These values are essentially similar to those found in the general population. We have also followed 10 cases of subclinical PSGN for 10–11 years and the prognosis is excellent. Studies in Australian aboriginal communities (Hoy et al., 1998) indicate that patients who had acute PSGN have an increased risk for albuminuria (adjusted odds ratio (OR) 6.1, 95% confidence interval (CI) 2.2–16.9) and haematuria (OR 3.7, 95% CI 1.8–8.0) in relation to controls who did not have acute PSGN. Finally, the long-term prognosis of acute PSGN may be influenced by the coexistence of other risk factors of chronic renal failure. The association of PSGN, diabetes, and metabolic syndrome are likely responsible for the high incidence of end-stage renal disease in aboriginal communities in Northern Australia (White et al., 2001; Hoy et al., 2012). It is interesting that persisting arterial stiffness, as determined by brachial–ankle pulse wave velocity, is found in patients with acute PSGN who develop chronic renal disease (Yu et al., 2011).

References

Ahn, S. Y. and Ingulli, E. (2008). Acute poststreptococcal glomerulonephritis: an update. *Curr Opin Pediatr*, 20, 157–62.

American Academy of Pediatrics, Committee on Infectious Diseases. (2000). Group A streptococcal infections. In *The Red Book*, pp. 526–92. Elk Groove Village, II: American Academy of Pediatrics.

Anthony, B. F., Kaplan, E. L., Wannamaker, L. W., *et al.* (1969). Attack rates of acute nephritis after type 49 streptococcal infections of the skin and of the respiratory tract. *J Clin Invest*, 48, 1697–704.

Ardiles, L. G., Valderrama, G., Moya, P., *et al.* (1997). Incidence and studies on antigenic specificities of antineutrophil-cytoplasmic autoantibodies (ANCA) in poststreptococcal glomerulonephritis. *Clin Nephrol*, 47, 1–5.

Asami, T., Tanaka, A., Gunji, T., *et al.* (1985). Elevated serum and urine sialic acid levels in renal diseases of childhood. *Clin Nephrol*, 23, 112–19.

Bach, J. F., Chalons, S., Forier, E., *et al.* (1966). 10-year educational programme aimed at rheumatic fever in two French Caribbean Islands. *Lancet*, 347, 644–8.

Baldwin, D. S., Gluck, M. C., Schacht, R. G., *et al.* (1974). The long-term course of poststreptococcal glomerulonephritis. *Ann Intern Med*, 80, 342–58.

Balter, S., Benin, A., Pinto, S. W., *et al.* (2000). Epidemic nephritis in Nova Serrana, Brazil. *Lancet*, 355, 1776–80.

Batsford, S. R., Mezzano, S., Mihatsch, M., *et al.* (2005). Is the nephritogenic antigen in poststreptococcal glomerulonephritis pyrogenic exotoxin B (SPE B) or GAPDH? *Kidney Int*, 68, 1120–29.

Becker, C. G. and Murphy, G. E. (1968). The experimental induction of glomerulonephritis like that in man by infection with Group A streptococci. *J Exp Med*, 127, 1–23.

Beres, S. B., Sesso, R., Pinto, S. W., *et al.* (2008). Genome sequence of a Lancefield group C Streptococcus zooepidemicus strain causing epidemic nephritis: New information about an old disease. *PLoS ONE*, 3, e3026.

Berge, A., Kihlberg, B. M., Sjöholm, A. G., *et al.* (1997). Streptococcal protein H forms soluble complement-activating complexes with IgG, but inhibits complement activation by IgG-coated targets. *J Biol Chem*, 272, 20774–81.

Burova, L. A., Nagornev, V. A., Pigarevsky, P. V., *et al.* (1998). Triggering of renal tissue damage in the rabbit by IgG Fc receptor-positive group A streptococci. *APMIS*, 106, 277–87.

Burova, L., Pigarevsky, P., Seliverstova, V., *et al.* (2012). Experimental poststreptococcal glomerulonephritis elicited by IgG Fc-binding M family proteins and blocked by IgG Fc fragment. *APMIS*, 120, 221–30.

Carapetis, J. R., Steer, A. C., Mullholand, E. K., *et al.* (2005). The global burden of group A streptococcal diseases. *Lancet Infect Dis*, 5, 685–94.

Casey, J. R. and Pichichero, M. E. (2004). Meta-analysis of cephalosporin versus penicillin treatment of group A streptococcal tonsillopharyngitis in children. *Pediatrics*, 113, 866–82.

Cebul, D. R. and Poses, R. M. (1986). The comparative cost-effectiveness of statistical decision rules and experienced physicians in pharyngitis management. *JAMA*, 256, 3353–7.

Choi, S. H., Zhang, X., and Stinton, M. W. (1995). Dynamics of streptococcal histone retention by mouse kidneys. *Clin Immunol Immunopathol*, 6, 68–74.

Councilman, W. T. (1898). Acute interstitial nephritis. *J Exp Med*, 3, 393–420.
Currie, B. J. and Brewster, D. R. (2001). Childhood infections in the tropical north of Australia. *J Paediatr Child Health*, 37, 326–30.
D'Agati, V. D., Jennette, J. C., and Silva, F. G. (2005). *Non-Neoplastic Kidney Diseases. Atlas of Nontumor Pathology, First Series, Fascicle 4*, pp. 269–96. Washington, DC: American Registry of Pathology.
Dochez, A. R. and Sherman, L. (1924). The significance of Streptococcus hemolyticus in scarlet fever and the preparation of a specific antiscarlatinal serum by immunization of the horse to Streptococcus hemolyticus scarlatinae. *JAMA*, 2, 542–4.
Dodge, W. F., Spargo, B. F., and Travis, L. B. (1967). Occurrence of acute glomerulonephritis in sibling contacts of children with sporadic acute glomerulonephritis. *Pediatrics*, 40, 1028–30.
Duvic, C., Desramé, J., Hérody, M., *et al.* (2000). Acute poststreptococcal glomerulonephritis associated with thrombotic microangiopathy in an adult. *Clin Nephrol*, 54, 169–73.
Francis, A. J., Nimmo, G. R., Efstratiou, A., *et al.* (1993). Investigation of milk-borne Streptococcus zooepidemicus infection associated with glomerulonephritis in Australia. *J Infect*, 27, 317–23.
Frémeaux-Bacchi, V., Weiss, L., Demouchy, C., *et al.* (1994). Hypocomplementaemia of poststreptococcal acute glomerulonephritis is associated with a C3 nephritic factor (C3NeF) IgG autoantibody activity. *Nephrol Dial Transplant*, 9, 1747–50.
Gallo, G. R., Feiner, H. D., Steele, J. M. Jr., *et al.* (1980). Role of intrarenal vascular sclerosis in progression of poststreptococcal glomerulonephritis. *Clin Nephrol*, 13, 449–57.
Haas, M. (2003). Incidental healed postinfectious glomerulonephritis: a study of 1012 renal biopsy specimens examined by electron microscopy. *Hum Pathol*, 34, 3–10.
Heptinstall, R. H. (1974). *Pathology of the Kidney* (2nd ed.). Boston, MA: Little, Brown.
Hoy, W. E., Mathews, J. D., McCredie, D. A., *et al.* (1998). The multidimensional nature of renal disease: rates and association of albuminuria in an Australian Aboriginal community. *Kidney Int*, 54, 1296–304.
Hoy, W. E., White, A. V., Dowling, A., *et al.* (2012) Post-streptococcal glomerulonephritis is a strong risk factor for chronic kidney disease in later life. *Kidney Int*, 81, 1026–32.
Johnston, F., Carapetis, J., Patel, M. S., *et al.* (1999). Evaluating the use of penicillin to control outbreaks of acute poststreptococcal glomerulonephritis. *Pediatr Infect Dis J*, 18, 327–32.
Kasahara, T., Hayakawa, H., Okubo, S., *et al.* (2001). Prognosis of acute poststreptococcal glomerulonephritis (APSGN) is excellent in children, when adequately diagnosed. *Pediatr Int*, 43, 364–7.
Kozyro, I., Perahud, I., Sadallah, S., *et al.* (2006). Clinical value of autoantibodies against C1q in children with glomerulonephritis. *Pediatrics*, 117, 1663–8.
Lung, J. M., and Mallory, S. B. (2000). A child with spider bite and glomerulonephritis: a diagnostic challenge. *Int J Dermatol*, 39, 287–9.
Marín, C., Mosquera, J., and Rodríguez-Iturbe, B. (1995). Neuraminidase promotes neutrophil, lymphocyte and macrophage infiltration in the normal rat kidney. *Kidney Int*, 47, 88–95.
Marshall, C. S., Cheng, A. C., Markey, P. G., *et al.* (2011). Acute post-streptococcal glomerulonephritis in the Northern Territory of Australia: A review of 16 years data and comparison with the literature. *Am J Trop Med Hyg*, 85, 703–10.
Matsell, D. G, Wyatt, R. J., and Glaber, L. W. (1994). Terminal complement complexes in acute poststreptococcal glomerulonephritis. *Pediatr Nephrol*, 8, 671–6.
Melby, P. C., Musick, W. D., Luger, A. M., *et al.* (1987). Poststreptococcal glomerulonephritis in the elderly. Report of a case and review of the literature. *Am J Nephrol*, 7, 235–40.
McIsaac, W. J., White, D., Tannenbaum, D., *et al.* (1998). A clinical score to reduce unnecessary antibiotic use in patients with sore throat. *Can Med Assoc J*, 158, 75–83.
Mezzano, S., Kunick, M., Olavarria, F., *et al.* (1993). Detection of platelet-activating factor activity in plasma of patients with streptococcal nephritis. *J Am Soc Nephrol*, 4, 235–42.
Mezzano, S., Burgos, M. E., Olavarría, F., *et al.* (1997). Immunohistochemical localization of IL-8 and TGF-beta in streptococcal glomerulonephritis. *J Am Soc Nephrol*, 8, 234–41.
Mezzano, S., Lopez, M. I., Olavarria, F., *et al.* (1991). Age influence on mononuclear phagocyte system Fc-receptor function in poststreptococcal nephritis. *Nephron*, 57, 16–22.
Montseny, J. -J., Meyrier, A., Kleinknecht, D., *et al.* (1995). The current spectrum of infectious glomerulonephritis: experience with 76 patients and review of the literature. *Medicine*, 74, 63–73.
Nasr, S. H., Markowitz, G. S., Stokes, M. B., *et al.* (2008). Acute postinfectious glomerulonephritis in the modern era: experience with 86 adults and review of the literatura. *Medicine*, 87, 21–32.
Nicholson, M. L., Ferdinand, L., Sampson, J. S., *et al.* (2000). Analysis of immunoreactivity to a Streptococcus equi subsp. zooepidemicus M-like protein to confirm an outbreak of poststreptococcal glomerulonephritis, and sequences of M-liked proteins from isolates obtained from different host species. *J Clin Microbiol*, 38, 4126–30.
Oda, T., Tamura, K., Yoshizawa, N., *et al.* (2008). Elevated urinary plasmin activity resistant to alpha2-antiplasmin in acute poststreptococcal glomerulonephritis. *Nephrol Dial Transplant*, 23, 2254–9.
Oda, T., Yamakami, K., Omasu, F., *et al.* (2005). Glomerular plasmin-like activity in relation to nephritis-associated plasmin receptor in acute poststreptococcal glomerulonephritis. *J Am Soc Nephrol*, 16, 247–54.
Oda, T., Yoshizawa, N., Yamakami, K., *et al.* (2010). Localization of nephritis-associated plasmin receptor in acute poststreptococcal glomerulonephritis. *Hum Pathol*, 41, 1276–85.
Orta, N. and Moriyón, J. C. (2001). Epidemiología de las enfermedades renales en niños en Venezuela. *Arch Venz Pueric Pediatr*, 64, 76–83.
Oshawa, I., Ohi, H., Endo, M., *et al.* (1999). Evidence of lectin complement pathway activation in postsreptococcal glomerulonephritis. *Kidney Int*, 56, 1158–9.
Pais, P. J., Kump, T., and Greenbaum, L. A. (2008). Delay in diagnosis in poststreptococcal glomerulonephritis. *J Pediatr*, 153, 560–4.
Parra G., Rodríguez-Iturbe, B., Batsford, S., *et al.* (1998). Antibody to streptococcal zymogen in the serum of patients with acute glomerulonephritis: amulticentric study. *Kidney Int*, 54, 509–17.
Parra, G., Platt, J. L., Falk, R. J., *et al.* (1984). Cell populations and membrane attack complex in glomeruli and patients with poststreptococcal glomerulonephritis: identification using monoclonal antibodies by indirect immunofluorescence. *Clin Exp Immunol Immunopathol*, 33, 324–32.
Parra, G., Romero, M., Henríquez-LaRoche, C., *et al.* (1994). Expression of adhesion molecules in poststreptococcal glomerulonephritis. *Nephrol Dial Transplant*, 9, 1412–4.
Pedreanez, A., Viera, N., Rincon, J., *et al.* (2006). Increased IL-6 in supernatant of rat mesangial cell cultures treated with erythrogenic toxin type B and its precursor isolated from nephritogenic streptococci. *Am J Nephrol*, 26, 75–81.
Pickering, M.C., D'Agati, V.D., Nester, C.M., *et al.* (2013). C3 glomerulopathy: consensus report. *Kidney Int*, 84, 1079–89.
Pinto, S. L. W., Sesso, R., Vasconcelos, E., *et al.* (2001). Follow-up of patients with epidemic postreptococcal glomerulonephritis. *Am J Kidney Dis*, 38, 249–55.
Poon-King, T., Bannan, J., Viteri, A., *et al.* (1993). Identification of an extracellular plasmin binding protein from nephritogenic streptococci. *J Exp Med*, 178, 759–63.
Poon-King, T., Mohammed, I., Cox, R., *et al.* (1967). Recurrent epidemic nephritis in South Trinidad. *N Engl J Med*, 277, 728–33.
Potter, E. V., Lipschultz, S. A., Abidh, S., *et al.* (1982). Twelve to seventeen-year follow-up of patients with poststreptococcal acute glomerulonephritis in Trinidad. *N Engl J Med*, 307, 725–9.
Powell, H. T., McCredie, D., and Rotenberg, F. (1980). Response to furosemide in acute renal failure. Dissociation of renin and diuretic responses. *Clin Nephrol*, 14, 55–9.
Rodríguez-Iturbe, B. (1984). Epidemic poststreptococcal glomerulonephritis. (Nephrology Forum). *Kidney Int*, 25, 129–36.
Rodríguez-Iturbe, B. and Batsford, S. (2007). Pathogenesis of poststreptococcal glomerulonephritis a century after Clemens von Pirquet. *Kidney Int*, 71, 1094–104.

Rodríguez-Iturbe, B. and Mezzano, S. (2005). Infections and kidney diseases: a continuing global challenge. In M. El Nahas (ed.) *Kidney Diseases in the Developing World and Ethnic Minorities*, pp. 59–82. London: Taylor & Francis, London.

Rodríguez-Iturbe, B., Katiyar, V.N., and Coello, J. (1981a). Neuraminidase activity and free sialic acid levels in the serum of patients with acute poststreptococcal glomerulonephritis. *N Engl J Med*, 304, 1506–10.

Rodríguez-Iturbe, B. and Musser, J.M. (2008). The current state of poststreptococcal nephritis. *J Am Soc Nephrol*, 19, 1855–64.

Rodríguez-Iturbe, B., Rubio, L., Garcia, R. (1981b). Attack rate of poststreptococcal glomerulonephritis in families. A prospective study. *Lancet*, 1, 401–3

Romero, M., Mosquera, J., Novo, E., *et al.* (1999). Erythrogenic toxin type B and its precursor isolated from streptococci induce leukocyte infiltration in normal rat kidneys. *Nephrol Dial Transplant*, 14, 1867–74.

Rosenberg, H. G., Vial, S. U., Pomeroy, J., *et al.* (1985) Acute glomerulonephritis in children. An evolutive morphologic and immunologic study of the glomerular inflammation. *Pathol Res Pract*, 180, 633–43.

Sarkissian, A., Papazian, M., Azatian, G., *et al.* (1997). An epidemic of acute postinfectious glomerulonephritis in Armenia. *Arch Dis Child*, 77, 342–4.

Seegal, D. and Earle, D. P. (1941). A consideration of certain biological differences between glomerulonephritis and rheumatic fever. *Am J Med Sci*, 201, 528–39.

Sethi, S., Fervenza, F.C., Zhang, Y., *et al.* (2012). Atypical postinfectious glomerulonephritis is associated with abnormalities in the alternative pathway of complement. *Kidney Int*, 83, 293–9.

Skattum, L., Akesson, P., Truedsson, L., *et al.* (2006). Antibodies against four proteins from a Streptococcus pyogenes serotype M1 strain and levels of circulating mannan-binding lectin in acute poststreptococcal glomerulonephritis. *Int Arch Allergy Immunol*, 140, 9–19.

Sorger, K., Gessler, U., Hubner, F.K., *et al.* (1982) Subtypes of acute postinfectious glomerulonephritis. Synopsis of clinical and pathological features. *Clin Nephrol*, 17, 114–28.

Tasic. V. and Polenakovic, M. (2000). Acute poststreptococcal glomerulonephritis following circumcision. *Pediatr Nephrol*, 15, 274–5.

Thongboonkerd, V., Luengpailin, J., Cao, J., *et al.* (2002). Fluoride exposure attenuates expression of Streptococcus pyogenes virulence factors. *J Biol Chem*, 277, 16599–605.

Tornroth, T. (1976) The fate of subepithelial deposits in acute poststreptococcal glomerulonephritis. *Lab Invest*, 35, 461–74

Vernon, K. A., Goicoechea de Jorge, E., Hall, A. E., *et al.* (2012). Acute presentation and persistent glomerulonephritis following streptococcal infection in a patient with heterozygous complement factor h-related protein 5 deficiency. *Am J Kidney Dis*, 60, 121–5.

Viera, N., Pedreanez, A., Rincon, J., *et al.* (2009). Streptococcal zymogen type B induces angiotensin II in mesangial cells and leukocytes. *Pediatr Nephrol*, 24, 1005–11.

Vogl, W., Renke, M., Mayer-Eichberger, D., *et al.* (1986). Long-term prognosis for endocapillary glomerulonephritis of poststreptococcal type in children and adults. *Nephron*, 44, 58–65.

Vogt, A., Batsford, S., Rodríguez-Iturbe, B., *et al.* (1983). Cationic antigens in poststreptococcal glomerulonephritis. *Clin Nephrol*, 20, 271–9.

Vogt, A., Schmiedeke, T., Stöckl, F., *et al.* (1990). The role of cationic proteins in the pathogenesis of immune complex glomerulonephritis. *Nephrol Dial Transplant*, 5 Suppl 1, 6–9.

Von Pirquet, C. (1910). Ergebn Inn Med Kinderheilk, 5, 459–539. [Translated into English in: von Pirquet, C. (1911). Allergy. *Arch Intern Med*, 7, 259–88, 382–436.]

Wasserzug, O., Valinsky, L., Klement, E., *et al.* (2009). A cluster of ecthyma outbreaks caused by a single clone of invasive and highly infective Streptococcus pyogenes. *Clin Infect Dis*, 48, 1213–9.

Webb, K. H., Needham, C. A., and Kurtz, S. R. (2000). Use of a high-sensitivity rapid strep test without culture confirmation of negative results: 2 years' experience. *J Fam Pract*, 49, 34–6.

White, A. V., Hoy, W. E., and McCredie, D. A. (2001). Childhood post-streptococcal glomerulonephritis as a risk factor for chronic renal disease in later life. *Med J Aust*, 174, 492–4.

Wiles, K. S., Lee, M., Brindle, R., *et al.* (2011). Rare mmune-mediated pneumonitis in association with post-streptococcal glomerulonephritis. *Nephrol Dial Transplant*, 26, 4140–2.

Yamakami, K., Yoshizawa, N., Wakabayashi, K., *et al.* (2000). The potential role for nephritis-associated plasmin receptor in acute poststreptococcal glomerulonephritis. *Methods*, 21, 185–97.

Yoshizawa, N., Yamakami, K., Fujino, M., *et al.* (2004). Nephritis-associated plasmin receptor and acute poststreptococcal glomerulonephritis: characterization of the antigen and associated immune response. *J Am Soc Nephrol*, 15, 1785–93.

Yu, M. C., Yu, M. S., Yu, M. K., *et al.* (2011). Acute reversible changes of brachial-ankle pulse wave velocity in children with acute poststreptococcal glomerulonephritis. *Pediatr Nephrol*, 26, 233–9.

Zaffanello, M., Cataldi, L., Franchini, M., *et al.* (2010). Evidence-based treatment limitations prevent any therapeutic recommendation for acute poststreptococcal glomerulonephritis in children. *Med Sci Monitor*, 16, RA79–84.

Zegers, R. H., Weigl, A., and Steptoe, A. (2009). The death of Wolfgang Amadeus Mozart: an epidemiologic perspective. *Ann Intern Med*, 151, 274–8.

Zhang, L., Ignatowski, T. A., Spengler, R. N., *et al.* (1999). Streptococcal histone induces murine macrophages to produce interleukin-1 and tumor necrosis factor alpha. *Infect Immunol*, 67, 6473–7.

CHAPTER 78

Immunoglobulin A-dominant post-infectious glomerulonephritis

Bernardo Rodriguez-Iturbe and Mark Haas

Introduction

The uncommon IgA-dominant variant of post-infectious glomerulonephritis has been particularly associated with infections with *Staphylococcus*, which may induce immune complex-mediated glomerulonephritis (GN) (see Chapter 77) as well as this variant. It was first described by Koyama et al. (1995) in association with methicillin-resistant *Staphylococcus aureus* (MRSA) infection that caused a severe form of GN, but the case for there being a specific type of nephritis associated with methicillin resistance is not strong (Usui et al., 2011).

The frequency of this condition is unclear but reports have appeared from several centres around the world and it represents 1.6% of the biopsies in adult patients in a single institution (Satoskar et al., 2006)

Clinical characteristics

The typical patient presents with acute renal injury and massive proteinuria and the renal biopsy not infrequently shows focal glomerular crescent formation (Haas et al., 2008). The defining characteristic is the immunoglobulin A (IgA) dominant or co-dominant immune deposition in the biopsy, frequently increased serum IgA levels (Wen and Chen, 2011), and specific T-cell receptor Vβ+ subsets in the serum. Staphylococcal superantigens have been implicated in the pathogenesis (Koyama et al., 1995). The clinical picture has been reviewed by Nasr and D'Agati (2011). IgA-dominant post-infectious GN is most frequent in older patients (mean age 60 years) and is more than three time more frequent in males than in females. The most common site of infection is skin, reported in 51% of patients in whom the site of infection was identified. Other frequent sites include surgical wounds and intravenous lines. *Staphylococcus aureus* was responsible in 94% of the reported cases, including MRSA. Gross haematuria was present in 20% of the cases, proteinuria in the nephrotic range was found in 51%, and rapidly progressive renal failure was frequent. Diabetes is a major risk factor and two-thirds of the patients who developed end-stage renal disease had diabetic nephropathy.

Complement levels are frequently low but may be normal. Since non-infectious IgA nephropathy only rarely presents with massive proteinuria and acute kidney injury, and usually shows normal complement levels, these findings may be useful in the differential diagnosis (Satoskar et al., 2006).

Pathogenesis

The pathogenesis of this GN is not entirely clear. There may be activation of a selective IgA response or, in some cases, an intense T-cell activation and T cells activate B cells to produce polyclonal IgA and IgG. The T-cell activation is the result of direct binding of staphylococcal superantigens to the major histocompatibility complex class II molecules in antigen presenting cells that then engage the Vβ+ T-cell receptor region. Koyama et al. (2004) have identified an antigen in *Staphylococcus aureus* that is co-localized with glomerular IgA deposits and developed an experimental model of the disease immunizing Balb/c mice with this antigen (Sharmin et al., 2004).

Pathology

There have been a number of reports of GN with IgA-containing and often IgA-dominant or co-dominant immune complex deposits in association with infections with *Staphylococcus* species, including methicillin-sensitive *S. aureus* (MSSA), MRSA, and *S. epidermidis* (Koyama et al., 1995; Nasr et al., 2003; Satoskar et al., 2006; Haas et al., 2008). The histologic features of these cases have been quite variable, including predominantly mesangial proliferative GN, membranoproliferative-type GN with or without crescents, in some instances resembling cryoglobulinaemic GN, and diffuse proliferative and exudative GN resembling acute post-streptococcal GN (Koyama et al., 1995; Nasr et al., 2003; Satoskar et al., 2006). Nasr et al. (2003) reported five of the latter cases, three following MSSA infections and two following *S. epidermidis* infections, each superimposed on underlying diabetic nephropathy; a similar case is illustrated in Figs 78.1 and 78.2. Notably, many of these reported cases, including those of Nasr et al. (2003), did not show prominent subepithelial 'humps' by electron microscopy.

A recent series of 13 cases of IgA-dominant post-infectious GN (Haas et al. 2008), identified based on morphologic features including subepithelial 'humps' with or without evidence of resorption, demonstrated the full gamut of pathologic features described for post-streptococcal GN (see Chapter 77). Two of the biopsies

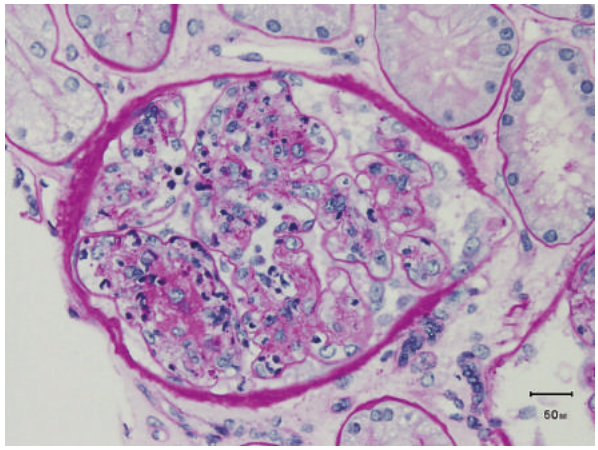

Fig. 78.1 Acute post-infectious (post-staphylococcal) glomerulonephritis superimposed on diabetic glomerulosclerosis. Note the prominent neutrophils, as well as the nodular expansion of the mesangial matrix. (Periodic acid–Schiff stain, original magnification 400×, scale bar 50 microns.)

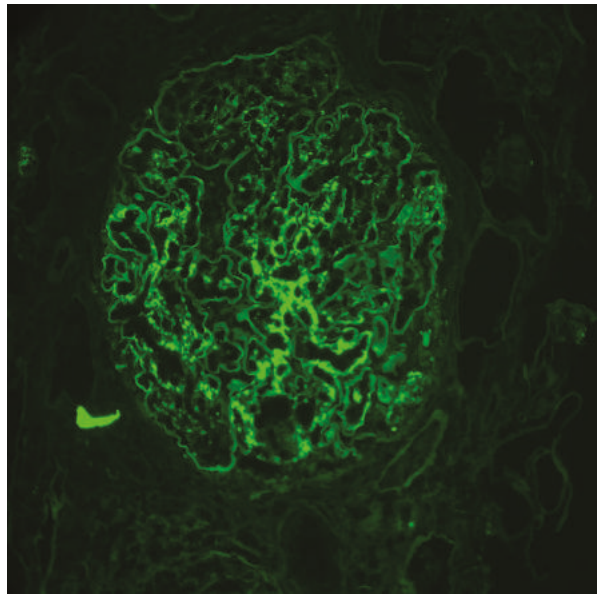

Fig. 78.2 Subacute post-staphylococcal glomerulonephritis superimposed on diabetic glomerulosclerosis: immunofluorescence microscopy for IgA. There is granular staining in the peripheral capillary walls and mesangial areas; the overall appearance is that of a 'starry sky' pattern transitioning into a mesangial pattern (see also Figs 77.4 and 77.5 in Chapter 77). This biopsy showed strong staining for IgA and C3, with weak and segmental staining for IgM and minimal staining for IgG. In the background at right, the underlying nodular expansion of the mesangial matrix can be appreciated. (FITC conjugated anti-human IgA, original magnification 400×.)

showed diffuse proliferative and exudative GN with prominent subepithelial 'humps' (acute post-infectious GN), three showed diffuse proliferative GN with only occasional neutrophils and subepithelial 'humps' showing evidence of resorption (as shown in Chapter 77, Figs 77.8 and 77.9; subacute), and the remaining eight showed mainly mesangial proliferative changes with largely resorbed subepithelial deposits, often localized to the mesangial 'waist' region of glomeruli (as shown in Chapter 77, Figs 77.2 and 77.10; resolving or persistent). Five of the 13 patients were diabetic and three had diabetic nephropathy, and seven had documented infections prior to the onset of GN (three MRSA, three MSSA, one hepatitis C; two additional patients were HIV positive). IgA was the dominant immunoglobulin present in all cases, with five biopsies showing staining for IgG and 10 for IgM; C3 was present in all cases with a mean staining intensity approximately equivalent to that for IgA. Biopsies with histologic changes of acute or subacute post-infectious GN showed a 'starry sky' pattern of IgA and C3 staining by immunofluorescence (Fig. 78.2), while those with resolving/persistent histology had mainly a mesangial pattern (Haas et al., 2008).

Distinction from IgA nephropathy

While acute, IgA-dominant post-infectious GN is rather easily diagnosed by light microscopy and immunofluorescence alone, subacute and resolving/persistent cases often cannot be distinguished from IgA nephropathy without electron microscopy. This is particularly true with cases of resolving/persistent IgA-dominant post-infectious GN, which show predominantly mesangial proliferative changes histologically, a mesangial pattern of IgA and C3 deposits by immunofluorescence, and in our experience often lack a clear infection history (Haas et al. 2008). As with IgA nephropathy, C1q staining by immunofluorescence is usually absent in IgA-dominant post-infectious GN. The latter typically shows equivalent staining intensity for kappa and lambda light chains, while some cases of IgA nephropathy exhibit a clear lambda predominance (Lai et al., 1986; Jennette, 1988) which when present can be helpful in resolving this differential diagnosis. However, the most definitive method for distinguishing resolving/persistent IgA-dominant post-infectious GN from IgA nephropathy remains the identification of prominent although partially or largely resorbed subepithelial deposits by electron microscopy, with at least some of these localized to the mesangial 'waist' region of glomeruli.

Treatment

Antibiotic treatment of staphylococcal infection may be associated with recovery of renal function. Steroid or immunosuppressive treatment is contraindicated if infection is present but it has been used in selected cases with protracted azotaemia when active infection is no longer present (Okuyama et al., 2008; Chen and Wen, 2011).

References

Chen, Y. R. and Wen, Y. K. (2011). Favorable outcome of crescentic IgA nephropathy associated with methicillin-resistant Staphylococcus aureus infection. *Renal Fail*, 33, 96–100.

Haas, M., Racusen, L. C., and Bagnasco, S. M. (2008). IgA-dominant postinfectious glomerulonephritis: a report of 13 cases with common ultrastructural features. *Hum Pathol*, 39, 1309–16.

Jennette, J. C. (1988). The immunohistology of IgA nephropathy. *Am J Kidney Dis*, 12, 348–52.

Koyama, A., Kobayashi, M., Yamaguchi, N., *et al.* (1995). Glomerulonephritis associated with MRSA infection: a possible role of bacterial superantigen. *Kidney Int*, 47, 207–16.

Koyama, A., Sharmin, S., Sakurai, H., *et al.* (2004). Staphylococcus aureus cell envelope antigen is a new candidate for the induction of IgA nephropathy. *Kidney Int*, 66, 121–32.

Lai, K. -N., Chan, K. W., Lai, F. M. -M., *et al.* (1986). The immunochemical characterization of the light chains in the mesangial IgA deposits in IgA nephropathy. *Am J Clin Pathol*, 85, 548–51.

Nasr, S. H. and D'Agati, V. D. (2011). IgA-dominant postinfectious glomerulonephritis: a new twist in an old disease. *Nephron Clin Pract*, 119, c18–25.

Nasr, S. H., Markowitz, G. S., Whelan, J. D., *et al.* (2003). IgA-dominant acute poststaphylococcal glomerulonephritis complicating diabetic nephropathy. *Hum Pathol*, 34, 1235–41

Okuyama, S., Wakui, H., Maki, N., *et al.* (2008). Successful treatment of post-MRSA infection glomerulonephritis with steroid therapy. *Clin Nephrol*, 70, 344–7.

Satoskar, A. A., Nadasdy, G., Plaza, J. A., *et al.* (2006). Staphylococcus infection-associated glomerulonephritis mimicking IgA nephropathy. *Clin J Am Soc Nephrol*, 1, 1179–86.

Sharmin, S., Shimizu, Y., Hagiwara, M., *et al.* (2004). Staphylococcus aureus antigens induce IgA-type glomerulonephritis in Balb/c mice. *J Nephrol*, 17, 504–11.

Usui, J., Kobayashi, M., Ebihara, I., *et al.* (2011). Methicillin-resistant Staphylococcus-aureus-associated glomerulonephritis on the decline: decreased incidence since the 1990s. *Clin Exp Nephrol*, 15, 184–6.

Wen, Y. K. and Chen, M. L. (2011). IgA-dominant postinfectious glomerulonephritis: not peculiar to staphylococcal infection and diabetic patients. *Renal Fail*, 33, 480–5.

CHAPTER 79

Glomerulonephritis associated with endocarditis, deep-seated infections, and shunt nephritis

Bernardo Rodriguez-Iturbe and Mark Haas

Glomerulonephritis associated with endocarditis

Introduction and epidemiology

Bacterial endocarditis may have an acute or subacute clinical presentation and is characterized by the formation of vegetations, composed of platelets, fibrin, inflammatory cells, and bacteria, on heart endothelial surfaces. The incidence of community-acquired native valve endocarditis in population studies in the United States and Western Europe has remained essentially unchanged in the last three decades. Indications for prophylactic antibiotic therapy have been reduced without increment in the incidence of infective endocarditis (Duval et al., 2012). Population studies indicate 1.7–6.2 cases per 100,000 person years (Tleyjeh et al., 2007). Not surprisingly, there is a decline in the number of cases associated with rheumatic heart disease and an increase in cases associated with valve surgery and intravenous drug abuse (Tleyjeh et al., 2005). The heart lesion found to predispose most frequently to endocarditis is mitral valve prolapse and the microorganism most frequently involved is *Staphylococcus aureus* (Mylonakis and Calderwood, 2001). The most important change in the epidemiology of bacterial endocarditis is the increased incidence related to healthcare interventions. Instead of being a disease that affects predominantly young adults with rheumatic heart disease, it presents now in older patients and in patients at risk, which include drug users, patients with prosthetic valves and implantable devices, and patients with HIV infection. *Staphylococcus aureus* and *Staphylococcus epidermidis* are the most common pathogens in hospital-acquired infective endocarditis while *Streptococcus* infections are more frequent in community acquired and native valve endocarditis (Furuno et al., 2011; Que and Moreillon, 2011); however, the incidence of infections by methicillin-resistant *Staphylococcus aureus* (MRSA) is increasing in community-acquired endocarditis (David and Daum, 2010).

In 1910, Löhlein described 'embolic' non-suppurative glomerulonephritis caused by bacterial endocarditis (Löhlein, 1910). Subsequent descriptions emphasized the immune complex pathogenesis of this disease, particularly in patients with *Streptococcus viridians* infections of rheumatic or congenital valve disease (Neugarten and Baldwin, 1984; Eknoyan, 1985). In recent reports, glomerulonephritis (GN) is present in 26% of patients with heart valve infection frequently associated with renal vasculitis. Localized infarcts (31%), many of which were septic, were also described. Interstitial nephritis, mostly attributable to antibiotic therapy (10%) and cortical necrosis (10%) were also significant autopsy findings (Majumdar et al., 2000). Immune deposits are sometimes present (Kirkpantur et al., 2007; Lee et al., 2007; Nasr et al., 2008) but may be absent (Majumdar et al., 2000). The increased incidence of bacterial endocarditis in association with healthcare interventions is particularly evident in patients treated with maintenance haemodialysis. Bacterial endocarditis is 20–60 times more frequent in these patients than in the general population and carries a near 50% mortality risk (Hoen, 2004; Rekik et al., 2009); infections with MRSA and vancomycin-resistant enterococci are associated with a poorer outcome (Leone and Suter, 2010). Emphasis has recently been placed on the need of strict infection control policies, prompt conversion to arteriovenous access from catheters and appropriate antibiotic prescriptions (Fitzgibbons et al., 2011).

Clinical features

The clinical picture of subacute bacterial endocarditis includes splinter haemorrhages, Osler nodules, and Janeway lesions but these manifestations are seen only occasionally. Heart murmurs, fever, anaemia, leucocytosis, elevated sedimentation rate, and purpura are frequent. In particular, purpura on the neck is highly suggestive of endocarditis. Patients at risk with fever should be evaluated for endocarditis since 38% of cases diagnosed at autopsy were not diagnosed clinically (Fernández Guerrero, et al., 2012).

In subacute forms of endocarditis, anorexia and weight loss are commonly observed. Infective endocarditis in haemodialysis patients usually is associated with infections originating from the vascular access, particularly in arteriovenous grafts or dialysis catheters in use for > 1 year (McCarthy and Steckelberg, 2000; Nori et al., 2006; and see Chapter 269).

The most frequently infected heart valves in haemodialysis patients are the mitral and aortic valves and the vegetations are best demonstrated with trans-oesophageal echocardiography (Tao et al., 2010). Systemic embolization with large emboli is usually associated with endocarditis caused by fungi or *Haemophilus*.

One-third of patients with bacterial endocarditis develop azotaemia and the risk increases with age, a history of hypertension, thrombocytopenia, and prosthetic valve infection (Conlon et al., 1998). Renal manifestations of GN in patients with bacterial endocarditis include microscopic haematuria and proteinuria. A rapidly progressive azotaemia is a feature of crescentic GN. A clinical presentation with nephrotic syndrome is distinctly unusual.

GN in a patient with bacterial endocarditis may be not easy to differentiate from renal involvement resulting from embolization or with interstitial nephritis due to antibiotic treatment. Large emboli may produce flank pain and haematuria while microscopic emboli produce microabscesses. Eosinophilia suggests antibiotic-induced interstitial nephritis but eosinophiluria is not a specific finding (see Chapter 6).

The serological findings frequently include decreased C3 and C4 levels (except in superantigen-mediated GN), high titres of rheumatoid factor, serum cryoglobulins, and occasionally positive anti-PR3 antineutrophil cytoplasmic antibodies (ANCAs) (Fukuda et al., 2006; Satake et al., 2011).

Aetiology and pathogenesis

Staphylococcus aureus is the most common aetiologic agent, followed by *S. epidermidis, Streptococcus viridians, S. pyogenes*, and *Enterococcus fecalis*. Less frequent are Gram-negative infections (*Escherichia coli, Proteus*). Experimental studies have confirmed that high-level bacteraemia is not required for endocarditis and a cumulative exposure to low-level bacteraemia is a genuine risk of endocarditis (Veloso et al., 2011). As mentioned above, there are several kidney lesions associated with bacterial endocarditis but the pathogenesis of GN in this condition involves the deposition of immune complexes formed with bacterial antigens (Yum et al., 1978) and, in some cases, the participation of superantigens directly activating T cells and inducing a polyclonal hypergammaglobulinaemia (Koyama et al., 1995; Yoh et al., 2000).

Pathology

Recent North American and European studies of acute post-infectious GN in adults have identified bacterial endocarditis as the underlying infectious process in approximately 10% of cases (Nasr et al., 2008; Montseny et al., 1995).

In the older literature, including autopsy studies from the pre-antibiotic era, focal GN was the most common pattern of glomerular disease associated with bacterial endocarditis, although diffuse GN (in some instances exudative) was not uncommonly seen (Baehr, 1912; Bell, 1932). However, in more recent studies two histologic patterns of GN predominate: diffuse endocapillary proliferative and exudative GN, similar to acute post-infectious GN resulting from infections at other sites such as the upper respiratory tract and the skin (see Fig. 77.1 in Chapter 77) and necrotizing and crescentic GN (Fig. 79.1), often resembling that seen in ANCA-associated vasculitis (ANCA-GN) (Majumdar et al., 2000; Nasr et al., 2008) (See Chapter 157). Notably, the latter form of GN, which was pauci-immune by immunofluorescence similar to ANCA-GN, was seen in 11/16 patients with GN and confirmed bacterial endocarditis reported in a series from the United Kingdom by Majumdar et al. (2000), and this form was predominant in patients diagnosed both by renal biopsy and autopsy, although only one of five patients tested had a positive ANCA serology. Three patients had diffuse endocapillary proliferative and exudative GN, and two had MPGN-like lesions (Majumdar et al., 2000). GN with crescents was also seen in 7 out of 10 patients with bacterial endocarditis from the French series (all biopsies) of Montseny et al. (1995), although the GN in these cases tended not to resemble ANCA-GN as in most instances endocapillary as well as extracapillary hypercellularity was present, and immunofluorescence studies showed deposits of complement in most cases, with or without accompanying immunoglobulin. The three remaining cases showed diffuse endocapillary proliferative and exudative GN (Montseny et al., 1995). In the biopsy series of Nasr et al. (2008) from the United States, 8 out of 10 patients with infective endocarditis had diffuse endocapillary proliferative and exudative GN, while 2 out of 10 (one with a positive C-ANCA) has necrotizing/crescentic GN, like ANCA-GN without significant endocapillary proliferation. In the latter cases, immune complex deposits were limited to the mesangium (Nasr et al., 2008). The reason for the differences between studies is not entirely clear, although focal GN seen in older autopsy series could represent partially resolved lesions detected later in the disease course than lesions diagnosed by renal biopsy. The high fraction of patients with pauci-immune necrotizing/crescentic GN in the series of Majumdar et al. (2000) was seen in both patients diagnosed by renal biopsy and at autopsy, and does not appear to be related to concurrent ANCA vasculitis. Still, while the fraction of patients with this form of glomerular pathology in this study was higher than in other studies, it should be emphasized that other studies have reported lesions resembling ANCA-GN in patients with bacterial endocarditis (Morel-Maroger et al., 1972; Boulton-Jones et al., 1974; Neugarten and Baldwin, 1984; Nasr et al., 2008), and immune complexes do not appear to account for all glomerular lesions in these patients.

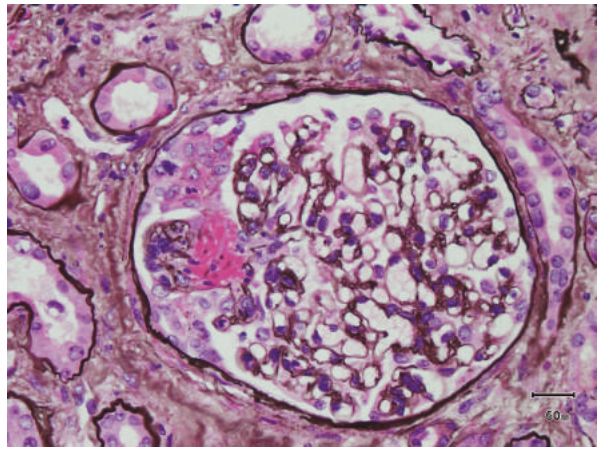

Fig. 79.1 Pauci-immune necrotizing and crescentic glomerulonephritis. This biopsy is from a patient with ANCA-associated vasculitis. The silver stain, which stains the basement membrane matrix black, shows segmental necrosis of the glomerular tuft with pink-staining fibrin, and disruption of the glomerular basement membrane. Adjacent to the area of necrosis is an early cellular crescent. Note the lack of hypercellularity in portions of the glomerulus not involved by necrosis or crescent formation; this latter feature is typical of pauci-immune necrotizing and crescentic glomerulonephritis and contrasts with the hypercellularity of crescentic forms of immune complex-mediated glomerulonephritides, including post-streptococcal glomerulonephritis. (Jones silver methenamine stain, original magnification 400×, scale bar 50 microns.)

Treatment and outcome

Complete eradication of the infection usually requires 4–6 weeks of antibiotic treatment. Serological abnormalities are usually corrected in this time but proteinuria, haematuria, and mild elevation of serum creatinine may persist for several months. As in other varieties of crescentic GN, patients with rapidly progressive GN have been treated with 'pulse' steroid therapy and plasmapheresis (Couzi et al., 2004; Koya et al., 2004) but the value of these treatments remains unproven. Mortality of patients with bacterial endocarditis is as high as 36% in patients who develop kidney failure (Baddour et al., 2005). Plasmapheresis has been anecdotally reported to improve crescentic GN resulting from bacterial endocarditis (Daimon et al., 1998), but this and any other potential immunosuppressive manoeuvres carry significant additional risks in this patient group.

Glomerulonephritis associated with ventriculoatrial shunt infections

Shunt nephritis is now exceptionally rare, but a similar syndrome may occur with infections of other long term intravascular devices such as central vein catheters or pacing wires.

Atrioventricular and peritoneoventricular shunts are used for alleviating the intracranial pressure in congenital and acquired hydrocephalus. Previous studies indicate that 30% of the atrioventricular shunts become infected from 2 months to many years after insertion (Haffner et al., 1997; Kubota et al., 2001). However, in recent studies, the incidence of infection in cerebrospinal fluid shunts in adults appears to be considerably less. A recent prospective 8-year surveillance study found that 6.1% of adult patients developed shunt infection (Korinek et al., 2011).

The initial description of a patient with infected atrioventricular shunt who developed nephrotic syndrome was made by Black et al. (1965). It is now recognized that GN may occur in 1–2% of infected atrioventricular shunts; in contrast, this complication is seldom seen with infected ventriculoperitoneal shunts. The infecting organisms are usually *Staphylococcus epidermidis* (75% of the cases) and *S. aureus* and less frequently *Propionibacterium acne*, diphtheroids, *Pseudomonas*, and *Serratia* species.

The clinical picture of infected ventriculoatrial or ventriculoperitoneal shunts includes low-grade fever, hepatosplenomegaly, arthralgias, weight loss, anaemia, and skin rash, with or without increased intracranial pressure. The diagnosis may sometimes be difficult by standard methods and when cultures are negative. Eosinophilia in the cerebrospinal fluid, which is a sign of malfunctioning shunt (Heidemann et al., 2010; Rehman et al., 2011) and positron emission tomography may be of help in the diagnosis (Rehman et al., 2011). GN, when present, is manifested by microscopic haematuria and proteinuria, frequently in the nephrotic range. The full picture of nephrotic syndrome occurs in 25% of the cases. Serological findings include high rheumatoid factor titres, cryoglobulinaemia, depressed serum complement levels, and, in some patients, positive proteinase 3 ANCA titres (Dobrin et al., 1975; ter Borg et al., 1991; Iwata et al., 2004).

Pathology

The initial description of shunt nephritis noted a 'lobular proliferative' GN (Black et al., 1965). From a number of published series since that time (Arze et al., 1983; Vella et al., 1995; Haffner et al., 1997; Ozcan et al., 2001; D'Agati et al., 2005), it is now well recognized that the most common histologic pattern of GN associated with infected ventriculoatrial shunts is an MPGN (type I) pattern, with diffuse, global endocapillary hypercellularity and glomerular capillary wall thickening with double contours of the glomerular basement membrane (GBM) appreciated on periodic acid–Schiff (PAS) and silver methenamine stains (Fig. 79.2). Less commonly, other patterns of GN may be seen, including diffuse endocapillary proliferative and exudative GN, and predominantly mesangial proliferative GN that may be diffuse or focal. While crescents may be seen, the presence of these in > 50% of glomeruli is rare (D'Agati et al., 2005).

Immunofluorescence microscopy shows immune complex deposits along glomerular capillary walls that typically contain immunoglobulin (Ig)-M and C3, frequently C1q, and more variably IgG and IgA (D'Agati et al., 2005). Interestingly, while the most common underlying infection is with staphylococcal species, mainly *S. epidermidis* (Vella et al., 1995; Haffner et al., 1997), staining for IgA, when present, is typically of lower intensity than that for IgM and IgG (D'Agati et al., 2005). This contrasts with IgA-dominant post-infectious GN associated with staphylococcal infections (see Chapter 78) and together with the different histologic patterns would indicate different pathogenic mechanisms for these lesions. Electron microscopy in the majority of cases of shunt nephritis shows immune complex deposits with a distribution consistent with an MPGN-like lesion, mainly in mesangial and subendothelial locations, the latter often associated with GBM duplication (Vella et al., 1995; D'Agati et al., 2005). Subepithelial 'humps' are seen in a minority of cases, including those with endocapillary proliferative and exudative GN, and in biopsies with mesangial proliferative GN deposits may be restricted to mesangial areas (D'Agati et al., 2005). Bacterial antigens have been demonstrated in the glomeruli (Dobrin et al., 1975).

Treatment and outcomes

Treatment consists of antibiotic therapy and removal of the infected atrioventricular shunt that is substituted at a later date by a ventriculoperitoneal shunt. If dialysis is necessary, haemodialysis is the modality indicated to avoid potential peritoneal infection that

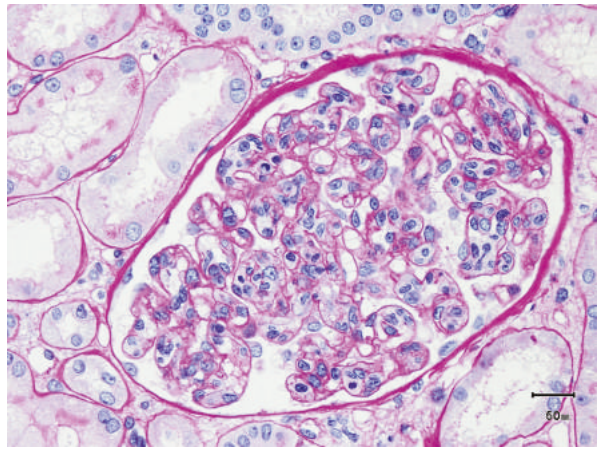

Fig. 79.2 Shunt nephritis. There is a membranoproliferative-type glomerulonephritis with endocapillary hypercellularity, double contours of the glomerular capillary basement membrane, and lobular accentuation. (PAS stain, original magnification 400×, scale bar 50 microns.)

carries the risk of meningitis in patients with ventriculoperitoneal shunts. Complete recovery is the outcome in more than half of the patients but persistent urinary abnormalities and end-stage renal failure are reported in 22% and 6% of the patients, respectively (Haffner et al., 1997).

Glomerulonephritis associated with deep-seated infections (abscesses, osteomyelitis)

Osteomyelitis, intra-abdominal abscesses, pneumonia, and *dental abscesses* are sometimes associated with glomerulonephritis. Osteomyelitis and intra-abdominal abscesses are generally present for several months prior to renal disease that can manifest with mild urinary abnormalities, rapidly progressive azotaemia or, more frequently, by nephrotic syndrome (Beaufils et al., 1976; Ho et al., 2008). In contrast with other infection-related GN, serum complement levels are often normal. Hypergammaglobulinaemia is sometimes present. Treatment of the infection, if started early, improves the renal disease.

Lobar pneumonia caused by *Streptococcus pneumoniae* has been reported to cause GN usually manifested by proteinuria and haematuria (Phillips et al., 2005; Hoshino et al., 2007). The pathogenesis is mediated by immune complexes and pneumococcal antigens (type 14) that have been localized in the glomeruli. The bacterial capsular antigen is capable of activating the alternative complement pathway. In some cases of pneumococcal pneumonia the patient may develop haemolytic-uraemic syndrome due to unmasking of the Thomsen–Friedenreich antigen by the bacterial neuraminidase (Krysan et al., 2001) (see Chapter 174).

Pathology

A number of different histologic patterns of GN have been reported with these infections, although the most common appears to be diffuse proliferative GN, often with crescents (Beaufils et al., 1976; Whitworth et al., 1976). Prominent monocytic infiltration of glomeruli has been reported, albeit not to the extent seen with cryoglobulaemic GN (Magil, 1984). Membranoproliferative-type GN has been infrequently reported, while a predominantly mesangial proliferative pattern may indicate partial resolution. Immunofluorescence studies, even in diffuse proliferative lesions with crescents, tend to show glomerular capillary wall and mesangial deposits containing mainly C3, with little or no specific staining for immunoglobulins (Beaufils et al., 1976; D'Agati et al., 2005).

References

Arze, R. S., Rashid, H., Morley, R., *et al.* (1983). Shunt nephritis. Report of two cases and review of the literature. *Clin Nephrol*, 19, 48–53.

Baddour, L. M., Wilson, W. R., Bayer, A. S. *et al.* (2005). Infective endocarditis: diagnosis, antimicrobial therapy, and management of complications. A statement for healthcare professionals from the Committee on Rheumatic Fever, Endocarditis, and Kawasaki Disease, Council on Cardiovascular Disease in the Young and the Councils on Clinical Cardiology, Stroke, and Cardiovascular Surgery and Anesthesia, American Heart Association: endorsed by the Infectious Disease Society of America. *Circulation,* 111, e394–434.

Baehr, G. (1912). Glomerular lesions of subacute bacterial endocarditis. *J Exp Med*, 15, 330–47.

Beaufils, M., Morel-Maroger, L., Sraer, J. D., *et al.* (1976). Acute renal failure of glomerular origin during visceral abscesses. *N Engl J Med*, 295, 185–9.

Bell, E. T. (1932). Glomerular lesions associated with endocarditis. *Am J Pathol*, 8, 639–63.

Black, J. A., Chaacombe, D. N., and Ockenden, B. G. (1965). Nephrotic syndrome associated with bacteraemia after shunt operations for hydrocephalus. *Lancet*, 2, 921–4.

Boulton-Jones, J. M., Sissons, J. G. P., Evans, D. J., *et al.* (1974). Renal lesions of subacute infective endocarditis. *Br Med J*, 2, 11–14.

Conlon, P. J., Jefferies, F., Krigman, H. R., *et al.* (1998). Predictors of prognosis and risk of acute renal failure in bacterial endocarditis. *Clin Nephrol*, 49, 96–101.

Couzi, L., Morel, D., Deminiére, C., *et al.* (2004). An unusual endocarditis-induced crescentic glomerulonephritis treated by plasmapheresis. *Clin Nephrol*, 62, 461–4.

D'Agati, V. D., Jennette, J. C., and Silva, F. G. (2005). Non-neoplastic kidney diseases. In *Atlas of Nontumor Pathology* (First Series, Fascicle 4), pp. 269–96. Washington, DC: American Registry of Pathology.

Daimon, S., Mizuno, Y., Fujii, S., *et al.* (1998). Infective endocarditis-induced crescentic glomerulonephritis dramatically improved by plasmapheresis. *Am J Kidney Dis*, 32, 309–13.

David, M. Z. and Daum, R. S. (2010). Community-associated methicillin-resistant Staphylococcus aureus: epidemiology and clinical consequences of an emerging epidemic. *Clin Microbiol Rev*, 23, 616–87.

Dobrin, R. S., Day, N. K., Quie, P. G., *et al.* (1975). The role of complement, immunoglobulin and bacterial antigens in coagulase-negative Staphylococcus shunt nephritis. *Am J Med*, 59, 660–73.

Duval, X., Delahaye, F., Alla, F., *et al.* (2012). Temporal trends in infective endocarditis in the context of prophylaxis guideline modifications: three successive population-based surveys. *J Am Coll Cardiol*, 29, 1968–76.

Eknoyan, G. (1984). Renal complications of bacterial endocarditis. *Am J Nephrol*, 5, 457–69.

Fitzgibbons, L. N., Puls, D. L., Mackay, K., *et al.* (2011). Management of gram-positive coccal bacteremia and hemodialysis. *Am J Kidney Dis*, 57, 624–40.

Fernández Guerrero, M. L., Álvarez, B., Manzarbeitia, F., *et al.* (2012). Infective endocarditis at autopsy: a review of pathologic manifestations and clinical correlates. *Medicine*, 91, 152–64.

Fukuda, M., Motokawa, M., Usami, T., *et al.* (2006). PR3-ANCA-positive crescentic necrotizing glomerulonephritis accompanied by isolated pulmonic valve infective endocarditis, with reference to previous reports of renal pathology. *Clin Nephrol*, 66, 202–9.

Furuno, J. P., Johnson, J. K., Schweizer, M. L., *et al.* (2011). Community-associated methicillin-resistant Staphylococcus aureus bacteremia and endocarditis among HIV patients: a cohort study. *BMC Infect Dis*, 11, 298.

Haffner, D., Schinderas, F., and Aschoff, A. (1997). The clinical spectrum of shunt nephritis. *Nephrol Dial Transplant*, 12, 1143–8.

Heidemann, S. M., Fiore, M., Sood, S., *et al.* (2010). Eosinophil activation in the cerebrospinal fluid of children with shunt obstruction. *Pediatr Neurosur*, 46, 255–8.

Ho, C. I., Wen, Y. K., and Chen, M. L. (2008). Glomerulonephritis with acute renal failure related to osteomyelitis. *Journal of Chinese Medical Association*, 71, 315–17.

Hoen, B. (2004). Infective endocarditis: a frequent disease in dialysis patients. *Nephrol Dial Transplant*, 19, 1360–2.

Hoshino, C., Satoh, N., Sugawara, S., *et al.* (2007). Community-acquired Staphylococcus aureus pneumonia accompanied by rapidly progressive glomerulonephritis and hemophagocytic syndrome. *Intern Med*, 46, 1047–53.

Iwata, Y., Ohta, S., Kawai, K., *et al.* (2004) Shunt nephritis with positive titers for ANCA specific for proteinase 3. *Am J Kidney Dis*, 43, e11–16.

Kirkpantur, A., Altinbas, A., Arici, M., *et al.* (2007). Enterococcal endocarditis associated with crescentic glomerulonephritis. *Clin Exp Nephrol*, 11, 321–5.

Korinek, A. M., Fulla-Oller, L., Boch, A. L., *et al.* (2011). Morbidity of ventricular cerebrospinal fluid shunt surgery in adults an 8-year study. *J Neurosurg Sci*, 55, 161–3.

Koya, D., Shibuya, K., Kikkawa, R., *et al.* (2004). Successful recovery of infective endocarditis-induced rapidly progressive glomerulonephritis by steroid therapy combined with antibiotics: a case report. *BMC Nephrol*, 5, 18.

Koyama, A., Kobayashi, M., Yamaguchi, N., *et al.* (1995). Glomerulonephritis associated with MRSA infection: a possible role of bacterial superantigen. *Kidney Int*, 47, 207–16.

Krysan, D. J. and Flynn, J. T. (2001). Renal transplantation after Streptococcus pneumoniae-associated hemolytic uremic syndrome. *Am J Kidney Dis*, 37, E15.

Kubota, M., Sakata, Y., Saeki, N., *et al.* (2001). A case of shunt nephritis diagnosed 17 years after ventriculoatrial shunt implantation. *Clin Neurol Neurosurg*, 103, 245–6.

Landry, D. L., Braden, G. L., Gobeille, S. L., *et al.* (2010). Emergence of gentamicin-resistant bacteremia in hemodialysis patients receiving gentamicin lock catheter prophylaxis. *Clin J Am Soc Nephrol*, 5, 1799–804.

Lee, L. C., Lam, K. K., Lee, C. T., *et al.* (2007). 'Full house' proliferative glomerulonephritis: an unreported presentation of subacute infective endocarditis. *J Nephrol*, 20, 745–9.

Leone, S. and Suter, F. (2010). Severe bacterial infections in haemodialysis patients. *Infezione Medicale*, 18, 79–85.

Löhlein, M. (1910). Über hämorrhagische Nierenalffek bei chronischer ulzeröser Endokarditis (embolische nichteiterige Herdnephritis). *Medizinische Klinik*, 6, 375–9.

Magil, A. B. (1984). Monocytes and glomerulonephritis associated with remote visceral infection. *Clin Nephrol*, 22, 169–75.

Majumdar. A., Chowdhary, S., Ferreira, M. A. A. S., *et al.* (2000). Renal pathological findings in infective endocarditis. *Nephrol Dial Transplant*, 15, 1782–7.

McCarthy, J. T. and Steckelberg, J. M. (2000). Infective endocarditis in patients receiving long-term hemodialysis. *Mayo Clin Proc*, 75, 1008–14.

Montseny, J. -J., Meyrier, A., Kleinknecht, D., *et al.* (1995). The current spectrum of infectious glomerulonephritis: experience with 76 patients and review of the literature. *Medicine*, 74, 63–73.

Morel-Maroger, L., Sraer, J. D., Herreman, G., *et al.* (1972). Kidney in subacute endocarditis. Pathological and immunofluorescent findings. *Arch Pathol*, 94, 205–13.

Mylonakis, E. and Calderwood, S.B. (2001). Infective endocarditis in adults. *N Engl J Med*, 345, 1318–30.

Nasr, S. H., Markowitz, G. S., Stokes, M. B., *et al.* (2008). Acute postinfectious glomerulonephritis in the modern era: experience with 86 adults and review of the literatura. *Medicine*, 87, 21–32.

Neugarten, J. and Baldwin, D. S. (1984). Glomerulonephritis in bacterial endocarditis. *Am J Med*, 77, 297–304.

Nori, U. S., Manoharan, A., Thornby, J. I., *et al.* (2006). Mortality risk factors in chronic haemodialysis patients with infective endocarditis. *Nephrol Dial Transplant*, 21, 2184–90.

Ozcan, F., Sabel, M., Heering, P., *et al.* (2001). Glomerulonephritis secondary to chronic infection of a ventriculoatrial shunt. *Deutsche Medizinische Wochenschrift*, 126, 1229–32.

Phillips, J., Palmer, A. and Baliga, R. (2005). Glomerulonephritis associated with acute pneumococcal pneumonia: a case report. *Pediatr Nephrol*, 20, 1494–5.

Que, Y. A. and Moreillon P. (2011). Infective endocarditis. *Nat Rev Cardiol*, 8, 322–36.

Rehman, T., Chohan, M. and Yonas, H. (2011). Diagnosis of ventriculoperitoneal shunt infection using [F-18]-FDG PET: a case report. *J Neurosurg Sci*, 55, 161–3.

Rekik, S., Trabelsi, I., Hentati M., *et al.* (2009). Infective endocarditis in hemodialysis patients: clinical features, echocardiographic data and outcome: a 10-year descriptive analysis. *Clin Exp Nephrol*, 13, 350–4.

Satake, K., Ohsawa, I., Kobayashi, N., *et al.* (2011). Three cases of PR3-ANCA positive subacute endocarditis caused by attenuated bacteria (Propionibacterium, Gemella, and Bartonella) complicated with kidney injury. *Modern Rheumatol*, 21, 536–41.

Tao, J. L., Ma, J., Ge, G. L., *et al.* (2010). Diagnosis and treatment of infective endocarditis in chronic hemodialysis patients. *Chin Med Sci J*, 25,135–9.

Ter Borg, E. J., Van Rijswijk, M. H. and Kallenberg, C. G. (1991). Transient arthritis with positive tests for rheumatoid factor as presenting sign of shunt nephritis. *Ann Rheum Dis*, 50, 182–3.

Tleyjeh, I. M., Abdel-Latif, A., Rahbi, H., *et al.* (2007). A systematic review of population-based studies of infective endocarditis. *Chest*, 132, 1025–35.

Tleyjeh, I. M., Steckelberg, J. M., Murad, H. S., *et al.* (2005). Temporal trends in infective endocarditis: a population-based study in Olmsted County, Minnesota. *JAMA*, 293, 3022–8.

Vella, J., Carmody, M., Campbell, E., *et al.* (1995). Glomerulonephritis after ventriculo-atrial shunt. *QJM*, 88, 911–18.

Veloso, T. R., Amiguet, M., Rousson, V., *et al.* (2011). Induction of experimental endocarditis by continous low-grade bacteremia mimicking spontaneous bacteremia in humans. *Infect Immun*, 79, 2006–11.

Whitworth, J. A., Morel-Maroger, L., Mignon, F., *et al.* (1976). The significance of extracapillary proliferation. Clinicopathological review of 60 patients. *Nephron*, 16, 1–19.

Yum, M., Wheat, L. J., Maxwell, D., *et al.* (1978). Immunofluorescent localization of Staphylococcus aureus antigen in acute bacterial endocarditis nephritis. *Am J Clin Pathol*, 70, 832–5.

CHAPTER 80

Membranoproliferative glomerulonephritis and C3 glomerulopathy

Daniel P. Gale and Terry Cook

Historical perspective and nomenclature

The term membranoproliferative glomerulonephritis (MPGN) was first introduced in 1961 (Habib et al., 1961) and originally cases were subdivided purely on the basis of light microscopic appearances (Fig. 80.1) into the categories classical, lobular, crescentic, and focal.

With the introduction of electron microscopy and immunostaining during the following decade and the observation that electron-dense deposits were an almost invariable ultrastructural feature, MPGN was subclassified into three types based on the location of deposits. In type 1 there are deposits in the mesangium and between the endothelium and glomerular basement membrane (GBM)—subendothelial deposits (Fig. 80.2). Type 2 is defined by dense transformation of the *lamina densa* of the GBM and is alternatively known as dense deposit disease (DDD) (Fig. 80.3). Type 3 MPGN is a less well-defined condition with subendothelial, subepithelial, and variable intramembranous deposits. It was also recognized that, while types 1 and 3 MPGN were associated with the presence of glomerular immunoglobulin, complement C1q and complement C3 deposition, immunostaining in type 2 MPGN (DDD) was usually positive for C3 only, implicating a primary defect of complement alternative pathway regulation (see Fig. 80.4).

Current nomenclature

Although the subdivision of MPGN described above was clinically useful in identifying patients in whom a defect of complement regulation was likely, several factors have led to the current classification of MPGN into immune-complex MPGN and those cases that are examples of C3 glomerulopathy, according to the likely underlying pathophysiology (Fig. 80.5 and see algorithm in Fig 18.11).

- Recognition of the importance of diseases, such as hepatitis C, other viral infections, paraproteinaemia, and cryoglobulinaemia in causing glomerulonephritis. This reduced the number of patients for whom 'type 1 MPGN' was a diagnosis (rather than simply the kidney biopsy appearance).
- Appreciation that DDD was more commonly associated with light microscopic appearances that do not fulfil the criteria for MPGN (Walker et al., 2007).
- Realization that type 1 or type 3 MPGN can also occur without significant deposition of immunoglobulins, and that in these cases an underlying systemic defect of complement regulation can frequently be identified.

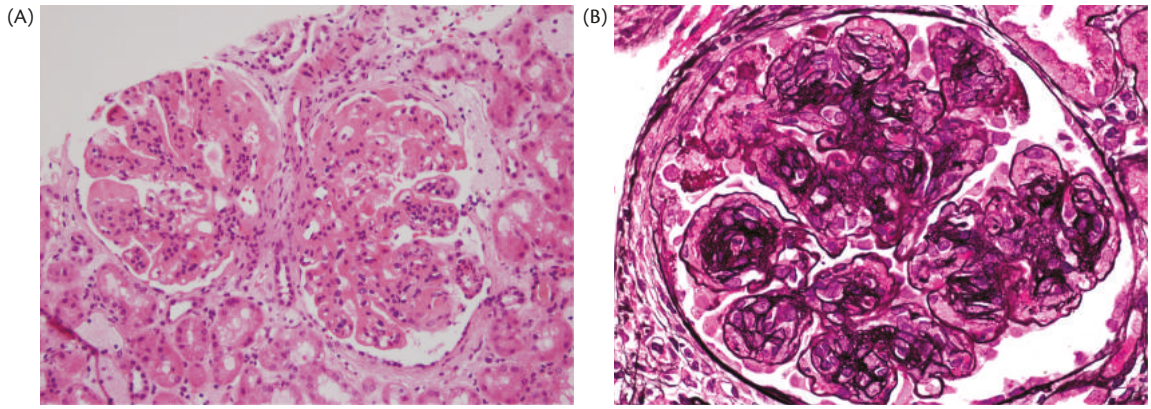

Fig. 80.1 Light microscopic appearances of MPGN. (A) H&E stained section. The glomeruli show enhanced lobulation, mesangial expansion and mesangial hypercellularity, and capillary wall thickening (B) Methenamine sliver-stained section. In the silver-stained section the double-contour appearance of the capillary walls is clearly seen.

Courtesy of Dr P. Walker.

These and other considerations led to the introduction of the term 'C3 glomerulopathy' which encompasses the disorders in which complement C3 accumulates in the glomerulus in the absence of significant immunoglobulin deposition. This is the hallmark of complement alternative pathway dysregulation and represents underlying aetiology, pathophysiology, and prognosis distinct from those associated with cases of proliferative glomerulonephritis (GN) in which immunoglobulins are present, whatever the morphology may be by light or electron microscopy.

With the recent development of therapeutic agents that directly modulate complement activity it is more clinically relevant to define these disorders by the underlying pathophysiology rather than the morphological appearances of the kidney biopsy.

Light microscopic appearances resembling MPGN are sometimes seen in patients with chronic thrombotic microangiopathies. In this context, rather than immunoglobulin and complement deposition with electron-dense deposits visible on electron microscopy, immunostains are typically negative and there is accumulation of electron-lucent, flocculent material (thought to be fibrin and its metabolites) beneath the endothelial cells. Thrombotic microangiopathies are discussed in Chapter 174. This differential diagnosis is also considered in Chapter 18, see Fig 18.11.

Epidemiology

The incidence of MPGN varies across different regions, with higher rates documented in developing countries—an observation usually attributed to the relative prevalence of infectious disease (see below). In the United Kingdom, MPGN is more common in children and young adults and is thought to account for approximately 2% of kidney biopsies, although it is reported in up to 10% of biopsies performed to investigate nephrotic syndrome. Longitudinal evidence suggests that the rates of MPGN are declining (Covic et al., 2006; Hanko et al., 2009; Woo et al., 2010), possibly as a result of reductions in chronic infectious disease and perhaps also due to better treatment and diagnosis of autoimmune conditions.

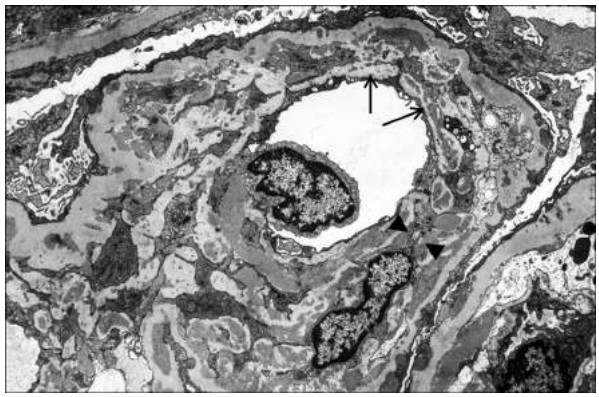

Fig. 80.2 Electron micrograph of a case of immune complex-associated MPGN type 1. There are many subendothelial and mesangial deposits and rare small subepithelial deposits. There is reduplication of the glomerular basement membrane and mesangial cell interposition.

Immune complex membranoproliferative glomerulonephritis

MPGN associated with immunoglobulin deposition can occur in a very wide range of diseases—usually those in which there is increased or abnormal immunoglobulin production. In up to one-third of cases immune complexes are detectable in the circulation, but many people with such immune complexes do not develop glomerulonephritis.

The aetiologies of immune complex MPGN can be divided into infectious, autoimmune, or neoplastic. In these conditions, aberrant production of immunoglobulins can lead to accumulation in the glomerulus with consequent complement activation, inflammation and damage to the kidney. The fact that most people with immunological activation do not develop renal disease suggests that other factors (perhaps relating to characteristics of the antigen or immunoglobulins, or to variation in complement regulators or local glomerular architecture) determine which patients will develop renal disease.

Infection-related membranoproliferative glomerulonephritis

Chronic infection was probably the most common cause of MPGN historically, and still accounts for a significant proportion of cases—especially in regions where infectious disease is common. Bacterial infection-related GN is reviewed in Chapter 76. Generation of antibody–antigen complexes leads to deposition in the glomerulus, local complement activation, and MPGN. Although overall the risk of MPGN with infection is low, some infections, such as endocarditis and viral hepatitis (with or without associated cryoglobulinaemia) seem more likely to result in this renal lesion. *Schistosoma mansoni* infection (see Chapter 182) is a common cause of MPGN in endemic countries, and it has been

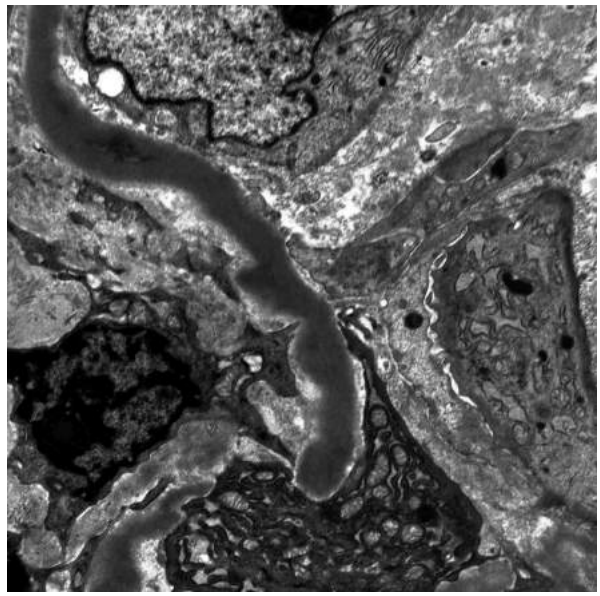

Fig. 80.3 Electron micrograph of the glomerulus from a case of dense deposit disease showing prominent osmiophilic transformation of the glomerular basement membrane.
Courtesy of Dr C. Nast.

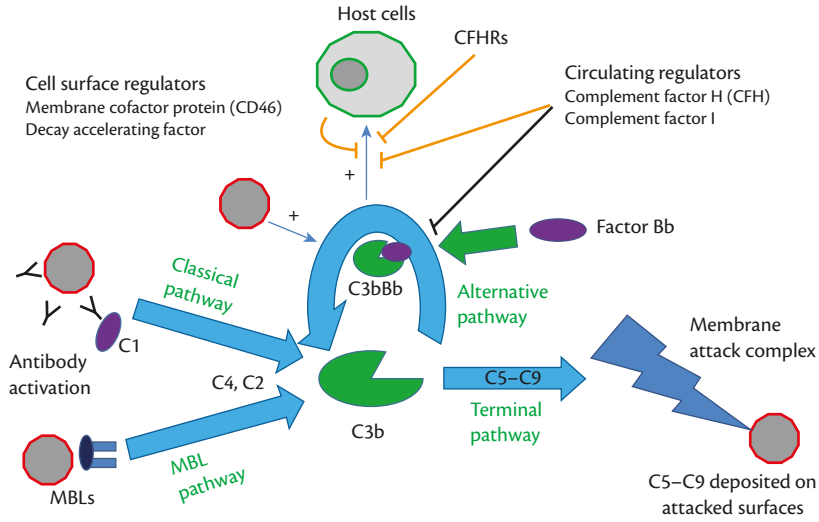

Fig. 80.4 The complement system. Complement is a cascade of circulating proteins (designated C1 to C9) that forms an important component of the innate immune system. It can be activated via the 'classical pathway' in which antibodies bind to antigen, and (via the recruitment of the C1 complex and other proteins) lead to the formation of a C3 convertase that catalyses the cleavage of C3 to generate C3b. This C3 convertase can also be generated by mannose binding lectins (MBLs) that recognize mannose residues present on the surface of bacteria (the 'MBL pathway') and there is also spontaneous 'tickover' activation of C3 in the circulation by hydrolysis. C3b binds to activated factor B to form C3bBb, a non-covalently bound alternative C3 convertase enzyme that catalyses the cleavage of C3 to generate more C3b in a positive feedback amplification loop known as the 'alternative pathway'. The C3bBb convertase can freely dissociate but is stabilized by a number of factors, including biological surfaces. C3b also forms part of a complex that catalyses the cleavage of C5 to activate the 'terminal pathway' that leads to lysis of a targeted cell. In order to prevent runaway activation and damage to host tissues, the alternative pathway is regulated by a number of cell-surface and circulating regulators, including complement factor I (CFI), membrane cofactor protein (CD46), and complement factor H (CFH). Regulation by CFH is modulated by some or all of the CFH-related proteins CFHRs 1–5. Genetic defects of these regulators can lead to diseases to which the kidney is particularly susceptible.

postulated that hepatosplenic involvement in the disease results in reduced clearance of immune complexes by Kupffer cells, leading to increased systemic (and therefore renal) exposure (Barsoum et al., 1988). Presentation of infection-associated MPGN is usually with haematuria, proteinuria, and renal dysfunction which may be progressive if the underlying cause does not resolve.

Classic post-streptococcal GN (see Chapter 77) is sometimes seen several days after resolution of an acute bacterial infection. Presentation is typically with haematuria, proteinuria (sometimes with nephrotic syndrome), and renal impairment. It is usually self-limiting, although supportive renal replacement therapy is occasionally needed. There are frequently detectable circulating antibodies against streptolysin O and DNase B, and hypocomplementaemia is common (Blyth et al., 2007). Because some antibodies and bacterial antigens are able to stabilize the alternative pathway C3 convertase, low C3 is not always accompanied by low C4. Kidney biopsy in post-infectious GN typically shows diffuse mesangial proliferation, neutrophil infiltration and large, hump-like subepithelial deposits on electron microscopy. Immunostains of the glomerulus are usually positive for IgG, IgM, C1q, and C3, although C3-predominant staining in this context is well recognized (Sethi et al., 2013). Distinguishing post-infectious GN from C3 glomerulopathy (see below) on a single biopsy is therefore not always possible, and lack of the typical clinical course and resolution should raise the suspicion of an underlying disorder of complement regulation.

Cryoglobulinaemic membranoproliferative glomerulonephritis

Cryoglobulins are immunoglobulins that reversibly precipitate at 4°C. Cryoglobulinaemia can arise as a result of an aberrant plasma cell clone (types 1 and 2 cryoglobulinaemia) or due to polyclonal production (type 3) and these are discussed in more detail in Chapter 151. Tests for rheumatoid factor are often positive and hypocomplementaemia is common in this condition. The association of cryoglobulins with infection is well recognized and they are detectable in 15–20% of people infected with HIV and up to 50% of those infected with hepatitis C. Cryoglobulinaemia may also occur in autoimmune diseases, most commonly Sjögren syndrome. Clinically significant disease resulting from cryoglobulinaemia is only seen in a small proportion of patients with detectable circulating cryoglobulins.

Deposition of cryoglobulins may occur anywhere in the body, leading to local activation of complement and thrombosis, and leading to Meltzer's clinical triad of palpable purpura, joint pains, and muscle weakness. Renal involvement usually manifests as proteinuria (with nephrotic syndrome in approximately one-fifth of patients), haematuria, and renal impairment. Around a third of patients have concurrent extrarenal disease at presentation, although renal involvement can be the presenting feature of hepatitis C infection, with over half of patients with this underlying cause having normal liver function tests at presentation.

Kidney biopsy in cryoglobulinaemic GN frequently shows MPGN, sometimes with very prominent hypercellularity due to macrophage infiltration into the glomeruli. There may also be florid accumulation of eosinophilic material in intraluminal thrombi, likely representing cryoprecipitate in the glomerular capillaries (Fig. 80.6). Small or medium vessel vasculitis is seen in approximately one-third of patients.

Progression of cryoglobulinaemic GN to end-stage renal disease is seen in approximately 10% patients, usually after at 10 years or

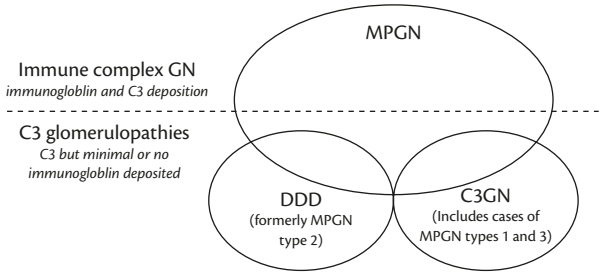

Fig. 80.5 Classification of proliferative glomerulonephritis. C3GN = C3 glomerulonephritis; DDD = dense deposit disease. (Compare with Fig. 18.11D)

more, and treatment is usually aimed at the underlying cause (e.g. clearing hepatitis C infection (see Chapter 186) or suppressing a plasma cell clone). Plasma exchange is sometimes used to reduce the cryoglobulin load (or 'cryocrit'), especially where there is hyperviscosity syndrome, but although good outcomes have been reported in case series, clinical trial data is lacking (Dammacco and Sansonno, 2013).

Monoclonal immunoglobulin G deposition and MPGN

Proliferative GN has also been described in association with monoclonal immunoglobulin G (IgG) deposition in the absence of detectable cryoglobulinaemia. This is sometimes associated with hypocomplementaemia and/or a monoclonal band detectable in the serum or urine. Published series indicate there is a low risk of development of subsequent haematological malignancy, at least within 2–3 years of diagnosis. However, it is also recognized that monoclonal gammopathy associated with established lymphocytic leukaemia can also result in MPGN with monoclonal immunoglobulin deposition, implying that thorough haematological assessment of patients presenting with monoclonal immunoglobulin deposition in the glomerulus is warranted. Follow-up data in proliferative GN with monoclonal IgG deposition is limited, but in the largest published series > 20% of patients progressed to end-stage renal disease over 30 months of follow-up (Nasr et al., 2009). Unsurprisingly, disease recurrence following transplantation is also described in this condition.

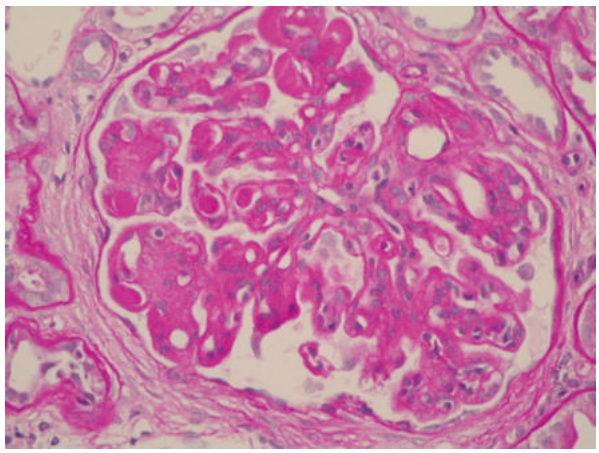

Fig. 80.6 Glomerulus in a case of cryoglobulinaemia showing prominent intraluminal thrombi (periodic acid–Schiff stain).

Autoimmune disease

The presence of circulating antibodies against autoantigens can result in immune complexes that can lead to glomerular disease. Most commonly, this results in proliferation confined to either mesangial regions or the glomerular capillaries. In this context, positive immunostaining for IgA (but not IgG or IgM) is diagnostic of underlying IgA nephropathy, and immunostaining for all immunoglobulins is suggestive of lupus nephritis (although a diagnosis of systemic lupus erythematosus properly relies on additional clinical and/or serological features). These diseases are discussed in Chapter 162. Occasionally, proliferative GN with staining for IgM but not IgG or IgA is seen, and this is sometimes termed 'IgM nephropathy'. Whether the IgM is relevant to disease pathogenesis in this context is not known.

Where an underlying autoimmune, infectious, or lymphoproliferative disorder is not identified these cases are now regarded as 'immune complex MPGN of unknown cause' rather than the former diagnostic categories of 'primary' or 'idiopathic' MPGN type 1 or type 3. Interestingly, an underlying disorder of complement regulation can frequently be identified in such cases (Servais et al., 2012) (see Fig. 80.2). This suggests that regarding the histomorphological and immunohistochemical findings as the 'gold standard' in the diagnosis in MPGN may not always be warranted and that a clinically useful diagnosis is most likely to come from a complete clinical, genetic, serological and pathological assessment of each patient.

Treatment of immune-complex associated MPGN

Where an underlying systemic disease (such as bacterial infection, hepatitis, or autoimmune condition) is identified its successful treatment is likely to ameliorate the renal disease. In addition, aggressive treatment of high blood pressure and proteinuria with angiotensin blockade is widely accepted to be beneficial. Where a cause is not identified there is very little trial data to guide treatment for MPGN. Outcomes in immune-complex MPGN seem to relate most closely to the degree of inflammation seen on the initial biopsy (Little et al., 2006) and therapy aimed at suppressing the immune system is sometimes used. Alternate day corticosteroids has been used with some evidence of efficacy in children with MPGN, and antiproliferative agents have shown efficacy in small observational studies, but controlled trials with long-term follow-up have not been published. Kidney Disease: Improving Global Outcomes (KDIGO) guidelines suggest, in the presence of frank nephrotic syndrome and declining function, that a trial of 6 months of corticosteroids and either cyclophosphamide or mycophenolate mofetil is reasonable to treat MPGN of unknown cause.

C3 glomerulopathy

Proliferative GN sometimes occurs with deposition of C3 but without significant quantities of immunoglobulins or C1q in the glomerulus. This pattern of immunostaining is characteristic of DDD (formerly MPGN type 2) (Fig. 80.7) where there is dense transformation of the basement membrane, but it is also seen in some cases where there are discrete electron-dense deposits in distributions that would formerly have been characterized as MPGN type 1 (subendothelial and mesangial) or type 3 (subepithelial as well as subendothelial and mesangial). These appearances are now termed

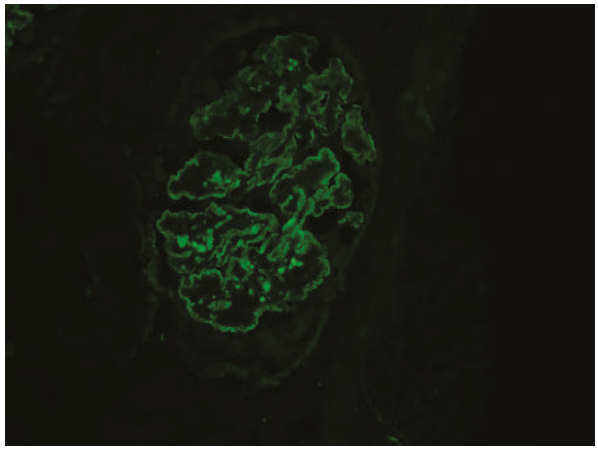

Fig. 80.7 Immunofluorescence for C3 in a case of dense deposit disease. There is staining along glomerular capillary walls and also granular mesangial staining.

C3 glomerulonephritis (C3GN). Together, DDD and C3GN are referred to as the C3 glomerulopathies and in the majority of cases a genetic or acquired defect of complement alternative pathway can be identified (Servais et al., 2012). Although an MPGN pattern is often seen, in some cases there may be a mesangial proliferative pattern or even normal glomeruli. Whatever the basic pattern of glomerular injury, there may also be variable segmental or global endocapillary hypercellularity and/or crescent formation.

Dense deposit disease

DDD is rare, occurring at a frequency of approximately 2–4 per million of the population. It is usually diagnosed in children (where it accounts for around 15–20% of MPGN) or, less commonly, young adults. Clinical presentation is usually with proteinuria which may be accompanied by the nephrotic syndrome and/or haematuria. The diagnosis is made by observing the characteristic highly osmiophilic dense transformation of the lamina densa of the GBM. Presentation is often in the aftermath of an infectious disease and there is usually progressive renal impairment, with 50% of patients requiring renal replacement therapy within 10 years of diagnosis. Prognosis is worse with greater age or greater degree of renal impairment at diagnosis. Recurrence following renal transplantation is common, with 50% grafts lost to recurrent disease at 5 years (Braun et al., 2005).

Aetiology of DDD

DDD is usually associated with systemic evidence of activation of the complement alternative pathway. In around 70% of patients, an autoantibody that recognizes the alternative pathway C3 convertase (termed a C3 nephritic factor, or C3NeF) is detectable. C3NeFs stabilize the convertase complex (C3bBb), leading to runaway activation of the alternative pathway in the circulation, usually resulting in very low C3 levels. C4 levels are typically unaffected as the classical pathway is not activated. In individuals in whom a C3NeF is not detected, antibodies that bind to both C3b and factor B, or antibodies that recognize complement factor H (CFH) have been reported (Chen et al., 2011).

Familial occurrence of DDD is exceedingly rare, but has been described in association with defects that result in fluid phase (as opposed to cell-surface) dysregulation of the complement alternative pathway. Examples include homozygous mutations of the complement regulator *CFH* gene (Licht et al., 2006) and heterozygosity for a particular activating mutation of *C3* (Martinez-Barricarte et al., 2010). Of note, fluid phase dysregulation of complement associated with DDD contrasts to mutations of *CFH* and other complement regulators that lead to complement dysregulation particularly at endothelial surfaces—these are most often described in association with atypical haemolytic uraemic syndrome (see Chapter 174).

In addition to these individual monogenic disorders, certain common allelic variants of *CFH* and its homologue complement factor H-related protein 5 (*CFHR5*) are more common in patients with DDD than in controls (Abrera-Abeleda et al., 2006). While these findings do not have direct implications for diagnostic or therapeutic decisions concerning individual patients, they do highlight the importance of the fine control of complement regulation in the disease.

Systemic features associated with DDD

Retinal drusen, which result from the accumulation of complement components in Bruch's membrane of the eye are also visible by fundoscopy in DDD. Visual loss may occur, as a consequence of retinal atrophy or occasional subretinal neovascular membrane formation, usually over several decades. Ophthalmological assessment is therefore recommended in patients with DDD.

Acquired partial lipodystrophy (loss of fat in the upper half of the body) is also associated with the presence of a C3NeF and is thought to result from complement-mediated damage to adipocytes caused by serum containing a C3NeF (Mathieson et al., 1993). Partial lipodystrophy can precede the renal disease and can sometimes be triggered following a minor infection.

C3 glomerulonephritis

Glomerular inflammation with C3 deposition, without significant immunoglobulin deposition, and in the absence of dense transformation of the basement membrane is termed C3 glomerulonephritis (C3GN). C3GN is rare, probably accounting for < 10% of proliferative GN and most commonly occurs in young adulthood, although can occur at any age. The term C3GN has only recently gained widespread acceptance, and it is believed that most cases would previously have been regarded as variants of MPGN types 1 or 3 (Fig. 80.8).

Presentation is with one or more of haematuria, proteinuria, or nephrotic syndrome and varies according to underlying aetiology. Although traditionally regarded as a benign disease, recent evidence suggests that outcomes are similar to those in DDD, with progressive renal impairment, usually over several years. Recurrence following transplantation is common in the disease, presumably because of the systemic nature of the underlying defect(s) of complement regulation.

Aetiology of C3GN

C3GN is usually sporadic but in published series potential defects of complement regulation are often present. A C3NeF is seen in approximately 40% of cases and mutations of the complement regulators *CFH*, complement factor I, and membrane cofactor protein (*MCP*) have also been described in sporadic cases (Servais et al., 2012). Interestingly, these mutations overlap with mutations also observed in patients with atypical haemolytic uraemic syndrome (see Chapter 174), implicating complement dysregulation particularly at surfaces in C3GN. These defects of complement regulation

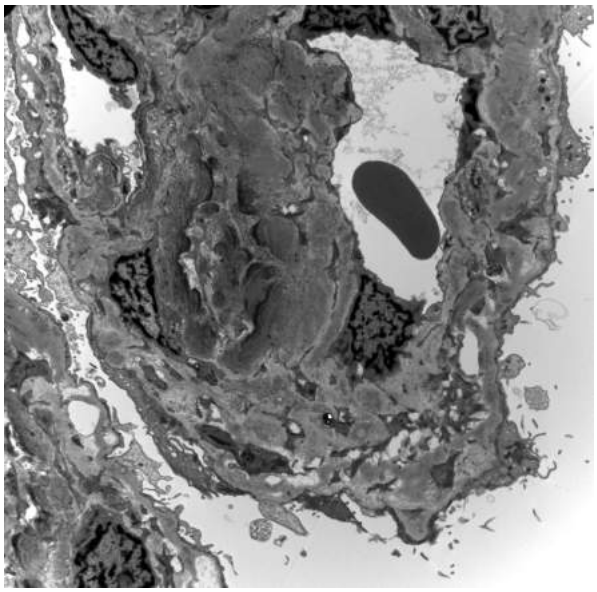

Fig. 80.8 Electron micrograph from a case of C3 glomerulonephritis. There is electron-dense material in the mesangium and the glomerular basement membrane is thickened with intramembranous deposits of variable density. This appearance has previously been referred to as MPGN type 3.

are summarized and compared with those identified in immune complex MPGN and DDD in Fig. 80.9.

In addition, an association exists between C3GN and paraproteinaemia (Zand et al., 2013), and it is important that this possibility is considered, especially in older patients. Familial forms of C3GN have been described in association with mutations and rearrangements of the *CFHR* genes, the most common of which, CFHR5 nephropathy, is endemic in Cypriots (Gale et al., 2010).

CFHR5 nephropathy

CFHR5 nephropathy is a highly penetrant monogenic disease with autosomal dominant inheritance. It is caused by heterozygous mutation of *CFHR5* leading to production of an elongated version of the protein due to duplication of the N-terminal 2 domains, denoted $CFHR5_{12123–9}$. The disease is endemic in Cyprus with an allele frequency of 1:6000 in this population. Kidney biopsy in the disease invariably shows C3GN and CFHR5 nephropathy is by far the commonest single known cause of this renal lesion.

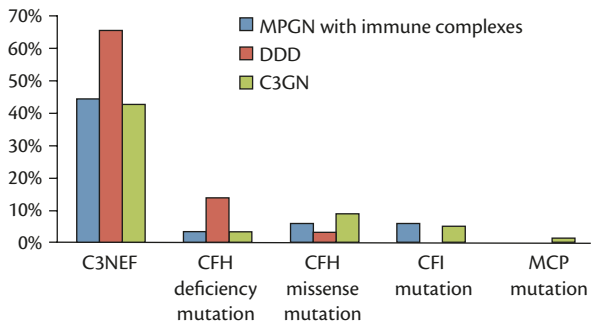

Fig. 80.9 Complement abnormalities in sporadic cases of MPGN and C3 glomerulopathy.
Data from Servais et al. (2012).

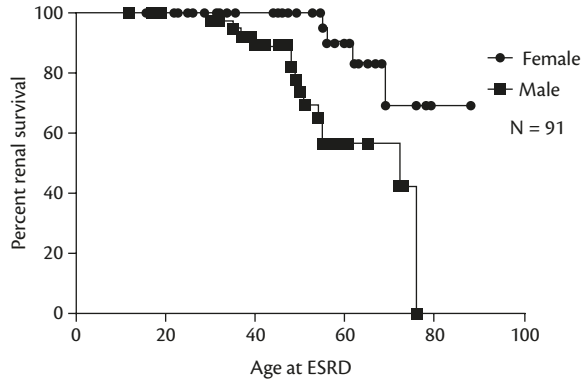

Fig. 80.10 Outcomes in CFHR5 nephropathy.
From Athanasiou et al. (2011).

The clinical features of CFHR5 nephropathy are distinct from those of C3GN due to other causes and are highly consistent: there is microscopic haematuria (present in > 90% of mutation carriers) and episodes of macroscopic haematuria occur at times of upper respiratory tract or other infections. There may also be acute kidney injury during these episodes, and the clinical resemblance to IgA nephropathy is striking. Proteinuria is usually only seen after the development of established chronic renal impairment and is usually low grade (i.e. < 1 g/day). Nephrotic syndrome has not been reported in this disease and extrarenal manifestations are not described. The association between disease flare and intercurrent infection, also seen in DDD, attests to the importance of environmental immunological exposures in modulating complement alternative pathway activity.

While microscopic haematuria and C3GN appear to be present in both sexes, episodes of macroscopic haematuria and renal dysfunction are markedly less common in females than males. Over 80% men with the disease have established renal dysfunction by the age of 50, whereas this is observed in < 20% of women (Athanasiou et al., 2011). A corollary of this is that end-stage renal disease is very much more likely in men with the disease, and this was borne out in a large cohort study where all the males but fewer than half the females had either died or required renal replacement therapy by the age of 80 (see Fig. 80.10).

The disease recurs following transplantation, but overall graft survival is not demonstrably affected, presumably because of the slow pace of progression.

Other mutations resulting in rearrangements of the *CFHR* genes and also leading to production of elongated versions of the proteins (e.g. a CFHR3-1 hybrid protein) have been described in association with autosomal dominant inheritance of C3GN in single families (Malik et al., 2012; Tortajada et al., 2013), strongly implicating the CFHR proteins in the modulation of complement activity in humans.

Investigation of C3 glomerulopathies

A kidney biopsy showing a C3 glomerulopathy (i.e. DDD or C3GN) should prompt investigation of alternative pathway regulation. Tests for paraproteinaemia, complement C3, and C4 are widely available and C3NeF measurement is available in regional or national reference centres. These tests should therefore be performed in all patients. In patients with C3 glomerulopathy who may have Cypriot

ancestry, a genetic test for the $CFHR5_{12123-9}$ mutation should be performed (this test is available at the Institute of Child Health in London, <http://www.labs.gosh.nhs.uk/laboratory-services/genetics/tests/cfhr5-nephropathy>). Additional tests such as serum CFH and CFI levels and tests for autoantibodies against factor B and CFH should be considered if a C3NeF is not identified.

Where these serological tests do not identify a likely cause, additional genetic testing by mutation and copy number variation screening of the genes for complement regulators, including *CFH, MCP, CFHR1-5, CFI, CFB*, and *C3* should be considered, especially if there is a family history of kidney disease. Interpretation of specialized serological and genetic tests is not always straightforward so referral to a centre with experience in this area may be advisable.

Treatment of C3 glomerulopathies

It is generally assumed that blood pressure control and angiotensin system blockade should be introduced to delay progression of renal damage in the presence of hypertension or proteinuria. Probably because of the rarity, slowly progressive course, and heterogeneity of aetiology of C3 glomerulopathies, there are currently no controlled trial data to guide the treatment of these conditions. As with immune complex MPGN, a kidney biopsy showing highly cellular or inflammatory appearances predicts a worse prognosis and may prompt the use of immunosuppressive therapy. Where a pathogenic antibody (e.g. a C3NeF) is detected or suspected, therapeutic strategies aimed at depleting antibody production (e.g. with an anti-CD20 monoclonal antibody, corticosteroids, or mycophenolate mofetil) have been employed, with some anecdotal evidence of success. Plasma exchange has been used to treat patients with a C3NeF, CFH deficiency, and CFHR5 nephropathy, but there are no controlled outcome data from these manoeuvres.

Eculizumab, a humanized monoclonal antibody directed against complement C5, is effective at blocking the terminal complement pathway in humans. Prospective trials showing efficacy in the complement-associated diseases paroxysmal nocturnal haemoglobinuria and atypical haemolytic uraemic syndrome have been published, but reports of its use in DDD and C3GN (in very small numbers of patients) have shown mixed and somewhat disappointing responses (Bomback et al., 2012).

Conclusions

Identification of proliferative GN by kidney biopsy should prompt a search as to the underlying cause. The presence of immunoglobulins suggests underlying increased or aberrant antibody production, which can have a variety of causes, including infection, autoimmune disease, and lymphoproliferative disease. The presence of complement C3 without significant immunoglobulin is diagnostic of a C3 glomerulopathy and suggests an underlying disorder of complement regulation. An approach to differential diagnosis based on histomorphology, immunohistochemistry, and electron microscopy is summarized in Fig. 80.11.

It has long been evident that immunological activation leading to excessive or aberrant antibody production can lead to MPGN. In this context there is evidence of the immunoglobulins and complement C3 in the kidney. Appreciation that in C3 glomerulopathy activation of the alternative complement pathway alone is also sufficient to cause MPGN in the absence of antibody production or deposition suggests that renal complement activation per se is central to the pathogenesis of these diseases, and this has been supported by experiments in animal models.

In this paradigm, MPGN is a consequence of complement activation, resulting from either immunological diseases that lead to the generation of antibody–antigen complexes, or from defects in the regulation of the complement system itself. In clinical practice, determining which process is driving the renal disease is instructive in determining the appropriate therapy: treatments aimed at combating infections or suppressing the adaptive immune system

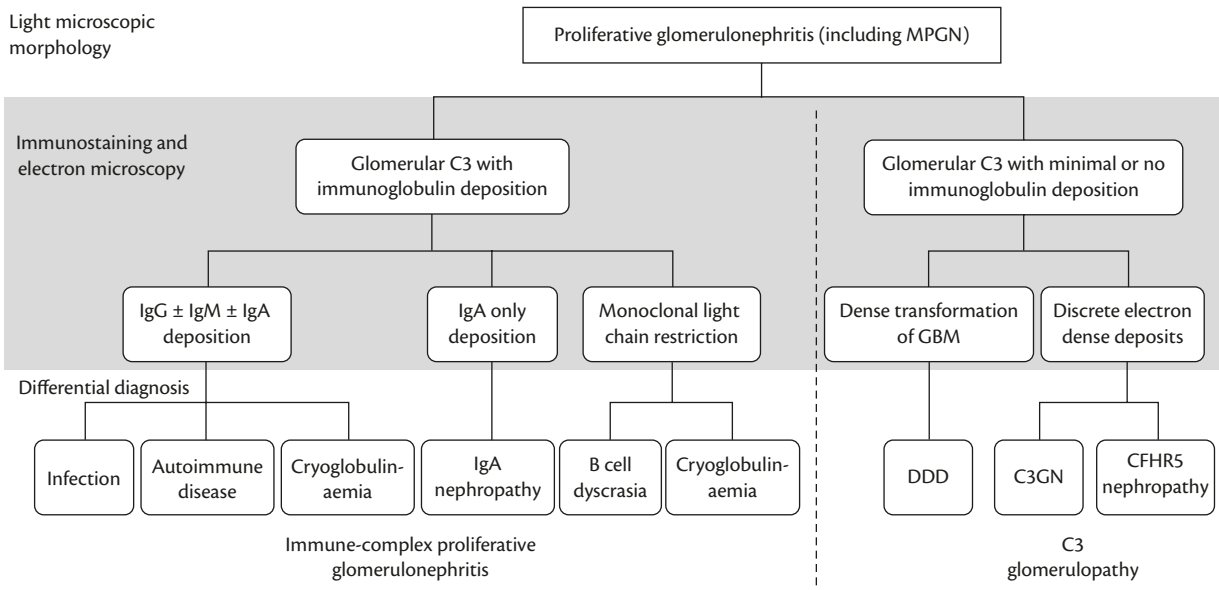

Fig. 80.11 Approach to the diagnosis of proliferative glomerulonephritis. DDD = dense deposit disease; C3GN, C3 glomerulonephritis. Compare the algorithm in Fig. 18.11 which is based on the appearance of basement membrane splitting (double contour).

(e.g. using cytotoxic or antiproliferative agents) are not necessarily effective in restoring normal regulation of complement alternative pathway activity. Currently available treatments are unsatisfactory but it is hoped that therapies, currently under development, that are able to block or modulate C3 activation may be efficacious in both C3 glomerulopathies and immune complex-driven GN.

Further reading

Bomback, A. S. and Appel, G. B. (2012). Pathogenesis of the C3 glomerulopathies and reclassification of MPGN. *Nat Rev Nephrol*, 8(11), 634–42.

Gale, D. P. and Maxwell, P. H. (2013). C3 glomerulonephritis and CFHR5 nephropathy. *Nephrol Dial Transplant*, 28(2), 282–8.

Pickering, M. C., D'Agati, V. D., Nester, C. M., *et al.* (2013). C3 glomerulopathy: consensus report. *Kidney Int*, 84(6), 1079–89.

Sethi, S., Nester, C. M., and Smith, R. J. (2012). Membranoproliferative glomerulonephritis and C3 glomerulopathy: resolving the confusion. *Kidney Int*, 81(5), 434–41.

Smith, R. J., Harris, C. L., and Pickering, M. C. (2011). Dense deposit disease. *Mol Immunol*, 48(14), 1604–10.

References

Abrera-Abeleda, M. A., Nishimura, C., Smith, J. L., *et al.* (2006). Variations in the complement regulatory genes factor H (CFH) and factor H related 5 (CFHR5) are associated with membranoproliferative glomerulonephritis type II (dense deposit disease). *J Med Genet*, 43(7), 582–9.

Athanasiou, Y., Voskarides, K., Gale, D. P., *et al.* (2011). Familial C3 glomerulopathy associated with CFHR5 mutations: clinical characteristics of 91 patients in 16 pedigrees. *Clin J Am Soc Nephrol*, 6(6), 1436–46.

Barsoum, R. S., Sersawy, G., Haddad, S., *et al.* (1988). Hepatic macrophage function in schistosomal glomerulopathy. *Nephrol Dial Transplant*, 3(5), 612–16.

Blyth, C. C., Robertson, P. W., and Rosenberg, A. R. (2007). Post-streptococcal glomerulonephritis in Sydney: a 16-year retrospective review. *J Paediatr Child Health*, 43(6), 446–50.

Bomback, A. S., Smith, R. J., Barile, G. R., *et al.* (2012). Eculizumab for dense deposit disease and C3 glomerulonephritis. *Clin J Am Soc Nephrol*, 7(5), 748–56.

Braun, M. C., Stablein, D. M., Hamiwka, L. A., *et al.* (2005). Recurrence of membranoproliferative glomerulonephritis type II in renal allografts: the North American Pediatric Renal Transplant Cooperative Study experience. *J Am Soc Nephrol*, 16(7), 2225–33.

Chen, Q., Muller, D., Rudolph, B., *et al.* (2011). Combined C3b and factor B autoantibodies and MPGN type II. *N Engl J Med*, 365(24), 2340–2.

Covic, A., Schiller, A., Volovat, C., *et al.* (2006). Epidemiology of renal disease in Romania: a 10 year review of two regional renal biopsy databases. *Nephrol Dial Transplant*, 21(2), 419–24.

Dammacco, F. and Sansonno, D. (2013). Therapy for hepatitis C virus-related cryoglobulinemic vasculitis. *N Engl J Med*, 369(11), 1035–45.

Gale, D. P., de Jorge, E. G., Cook, H. T., *et al.* (2010). Identification of a mutation in complement factor H-related protein 5 in patients of Cypriot origin with glomerulonephritis. *Lancet*, 376(9743), 794–801.

Habib, R., Michielsen, P., de Montera, E., *et al.* (1961). Clinical, microscopic and electron microscopic data in the nephrotic syndrome of unknown origin. In G. E. W. Wolstenholme and M. P. Cameron (eds.) *Ciba Foundation Symposium—Renal Biopsy: Clinical and Pathological Significance*, pp. 70–102. Chichester: John Wiley & Sons Ltd.

Hanko, J. B., Mullan, R. N., O'Rourke, D. M., *et al.* (2009). The changing pattern of adult primary glomerular disease. *Nephrol Dial Transplant*, 24(10), 3050–4.

Licht, C., Heinen, S., Józsi, M., *et al.* (2006). Deletion of Lys224 in regulatory domain 4 of Factor H reveals a novel pathomechanism for dense deposit disease (MPGN II). *Kidney Int*, 70(1), 42–50.

Little, M. A., Dupont, P., Campbell, E., *et al.* (2006). Severity of primary MPGN, rather than MPGN type, determines renal survival and post-transplantation recurrence risk. *Kidney Int*, 69(3), 504–11.

Malik, T. H., Lavin, P. J., Goicoechea de Jorge, E., *et al.* (2012). A hybrid CFHR3-1 gene causes familial C3 glomerulopathy. *J Am Soc Nephrol*, 23(7), 1155–60.

Martinez-Barricarte, R., M. Heurich, Vazquez-Martul, E., *et al.* (2010). Human C3 mutation reveals a mechanism of dense deposit disease pathogenesis and provides insights into complement activation and regulation. *J Clin Invest*, 120(10), 3702–12.

Mathieson, P. W., Wurzner, R., Oliveria, D. B., *et al.* (1993). Complement-mediated adipocyte lysis by nephritic factor sera. *J Exp Med*, 177(6), 1827–31.

Nasr, S. H., Satoskar, A., Markowitz, G. S., *et al.* (2009). Proliferative glomerulonephritis with monoclonal IgG deposits. *J Am Soc Nephrol*, 20(9), 2055–64.

Servais, A., L. Noel, H., Roumenina, L. T., *et al.* (2012). Acquired and genetic complement abnormalities play a critical role in dense deposit disease and other C3 glomerulopathies. *Kidney Int*, 82(4), 454–64.

Sethi, S., Fervenza, F. C., Zhang, Y., *et al.* (2013). Atypical postinfectious glomerulonephritis is associated with abnormalities in the alternative pathway of complement' *Kidney Int*, 83(2), 293–9.

Tortajada, A., Yebenes, H., Abarrategui-Garrido, C., *et al.* (2013). C3 glomerulopathy-associated CFHR1 mutation alters FHR oligomerization and complement regulation. *J Clin Invest*, 123(6), 2434–46.

Walker, P. D., Ferrario, F., Joh, K., *et al.* (2007). Dense deposit disease is not a membranoproliferative glomerulonephritis. *Mod Pathol*, 20(6), 605–16.

Woo, K. T., Chan, C. M., Mooi, C. Y., *et al.* (2010). The changing pattern of primary glomerulonephritis in Singapore and other countries over the past 3 decades. *Clin Nephrol*, 74(5), 372–83.

Zand, L., Kattah, A., Fervenza, F. C., *et al.* (2013). C3 glomerulonephritis associated with monoclonal gammopathy: a case series. *Am J Kidney Dis*, 62(3), 506–14.

CHAPTER 81

Fibrillary and immunotactoid glomerulopathy

Stephen M. Korbet, Melvin M. Schwartz, and Edmund J. Lewis

Introduction

In 1977, Rosenmann and Eliakim reported an unusual glomerular lesion in a 45-year-old woman presenting with the nephrotic syndrome and renal insufficiency (Rosenmann and Eliakim, 1977). Electron microscopy demonstrated electron-dense deposits with a high degree of organization in the form of fibrils which measured 10 nM in diameter. The deposits were associated with mesangial expansion and immune deposits of immunoglobulin (Ig)-G, IgM, and C3 in a mesangial pattern. Congo-red stain of the deposits was negative and there was no clinical or serologic evidence of a systemic disease. The deposits were interpreted to be 'amyloid-like' and it was speculated that they might represent a 'pre-amyloid' state. Shortly thereafter, Schwartz and Lewis (1980) reported a case of a 49-year-old man presenting with the nephrotic syndrome, with no evidence of systemic disease, who had a similar renal lesion: immune aggregates were associated with highly organized electron-dense deposits composed of microtubules. During 7 years of follow-up the patient progressed to renal failure but never demonstrated any clinical or serologic evidence of a systemic disease. In order to distinguish this lesion from other disorders with renal lesions having glomerular immune deposits associated with highly organized microtubular or fibrillary structures such as amyloidosis, cryoglobulinaemia, paraproteinaemias, and systemic lupus erythematosus, the term 'immunotactoid glomerulopathy' (ITG) was introduced, reflecting the immunoglobulin composition (immuno-) and polymeric morphology (tactoid) of the glomerular deposits.

Since these initial reports, > 300 cases of ITG have been reported. Various synonyms have been used to refer to the lesion described in these reports, including fibrillary glomerulonephritis (FGN), non-amyloidotic fibrillary glomerulopathy, amyloid-like glomerulopathy, and amyloid-stain-negative microfibrillary glomerulopathy, but we believe they all represent the same or a similar disease process. The unifying feature in all of the cases is the finding of highly organized ultrastructural deposits that appear to be composed of immunoglobulin and complement and are negative for amyloid by Congo-red stain.

Despite the increasing recognition of this lesion, ITG is an uncommon glomerulopathy found in < 1% of renal biopsies (Korbet et al., 1991; Iskander et al., 1992; Fogo et al., 1993; Pronovost et al., 1996; Brady, 1998; Rosenstock et al., 2003; Nasr et al., 2011). The clinical diagnosis of ITG is applied only after the exclusion of diseases known to be associated with organized glomerular immune deposits including amyloidosis, cryoglobulinaemia, paraproteinaemias, and systemic lupus erythematosus (SLE). Along with ITG, these disorders comprise the family of histopathologic lesions referred to as the 'fibrillary glomerulopathies' (Table 81.1 and Fig. 81.1). The disorders included in this classification are defined histochemically. In this schema, ITG represents one of the non-amyloid, immunoglobulin-mediated fibrillary glomerulopathies of which there is a differential diagnosis with diseases which must be excluded before the diagnosis of ITG is made. Many of the diseases associated with the fibrillary glomerulopathies have specific therapies and prognoses which differ significantly from that of ITG. As a result, it is critical that the clinician use a combined histologic, clinical and serologic approach in reaching the correct diagnosis (Fig. 81.1 and Table 81.2).

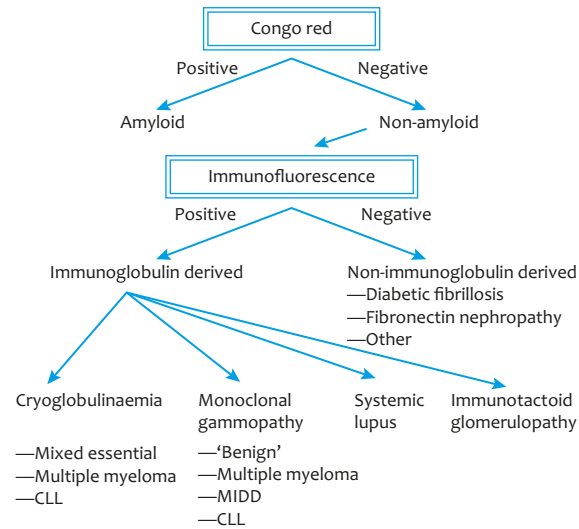

Fig. 81.1 Algorithm for the evaluation of a patient with a fibrillary glomerulopathy. CLL = chronic lymphocytic leukaemia; MIDD = monoclonal deposition disease.

Reproduced with permission from Korbet et al. (1994).

Table 81.1 Classification of the fibrillary glomerulopathies

Amyloid (Congo-red positive)
AL amyloid:
Primary
Multiple myeloma
AA amyloid:
Rheumatic diseases
Chronic suppurative and granulomatous inflammation
Tumours
Familial Mediterranean fever
Non-amyloid (Congo-red negative)
Immunoglobulin-derived fibrils:
Cryoglobulinaemias:
Mixed essential
Multiple myeloma
Chronic lymphocytic leukaemia
Monoclonal gammopathies:
'Benign'
Multiple myeloma
Monoclonal immunoglobulin deposition disease
Chronic lymphocytic leukaemia
Systemic lupus erythematosus
Immunotactoid (fibrillary) glomerulopathy
Non-immunoglobulin-derived fibrils:
Diabetes mellitus (diabetic fibrillosis)
Fibronectin nephropathy
Others

Clinical and laboratory features

Proteinuria is the presenting feature in patients with ITG and on clinical grounds there is nothing unique about the presentation or course of ITG that would allow one to distinguish this disorder from other primary glomerulopathies (Table 81.3). Patients with ITG range in age from 10 to 80 years but on average have been 44–57 years old (Korbet et al., 1991, 1994; Iskander et al., 1992; Pronovost et al., 1996; Brady, 1998; Rosenstock et al., 2003; Nasr et al., 2011). In excess of 90% of the patients are white, and the distribution between men and women is approximately equal. The level of proteinuria at presentation ranges from 0.4–25 g/day, with > 60% having the nephrotic syndrome. Hypertension and microscopic haematuria are common and present in > 65% and > 50% of patients, respectively, and > 45% of patients have some degree of renal insufficiency at the time of diagnosis, indicating the chronic and progressive nature of ITG. In two large reviews of ITG (Pronovost et al., 1996; Rosenstock et al., 2003) there was no difference in the prevalence of hypertension, haematuria, nephrotic syndrome, or renal insufficiency at presentation when the diagnosis of ITG was subdivided based on differences in fibril size (> 30 nM vs ≤ 30 nM) or arrangement (random vs parallel bundles).

The serologic evaluation for cryoglobulins and paraproteins (by immunoelectrophoresis or immunofixation of serum and urine) is, by our definition, negative in ITG and the serum complement levels are generally normal. However, up to 19% of patients have a low-titre of antinuclear antibodies, often in a speckled pattern (Korbet et al., 1985; Iskander et al., 1992; Pronovost et al., 1996; Rosenstock et al., 2003; Nasr et al., 2011). Nonetheless, patients with ITG do not have clinical SLE and in general, have no evidence of a systemic disease process.

ITG appears to represent a primary glomerulopathy as extrarenal manifestations associated with organized immunoglobulin deposits has been described in only four cases (Ozawa et al., 1991; Masson et al., 1992; Wallner et al., 1996; Sabatine et al., 2002). Liver involvement was described in two cases (Ozawa et al., 1991; Wallner et al., 1996) with lung (Masson et al., 1992) and cardiac involvement (Sabatine et al., 2002) found in the remaining two cases. Unlike amyloidosis and other forms of monoclonal immunoglobulin deposition diseases, deposits have not been demonstrated in clinically uninvolved organs studied at autopsy (Korbet et al., 1985; Satoskar et al., 2008).

While the overall prevalence of a lymphoproliferative malignancy in patients with ITG is low (≤ 3%), it has been suggested that lymphoproliferative disorders and dysproteinaemias are more frequently seen in patients whose deposits are comprised of larger (> 30 nM) microtubules (Alpers, 1992, 1993; Fogo et al., 1993; Rosenstock et al., 2003). A study by Pronovost et al. (1996) found that this observation is usually a result of the inclusion criteria used and when patients with paraproteinaemia are excluded, there is no difference in the prevalence of lymphoproliferative disease based on differences in fibril diameter (≤ 3% of patients). Furthermore, patients with ITG rarely go on to develop clinical or serologic evidence of a systemic disease or a dysproteinaemia (Korbet et al., 1991). Thus, the pathogenetic process in ITG primarily involves the glomeruli, which distinguishes the lesion from the other immunoglobulin-derived fibrillary glomerulopathies (Korbet et al., 1994).

Pathology

The primary pathology of ITG is almost exclusively confined to the glomeruli, reflecting the location of the microfibrils in the mesangium and the glomerular capillary walls (Korbet et al., 1991; Schwartz et al., 2002). By light microscopy, mesangial expansion by periodic acid–Schiff (PAS)-positive material with only a mild mesangial hypercellularity is almost always observed (Fig. 81.2). Glomerular capillary wall pathology is also common and may be focal or diffuse, and consists of thickening and complex staining patterns seen with methenamine silver-PAS (Jones) stain, including reticular patterns, spikes, and double contours (Fig. 81.3). In a study of 66 patients, Nasr et al. (2011) found that 71% had a mesangial proliferative, 15% a membranoproliferative, and 2% a membranous pattern of glomerulonephritis by light microscopy. Proliferative glomerulonephritis with cellular and fibrocellular crescents and segmental necrotizing lesions have been described in a few patients (Duffy et al., 1983; Alpers et al., 1987; Iskander et al., 1992; Brady 1998; Rosenstock et al., 2003; Nasr et al., 2011). However, we have not seen these lesions in ITG when systemic diseases such as cryoglobulinaemia, paraproteinaemia, and SLE have been excluded.

Table 81.2 Diagnostic features of fibrillary glomerulopathies

	Primary amyloid	Mixed essential cryoglobulinaemia	Light chain deposition disease	Systemic lupus	Immunotactoid glomerulopathy
Systemic symptoms	75%	75%	75%	100%	0%
Cryoglobulins	0%	100%	0%	50%	0%
Paraproteins	100%	75%	100%	0%	0%
ANA	0%	0%	0%	100%	20%
C3/C4	Normal	Low	Normal	Low	Normal
Microfibril diameter (nM)	8–10	6–62	10–15	8–25	10–49

Most importantly, the glomeruli, tubulointerstitium, and vessels are negative for amyloid by Congo red and thioflavin-T stains. Evaluation of extraglomerular structures demonstrates no specific vascular or tubulointerstitial lesions in ITG.

The principal findings by fluorescence microscopy are the presence of immunoglobulins and complement in a pattern that precisely reflects the glomerular mesangial and capillary wall pathology seen by light microscopy (Fig. 81.4) and the distribution of the fibrils by electron microscopy (Korbet et al., 1991; Schwartz et al., 2002; Rosenstock et al., 2003; Nasr et al., 2011). The capillary wall deposits are either diffuse and coarsely granular or discontinuous and pseudo-linear. Tubular basement membrane deposits have only rarely been described, but interstitial and vascular deposits, as determined by fluorescence microscopy, have not been observed. The immunoglobulin class is IgG in > 90% of cases, and the deposits usually contains both κ and λ light chains (Table 81.4). Despite the absence of a paraproteinaemia, monoclonal immunoglobulin deposits have been seen in approximately 20% of ITG cases studied with light chain antisera, and κ light chain restriction was present in the majority cases, usually in combination with an IgG heavy chain (Korbet et al., 1991; Nasr et al., 2011; Schwartz et al., 2002). Evaluations of IgG subgroups have demonstrated deposits comprised of both IgG1 and IgG4 but IgG2 and IgG3 were absent (Iskander et al., 1992; Rosenstock et al., 2003). Monoclonal IgG3κ was reported in one case, which was not associated with a paraproteinaemia, with 35 nM microtubular deposits (Schwartz and Lewis, 1980).

The ultrastructural appearance of ITG is characterized by the glomerular deposition of extracellular elongated, non-branching microfibrils/microtubules which have neither periodicity nor substructure. The microfibrils are seen in the same locations as the immune deposits seen by immunofluorescence microscopy suggesting that they are comprised of immunoglobulin and complement. Thus, the microfibrils are seen in the mesangium, the primary site of deposition and often also seen in the glomerular capillary wall. The amount of tactoidal material present in the glomerular capillary wall seems to correlate with the extent of glomerular damage. Most commonly, they are present within a thickened basal lamina, but they also are present beneath the epithelial cell where they form large deposits that alternate with projections of basement membrane (spikes). Occasionally, the deposits are seen in the subendothelial space and within the capillary lumen. When fibrils are subepithelial or subendothelial, new layers of basement membrane form over them and incorporate the fibrils into a thickened, irregular capillary wall. Extraglomerular fibrillar deposits have not been described in the interstitium or vasculature but tubular basement membrane involvement has been demonstrated in a few cases of ITG (Duffy et al., 1983; Korbet et al., 1985; Alpers et al., 1987; Korbet et al., 1991; Rosenstock et al., 2003; Nasr et al., 2011).

The size of the fibrils varies, but they are distinguished from amyloid by a larger diameter (Table 81.2). In most series the diameters have a mean value of 18–22 nM (Table 81.5 and Fig. 81.5).

Table 81.3 Presenting clinical features

	Korbet et al. (1991, 1994)	Pronovost et al. (1996)	Rosenstock et al. (2003)	Nasr et al. (2011)
N	62	186	61	66
Age range	10–80	10–81	28–81	19–81
Mean	44 ± 15	57 ± 2	53 ± 12	
Male	61%	47%	39%	45%
White	92%	90%	92%	95%
Hypertension	66%	70%	77%	71%
Proteinuria	100%	100%	100%	100%
Nephrotic	61%	72%	52%	55%
Haematuria	78%	71%	60%	52%
Renal insufficiency	47%	53%	69%	66%

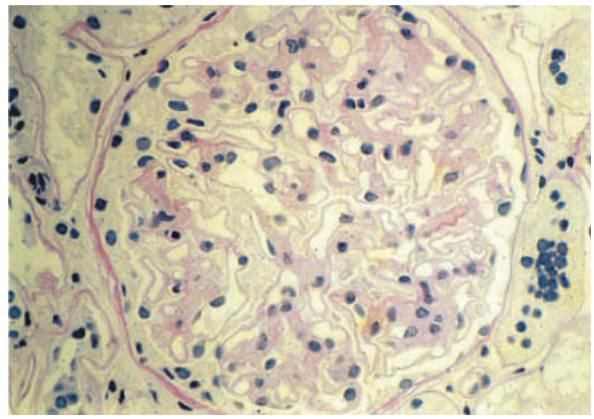

Fig. 81.2 Glomerulus with diffuse increase in mesangial matrix and normal capillary walls (PAS, ×100).

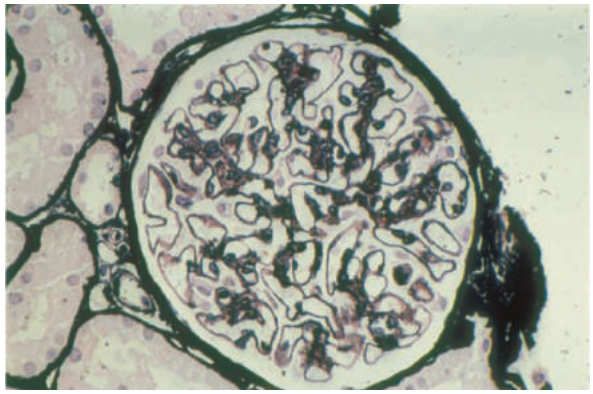

Fig. 81.3 The mesangium is diffusely expanded. The glomerular basement membrane has a complex appearance with diffuse thickening, focal spikes and a reticular pattern (Jones, ×100.)

However, the reported diameters have varied from slightly larger than amyloid (10–12 nM) to as large as 49 nM (Fig. 81.6). Even though there is variability of fibril size among cases, the fibrils in a given case are remarkably consistent in appearance wherever they appear in the glomerulus. The cross-sectional appearance varies from a solid dot to microtubules with either a thin or a thick wall. Examination of the fibrils at high magnification reveals a central core and a wall of varying thickness. Fibrils have a variable length and can appear long and straight or short and curved. They are usually present within a granular, electron-dense matrix suggesting that only part of the deposit is aggregated into fibrils. In most cases the microtubules are randomly arranged on cross-section with various elongated profiles seen in adjacent areas (Fig. 81.5). In other cases, the microtubules appear to be in tightly packed parallel bundles on cross-section, especially with larger fibrils, that have a paracrystalline appearance (Fig. 81.6).

It has been suggested that ITG should be separated into two categories based upon arbitrary ultrastructural criteria regarding fibril size and/or organization. The proponents of subdividing ITG suggest the diagnosis of ITG be reserved for cases with larger (> 30 nM), parallel microtubules, and that FGN be applied to cases with smaller (≤ 30 nM), randomly arranged fibrils (Alpers, 1992; Iskander et al., 1992; Fogo et al., 1993). The rationale used for this subdivision is that the different morphological categories have significant clinical implications (D'Agati et al., 1991; Iskander et al., 1992; Alpers, 1993; Fogo et al., 1993). Presently there is no compelling reason to separately diagnose ITG and FGN on the basis of morphology alone as it has not been demonstrated that the ultrastructural features have significant pathogenetic or clinical implications (Brady, 1998; Pronovost et al., 1996). Thus, we use the diagnosis of ITG to describe patients with both types of deposits, and reserve the term fibrillary glomerulopathy to denote the broader category of diseases (Fig. 81.1) that are characterized morphologically by fibrils seen by electron microscopy without regard to their biochemical composition (Churg and Venkataseshan, 1993; Korbet et al., 1994; Brady, 1998). While there continues to be debate on the issue of classification, what is agreed upon is the importance of distinguishing these patients from amyloidosis and being sure to assess patients for cryoglobulinaemia, a paraproteinaemia, and systemic lupus are these diagnoses carry important therapeutic and prognostic implications (Korbet et al., 2006; Alpers and Kowalewska, 2008).

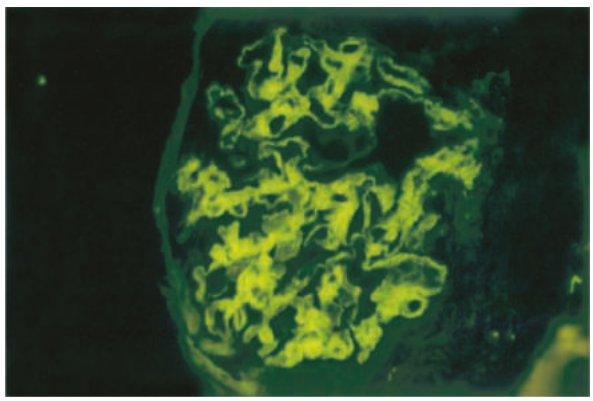

Fig. 81.4 Mesangial and peripheral capillary wall deposits of IgG. (Fluorescein isothiocynate conjugated rabbit anti-human immunoglobulin G (IgG), ×100.)

Table 81.4 Immunofluorescence features

	Schwartz et al. (2002)	Rosenstock et al. (2003)	Nasr et al. (2011)
IgG	94%	96%	100%
IgA	29%	30%	28%
IgM	60%	52%	47%
C3	96%	83%	92%
Kappa or lambda only	19%	3%	11%
Kappa and lambda	72%	96%	84%

Pathogenesis and pathophysiology

The term immunotactoid was chosen to stress the organized orientation of the deposits and their immunoglobulin composition (Korbet et al., 1985). Using immuno-electron microscopy, it has been shown that the fibrils in patients with ITG contain immunoglobulins (both heavy and light chains) and complement as well as amyloid P component but do not contain other amyloid-associated, basement membrane-associated (type IV collagen and heparan-sulphate proteoglycans) or microfibril-associated (fibronectin and fibrillin) proteins (Casanova et al., 1992; Yang et al., 1992). The presence of

Table 81.5 The range of microfibril diameter in ITG

Microfibril diameter (nM)	% of patients
< 12	13%
13–17	11%
18–22	44%
23–27	12%
28–32	14%
> 32	6%

Data from Korbet et al. (1994).

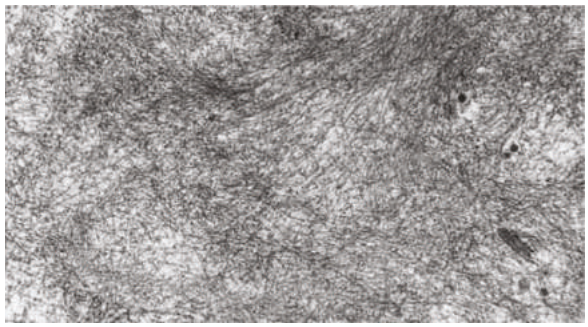

Fig. 81.5 Electron micrograph of glomerular fibrillar deposits showing a random arrangement and measuring 20 nM in diameter. (Uranyl acetate and lead citrate, ×32,000.)

amyloid P component raises the possibility that fibrillogenesis in ITG may be analogous to amyloidosis but without resulting in the critical β-pleated sheet formation.

In the usual physiologic environment, intact normal immunoglobulins do not crystallize readily. In ITG, the propensity to form microtubular structures or tactoids suggests that the deposits are composed of a uniform substructure with strong inter-molecular attraction. Therefore, one can speculate that the formation of immunotactoid deposits is the result of immune complexes having a uniform structure or an abnormal production of monoclonal immunoglobulins which perhaps have an unusual or abnormal structure. These may be produced in such small quantities that they escape detection with standard serologic evaluation as patients with ITG, by definition, do not have evidence of a circulating cryoglobulin or a paraprotein. The deposition of the immunoglobulins within the glomerulus along the filtration surface of the glomerular capillary wall may be a consequence of the unique environment created by the ultrafiltration of plasma (Korbet et al., 1985). The increased concentration of protein occurring along the glomerular capillary as a consequence of ultrafiltration may account for the tendency of the deposits to form exclusively within the kidney.

Structural alterations along the filtration surface of the glomerulus may also be important and predispose to fibril formation. In mice, absence of CD2 associated protein (CD2ap), a protein which binds to nephrin and is important in the function of the podocyte slit diaphragm, results in congenital nephrotic syndrome and glomerular ultrastructural pathology similar to that seen in ITG in humans (Shih et al., 1999; Li et al., 2000; Shaw, 2000; Kim et al., 2003). Thus, glomerular deposits in ITG may result from acquired defects in critical podocyte cellular functions involved in the clearance of filtered and retained immunoglobulin.

Although the cause of ITG is unknown, the heterogeneity of the immunopathology suggests that more than one aetiology is responsible for the production of fibrils with a common morphologic appearance. In this respect it may be similar to amyloid where it is well known that various disease states are capable of producing different proteins which have in common the capacity to form the highly organized beta pleated sheet structure. Since immunotactoids may be composed of either immune complexes or monoclonal proteins which are capable of forming tactoids or microtubules, the variability in the size and orientation of the tactoids from one patient to another, may be a result of concentration or biochemical composition of the protein similar to that described in cryoglobulinaemia. Alternatively, the variability in ultrastructural morphology among patients with ITG may be analogous to the morphologic heterogeneity in haemoglobin S described in the haemoglobinopathy of sickle cell anaemia (Eaton and Hofrichter, 1987). The morphology of deoxygenated haemoglobin S in sickle cell disease is dependent upon the concentration of Hb S and the rate of tactoid formation. Under circumstances where they form slowly, the tactoids are aligned in parallel forming a paracrystalline structure. In contrast, the more rapidly the tactoids are formed the more random the orientation to one another (Eaton and Hofrichter, 1987). Similarly, a patient with ITG has been described with biochemically identical fibrils in the glomeruli and in a serum precipitate that formed after 4 months in cold storage, however, the ultrastructural morphology differed significantly between the fibrils (Rostagno et al., 1996). The fibrils in the glomeruli were 15–20 nM in diameter while those in the serum precipitate were 90 nM. Thus, as in haemoglobin S, the variability in morphology observed in ITG may result from physio-chemical factors involved in fibrillogenesis.

The pathogenesis of ITG may be immunochemically diverse with the unifying feature being the ultrastructural organization of the deposits. In the appropriate setting, immune complexes or immunoglobulins are capable of forming fibrils or microtubules (tactoids) in the glomerular capillary wall or mesangium. Unfortunately, the disease(s) responsible for the production of the immune material in the tactoids of ITG has not been determined in the patients that have been described to date.

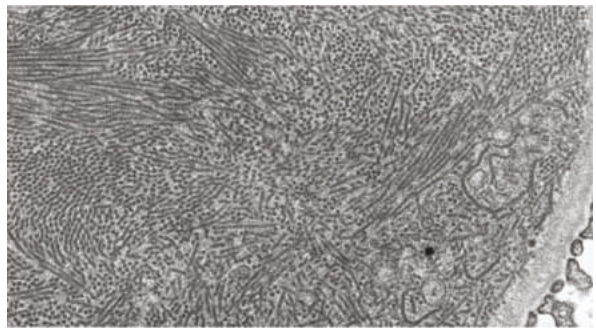

Fig. 81.6 Electron micrograph of glomerular microtubular deposits showing a parallel, packed arrangement and measuring 35 nM in diameter. (Uranyl acetate and lead citrate, ×32,000.)

Prognosis and treatment

The course of patients with ITG is one of progressive renal failure to the requirement of dialysis over 2–5 years in 50% of patients (Korbet et al., 1991; Iskander et al., 1992; Pronovost et al., 1996; Rosenstock et al., 2003; Nasr et al., 2011). The progression to end-stage renal disease (ESRD) is slower in those patients with predominantly mesangial proliferative or membranous lesions and more rapid in those with diffuse proliferative or membranoproliferative glomerular lesions (Rosenstock et al., 2003; Nasr et al., 2011). This is similar to other primary glomerulopathies but is distinct from that of the other fibrillary glomerulopathies (i.e. amyloid and monoclonal immunoglobulin deposition diseases) which experience a more rapid decline to ESRD (Korbet et al., 1994; Korbet and Schwartz, 2006). Clinical features at presentation which portend a poor renal prognosis include older age, hypertension, level of proteinuria,

Table 81.6 Post-transplant course in ITG

Reference	Patients (N)	Follow-up (years)	Recurrence	End-stage renal disease
Alpers et al. (1987)	1	5	1 (5 years)	0
Sturgil et al. (1989)	1	2	0	0
Korbet et al. (1990)	2	5, 6	1 (4.5 years)	1
Fogo et al. (1993)	2	??	0	0
Pronovost et al. (1996)	4	4–11	2 (? years)	1
Carles et al. (2000)	1	4	1 (1.5 years)	0
Samaniego et al. (2000)	4	3–13	2 (2 and 9 years)	1
Rosenstock et al. (2003)	2	4, 8	0	0
Czarnecki et al. (2009)/Nasr et al. (2011)	14	0.5–13	5 (0.25–7 years)	2
Total	31	2–13	12 (39%)	5 (16%)

nephrotic syndrome, and the level of renal insufficiency (Korbet et al., 1985, 1991; Pronovost et al., 1996; Rosenstock et al., 2003; Nasr et al., 2011). Pathologic features which portend a poor renal prognosis include the percentage of globally sclerotic glomeruli, the severity of tubular atrophy and interstitial fibrosis and the extent of glomerular deposits (Korbet et al., 1985; Rosenstock et al., 2003; Nasr et al., 2011).

The response to immunosuppressive treatment in nephrotic ITG patients has generally been poor. The use of prednisone (Dickenmann et al., 2002) and rituximab (Sathyan et al., 2009) has resulted in a remission of the nephrotic syndrome in a small number of patients with predominantly mesangial disease and well preserved renal function. However, the overall experience with treatment of ITG with steroids alone, steroids with immunosuppressive agents, and steroids with plasmapheresis have rarely (< 10% of patients) resulted in clinical remission of proteinuria or altered the progression to ESRD (Schwartz and Lewis, 1980; Alpers et al., 1987; Schifferli et al., 1987; D'Agati et al., 1991; Minami et al., 1997; Kurihara et al., 1998; Rosenstock et al., 2003; Nasr et al., 2011).

The overall patient survival of the patient with ITG is as one might expect with a primary glomerulopathy and no systemic disease. The survival at 1 year is 100% with > 80% of patients alive at 5 years (Korbet et al., 1985, 1991; Nasr et al., 2011)}. As a result, renal transplantation is a treatment consideration for ITG patients with ESRD.

Renal transplantation

The outcome of renal transplantation (Table 81.6) has been reported in 31 ITG patients with 2–13 years of post-transplant follow-up (Alpers et al., 1987; Sturgil et al., 1989; Korbet et al., 1990; Fogo et al., 1993; Carles et al., 2000; Samaniego et al., 2001; Rosenstock et al., 2003; Czarnecki et al., 2009; Nasr et al., 2011). Recurrence of ITG has been demonstrated in 39% of these patients from 0.25 to 9 years after transplantation, and in five cases this resulted in the loss of the graft. In the remaining patients with recurrent disease, renal function continued to be adequate after 5–13 years of follow-up. The rate of deterioration in renal function in patients with recurrent disease has been shown to be slower than with their original disease. One possible explanation could be the effect of immunosuppression (Pronovost et al., 1996). In those patients with recurrent disease, the ultrastructural morphology in the transplants was similar to that originally seen in the native kidneys (Alpers et al., 1987; Korbet et al., 1990; Carles et al., 2000; Samaniego et al., 2001). Thus, while recurrent disease does occur in ITG, it does not inevitably result in graft loss.

References

Alpers, C. E. (1992). Immunotactoid (microtubular) glomerulopathy: an entity distinct from fibrillary glomerulonephritis? *Am J Kidnet Dis*, 19, 185–91.

Alpers, C. E. (1993). Fibrillary glomerulonephritis and immunotactoid glomerulopathy: two entities, not one. *Am J Kidney Dis*, 22, 448–51.

Alpers, C. E. and Kowalewska, J. (2008). Fibrillary glomerulonephritis and immunotactoid glomerulopathy. *J Am Soc Nephrol*, 19(1), 34–7.

Alpers, C. E., Rennke, H. G., Hopper, J., *et al.* (1987). Fibrillary glomerulonephritis: An entity with unusual immunofluorescence features. *Kidney Int*, 31, 781–9.

Brady, H. R. (1998). Fibrillary glomerulopathy [clinical conference]. *Kidney Int*, 53(5), 1421–9.

Carles, X., Rostaing, L., Modesto, A., *et al.* (2000). Successful treatment of recurrence of immunotactoid glomerulopathy in a kidney allograft recipient. *Nephrol Dial Transplant*, 15(6), 897–900.

Casanova, S., Donini, U., Zucchelli, P., *et al.* (1992). Immunohistochemical distinction between amyloidosis and fibrillary glomerulopathy. *Am J Clin Pathol*, 97, 787–95.

Churg, J. and Venkataseshan, S. (1993). Fibrillary glomerulonephritis without immunoglobulin deposits in the kidney. *Kidney Int*, 44, 837–42.

Czarnecki, P. G., Lager, D. J., Leung, N., *et al.* (2009). Long-term outcome of kidney transplantation in patients with fibrillary glomerulonephritis or monoclonal gammopathy with fibrillary deposits. *Kidney Int*, 75(4), 420–7.

D'Agati, V., Sacchi, G., Truong, L., *et al.* (1991). Fibrillary glomerulopathy: defining the disease spectrum. *J Am Soc Nephrol*, 2, 591.

Dickenmann, M., Schaub, S., Nickeleit, V., *et al.* (2002). Fibrillary glomerulonephritis: early diagnosis associated with steroid responsiveness. *Am J Kidney Dis*, 40(3), E9.

Duffy, J. L., Khurana, E., Susin, M., *et al.* (1983). Fibrillary renal deposits and nephritis. *Am J Pathol*, 113, 279–90.

Eaton, W. and Hofrichter, J. (1987). Hemoglobin S gelation and sickle cell disease. *Blood*, 70, 1245–66.

Fogo, A., Qureshi, N., and Horn, R. G. (1993). Morphologic and clinical features of fibrillary glomerulonephritis versus immunotactoid glomerulopathy. *Am J Kidney Dis*, 22(3), 367–77.

Iskander, S. S., Falk, R. J., and Jennette, C. (1992). Clinical and pathological features of fibrillary glomerulonephritis. *Kidney Int*, 42, 1401–7.

Kim, J. M., Wu, H., Green, G., *et al*. (2003). CD2-associated protein haploinsufficiency is linked to glomerular disease susceptibility. *Science*, 300(5623), 1298–300.

Korbet, S. M., Rosenberg, B. F., Schwartz, M. M., *et al*. (1990). Course of renal transplantation in immunotactoid glomerulopathy. *Am J Med*, 89, 91–5.

Korbet, S. M. and Schwartz, M. M. (2006a). Multiple myeloma. *J Am Soc Nephrol*, 17(9), 2533–45.

Korbet, S. M., Schwartz, M. M., and Lewis, E. J. (1991). Immunotactoid glomerulopathy. *Am J Kidney Dis*, 17(3), 247–57.

Korbet, S. M., Schwartz, M. M., and Lewis, E. J. (1994). The fibrillary glomerulopathies. *Am J Kidney Dis*, 23(5), 751–65.

Korbet, S. M., Schwartz, M. M., and Lewis, E. J. (2006). Immuotactoid glomerulopathy (fibrillary glomerulonephritis). *Clin J Am Soc Nephrol*, 1(6), 1351–6.

Korbet, S. M., Schwartz, M. M., Rosenberg, B. F., *et al*. (1985). Immunotactoid glomerulopathy. *Medicine*, 64(4), 228–43.

Kurihara, I., Saito, T., Sato, H., *et al*. (1998). Successful treatment with steroid pulse therapy in a case of immunotactoid glomerulopathy with hypocomplementemia. *Am J Kidney Dis*, 32(1), E4.

Li, C., Ruotsalainen, V., Tryggvason, K., *et al*. (2000). CD2AP is expressed with nephrin in developing podocytes and is found widely in mature kidney and elsewhere. *Am J Physiol Renal Physiol*, 279, F785–92.

Masson, R. G., Rennke, H. G., and Gottlieb, M. N. (1992). Pulmonary hemorrhage in a patient with fibrillary glomerulonephritis. *N Engl J Med*, 326, 36–9.

Minami, J., Ishimitsu, T., Inenaga, T., *et al*. (1997). Immunotactoid glomerulopathy: report of a case. *Am J Kidney Dis*, 30(1), 160–3.

Nasr, S. H., Valeri, A. M., Cornell, L. D., *et al*. (2011). Fibrillary glomerulonephritis: a report of 66 cases from a single institution. *Clin J Am Soc Nephrol*, 6(4), 775–84.

Ozawa, K., Yamabe, H., Fukushi, K., *et al*. (1991). Case report of amyloid-like glomerulopathy with hepatic involvement. *Nephron*, 58, 347–50.

Pronovost, P. H., Brady, H. R., Gunning, M. E., *et al*. (1996). Clinical features, predictors of disease progression and results of renal transplantation in fibrillary/immunotactoid glomerulopathy. *Nephrol Dial Transplant*, 11, 837–42.

Rosenmann, E. and Eliakim, M. (1977). Nephrotic syndrome associated with amyloid-like glomerular deposits. *Nephron*, 18, 301–8.

Rosenstock, J. L., Markowitz, G. S., Valeri, A. M., *et al*. (2003). Fibrillary and immunotactoid glomerulonephritis: Distinct entities with different clinical and pathologic features. *Kidney Int*, 63(4), 1450–61.

Rostagno, A., Vidal, R., Kumar, A., *et al*. (1996). Fibrillary glomerulonephritis related to serum fibrillar immunoglobulin-fibrinectin complexes. *Am J Kidney Dis*, 28, 676–84.

Sabatine, M. S., Aretz, H. T., Fang, L. S., *et al*. (2002). Images in cardiovascular medicine. Fibrillary/immunotactoid glomerulopathy with cardiac involvement. *Circulation*, 105(15), e120–e121.

Samaniego, M., Nadasdy, G. M., Laszik, Z., *et al*. (2001). Outcome of renal transplantation in fibrillary glomerulonephritis. *Clin Nephrol*, 55(2), 159–66.

Sathyan, S., Khan, F. N., and Ranga, K. V. (2009). A case of recurrent immunotactoid glomerulopathy in an allograft treated with rituximab. *Transplant Proc*, 41(9), 3953–5.

Satoskar, A. A., Calomeni, E., Nadasdy, G., *et al*. (2008). Fibrillary glomerulonephritis with splenic involvement: a detailed autopsy study. *Ultrastruct Pathol*, 32(3), 113–21.

Schifferli, J. A., Merot, Y., and Chatelanat, F. (1987). Immunotactoid glomerulopathy with leucocytoclastic skin vasculitis and hypocomplementemia: a case report. *Clin Nephrol*, 27, 151–5.

Schwartz, M. M., Korbet, S. M., and Lewis, E. J. (2002). Immunotactoid glomerulopathy. *J Am Soc Nephrol*, 13(5), 1390–7.

Schwartz, M. M. and Lewis, E. J. (1980). The quarterly case: nephrotic syndrome in a middle-aged man. *Ultrastruct Pathol*, 1, 575–82.

Shaw, A. S. (2000). Congenital nephrotic syndrome in mice lacking CD2-associated protein. *J Am Soc Nephrol*, 11, 19.

Shih, N. Y., Li, J., Karpitskii, V., *et al*. (1999). Congenital nephrotic syndrome in mice lacking CD2-associated protein. *Science*, 286, 312–5.

Sturgil, B. C. and Bolton, W. K. (1989). Non-amyloidotic fibrillary glomerulopathy. *Kidney Int*, 35, 233.

Wallner, M., Prischl, F. C., Hobling, W., *et al*. (1996). Immunotactoid glomerulopathy with extrarenal deposits in the bone, and chronic cholestatic liver disease. *Nephrol Dial Transplant*, 11, (8) 1619–24.

Yang, G. C. H., Nieto, R., Stachura, I., *et al*. (1992). Ultrastructural immunohistochemical localization of polyclonal IgG, C3, and amyloid P component on the Congo red-negative amyloid-like fibrils of fibrillary glomerulopathy. *Am J Pathol*, 141, 409–19.

CHAPTER 82

Drug-induced and toxic glomerulopathies

Alexander Woywodt and Diana Chiu

Introduction

The kidney is frequently the site of injury from drugs and its metabolites, as highly protein-bound substances are unbound and tubular concentrations are often much higher than those seen in plasma or tissues (see Chapter 362). Many offending substances therefore cause damage, primarily to tubular cells, as some concentrated solutes in the tubular fluid reach urinary:plasma concentration ratios in excess of 1000:1. Furthermore, tubular cells host a multitude of transport mechanisms and receptors, which render them even more vulnerable to injury as is illustrated in the role of megalin in aminoglycoside-induced nephrotoxicity. Therefore, drug-induced nephrotoxicity is in its overwhelming majority due to lesions of the tubules and interstitium, followed by drug-induced endothelial and vascular syndromes, such as drug-induced thrombotic microangiopathy.

In comparison, drug-induced glomerulopathy is relatively rare and also much less appreciated by many clinicians. However, the idea that many drugs and substances are capable of inducing glomerular lesions is very old and substances such as the aminonucleoside puromycin or phorbol myristate acetate (PMA) and Adriamycin® (doxorubicin) (Lee and Harris, 2011) have been used for decades to induce glomerular damage in animal models.

A broad variety of histological lesions have been described in drug-induced glomerulopathy (Izzedine et al., 2006). Large-scale studies are lacking but the commonest disease entity seen in our clinical practice is probably minimal change disease (MCD) associated with non-steroidal anti-inflammatory drug (NSAID) use. Drug-induced focal and segmental glomerulosclerosis (FSGS) and membranous GN (MGN) are rare, at least in our practice, although historically MGN associated with gold treatment used to be common while this substance was still in widespread use for rheumatoid arthritis (Hill, 1986). Other forms of drug-induced glomerulopathy are rarer still.

Some drugs are almost exclusively linked to one particular histological lesion, for example, pamidronate, which is associated with the collapsing variant of FSGS. Other substances, such as the NSAIDs, penicillamine, or heroin, are capable of causing a variety of glomerular lesions. Table 82.1 provides an overview of lesions and commonly implicated drugs and toxins. Drug-induced thrombotic microangiopathy (see Chapter 174), glomerular disease due to calcineurin inhibitors (see Chapter 362), and the putative role of hydrocarbons in renal disease associated with antibodies to the glomerular basement membrane (see Chapter 74) are all discussed elsewhere. In the following, we will first discuss common forms of drug-induced glomerulopathy by histological phenotype. We will then review other forms of glomerulopathy, which are associated with individual substances or substance classes that do not fit into any of the histological types of glomerulonephritis discussed already. This will include glomerulopathy due to inhibitors of the mammalian target of rapamycin (mTOR) and due to therapeutic inhibition of vascular endothelial growth factor (VEGF).

Drug-induced minimal change glomerulopathy

MCD is one of the more common forms of drug-induced glomerulopathy and NSAIDs are often implicated, among a variety of other drugs (Table 82.1).

The spectrum of NSAID-induced nephrotoxicity is well characterized. It also includes peripheral oedema, acute interstitial nephritis, papillary necrosis, tubular damage, and acute kidney injury (see Chapter 362). NSAID-induced MCD is well described and some 100 cases have been reported in the literature. In a large series of adult patients with MCD as many as 10% were associated with NSAID use (Warren et al., 1989).

Various mechanisms have been implicated (Izzedine et al., 2006). Interestingly, NSAID can also reduce proteinuria (Alavi et al., 1986) as is illustrated by their historic or last-ditch use in the treatment of nephrotic syndrome (Arisz et al., 1976; Alavi et al., 1986 and see Chapter 52).

All NSAIDS and atypical variant substances, such as piroxicam, or cyclooxygenase (COX)-2 inhibitors seem to be capable of causing MCD (Izzedine et al., 2006). The duration of NSAID use prior to the onset of proteinuria is variable, ranging from 2 weeks to 18 months. In our experience, many patients with NSAID-induced MCD will have used the drug for a considerable period of time. MCD associated with NSAIDs may or may not remit after stopping the offending drug.

Data as to who may benefit from steroid treatment are currently lacking; the optimum dose and duration of treatment are also unclear. Some authors have suggested a 2-month regimen, that is, a shorter treatment than is usually advocated for MCD not associated with NSAID (Izzedine et al., 2006).

The different renal lesions associated with NSAID use are not mutually exclusive. Indeed MCD and acute interstitial nephritis often coexist (Clive and Stoff, 1984; Warren et al., 1989) (Fig. 82.1). The same observation has been described for celecoxib (Alper

Table 82.1 Drug-induced glomerulopathy: histological lesions, and commonly implicated drugs

Lesion	Commonly implicated drug
Minimal change glomerulonephritis (Izzedine et al., 2006)	D-penicillamine (Herve et al., 1980), Gold (Francis et al., 1984; Wolters et al., 1987), interferon alpha (Dizer et al., 2003), interferon beta (Nakao et al., 2002), lithium, mercury (Tang et al., 2006), NSAIDS (Warren et al., 1989; Izzedine et al., 2006), including atypical NSAIDs, such as piroxicam (Fellner, 1985), pamidronate (Barri et al., 2004), and sulfasalazine (Molnar et al., 2010)
Focal and segmental glomerulosclerosis (Izzedine et al., 2006)	Pamidronate (Markowitz et al., 2001; Kunin et al., 2004), probably also alendronate (Pascual et al., 2007), anabolic steroids (Herlitz et al., 2010), interferon alpha, beta, and gamma (Markowitz et al., 2010), sirolimus (Dogan et al., 2011), valproic acid (Ackoundou-N'guessan et al., 2007)
Collapsing variant NOS Perihilar variant Tip lesion	Lithium (Markowitz et al., 2000), heroin (or an adulterant), particularly in black patients (Rao et al., 1974), interferon alpha (Traynor et al., 1994), mTOR inhibitors (Letavernier et al., 2007; Izzedine et al., 2009), norfloxacin (Traynor et al., 1996), toluene (Bosch et al., 1988) Anabolic steroids (Herlitz et al., 2010) NSAID (Sekhon et al., 2005)
Membranous glomerulonephritis (Izzedine et al., 2006)	Adalimumab (den Broeder et al., 2003), aprotinine (Boag et al., 1985), captopril (Hoorntje et al., 1979), celecoxib (Markowitz et al., 2003b), diuretics, gold (Francis et al., 1984; Hall et al., 1987), lithium (Phan et al., 1991), mercury (Li et al., 2010; George, 2011), NSAIDs and COX-2 inhibitors (Markowitz et al., 2003b), penicillamine (Neild et al., 1979), probenecid (Izzedine et al., 2007), methiamazole (Reynolds and Bhathena, 1979), TNF inhibitors (Stokes et al., 2005)
Membranoproliferative glomerulonephritis	Heroin, particularly in Caucasian patients (Jaffe and Kimmel, 2006)
Crescentic glomerulonephritis and vasculitis (Izzedine et al., 2006) ANCA positive (mostly pANCA with MPO specificity) ANCA negative	Allopurinol (Choi et al., 1998), D-penicillamine (Nanke et al., 2000; Bienaime et al., 2007), hydralazine (Dobre et al., 2009; Kalra et al., 2012), minocycline (pANCA with specificities for cathepsin G (Elkayam et al., 1998), elastase (Elkayam et al., 1998), and bactericidal permeability increasing protein (Elkayam et al., 1998) rather than against MPO and anecdotal cases of cANCA with PR3 specificity (Sethi et al., 2003), propylthiouracil (Yu et al., 2007), TNF inhibitors (Stokes et al., 2005), levamisole (Simms et al., 2008; Zwang et al., 2011) Adalimumab (Fournier et al., 2009), foscarnet (Trolliet et al., 1995), isoniazid (Brik et al., 1998), D-penicillamine (Ntoso et al., 1986), penicillin, phenylbutazone (Leung et al., 1985), rifampicin (Ogata et al., 1998), TNF alpha antagonists (Simms et al., 2008), thiazides
Lupus nephritis (all subtypes) (Izzedine et al., 2006)	Alpha-methyldopa, procainamide (Sheikh et al., 1981; McLaughlin et al., 1998), hydralazine (Shapiro et al., 1984), quinidine (Alloway and Salata, 1995), TNF inhibitors (Neradova et al., 2009) and others (Stokes et al., 2007; Chang and Gershwin, 2011).
Others/unclassified forms of glomerulopathy	Chloroquine and hydroxychloroquine (Bracamonte et al., 2001; Woywodt et al., 2007), cocaine (Jaffe et al., 2006), foscarnet (Goldfarb and Coe, 1998; Maurice-Estepa et al., 1998), various herbs and natural products (Blowey, 2005), heroin (Jaffe et al., 2006), mercury (George, 2011), mTOR inhibitors (Bertoni et al., 2009), uranium (Vicente-Vicente et al., 2010), VEGF inhibitors (George et al., 2007; Stokes et al., 2008; Izzedine et al., 2010; Manjunath et al., 2011)

et al., 2002). This is a common clinical pitfall in that the presence of active urinary sediment together with the nephrotic syndrome may tempt the clinician to exclude MCD from the differential diagnosis. Fever, rash, and eosinophilia are usually absent (Izzedine et al., 2006) although in our experience serum immunoglobulin (Ig)-E is often elevated.

Many other drugs are also associated with MCD (Table 82.1). The second most commonly implicated drug is probably lithium, although large studies into the incidence of drug-associated MCD are lacking. Lithium nephropathy usually spares the glomeruli and instead features chronic tubulointerstitial nephritis with tubular cysts, polydipsia/polyuria, and impaired renal concentrating ability (see Chapter 362). In comparison little is known about glomerular damage due to lithium (Markowitz et al., 2000). However, a number of cases of lithium-induced MCD have been reported, often featuring rapid remission of nephrotic syndrome after drug withdrawal (Tam et al., 1996). There are also reports of relapse after reintroduction (Aliabadi et al., 2008). Wood and colleagues in 1989 reviewed nine cases and also reported a favourable outcome after cessation of lithium although two patients required steroid treatment to attain remission (Wood et al., 1989). Of note, FSGS in association with lithium has been reported as well (Markowitz et al., 2000), suggesting that lithium exerts direct effects on podocytes (Markowitz et al., 2000) The mechanism of this effect, however, is as yet controversial and poorly defined. Dai and colleagues demonstrated that lithium causes β-catenin activation, causing podocyte injury (Dai et al., 2009) whereas Tam and co-workers postulated an involvement of the phosphoinositol pathway (Tam et al., 1996).

Drug-induced focal and segmental glomerulosclerosis

FSGS has become a common histological diagnosis in adults with nephrotic syndrome and now represents the most common primary glomerular disease underlying end-stage renal disease (ESRD) in the United States (Kitiyakara et al., 2004). Recent years have seen not only a marked increase in the incidence of FSGS but also a revised classification of the disease (see Chapter 57). Among a variety of secondary forms, the drug-induced variant of FSGS is rare. Pamidronate (Fig. 82.2), lithium, anabolic steroids, and heroin are most commonly implicated (Table 82.1).

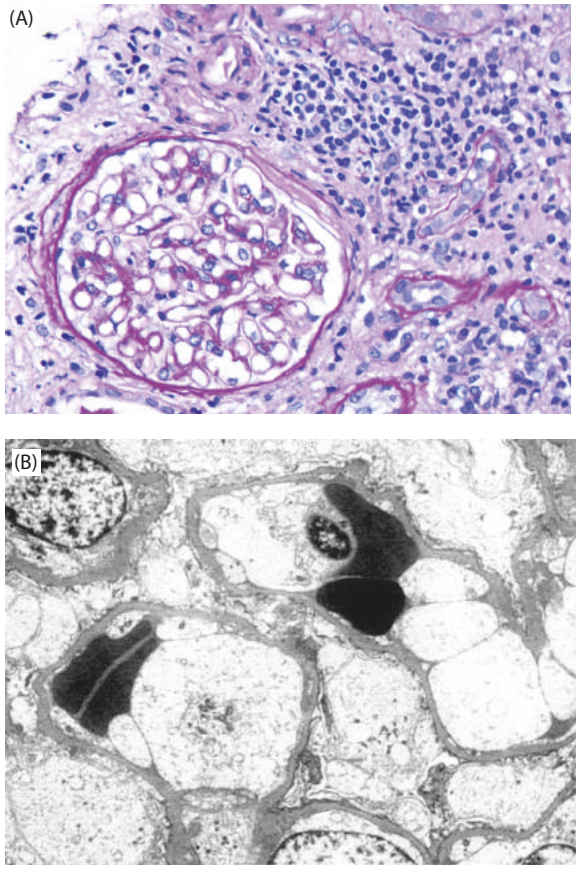

Fig. 82.1 Renal biopsy obtained from a 73-year-old patient who presented with nephrotic syndrome (proteinuria 30 g/L) and renal impairment (serum creatinine 375 µmol/L) after a long history of using various NSAIDs, most recently naproxen. (A) Light microscopy shows normal glomeruli with an infiltrate composed of lymphocytes and occasional eosinophils. (B) Electron microscopy shows widespread fusion of podocyte foot processes. The diagnosis is NSAID-associated minimal change disease with concurrent acute interstitial nephritis. The patient made a full recovery with steroid treatment and renal function returned to normal.
Courtesy of Dr Beena Nair, Department of Pathology, Royal Preston Hospital, and Dr Ajay Dhaygude, Department of Nephrology, Royal Preston Hospital.

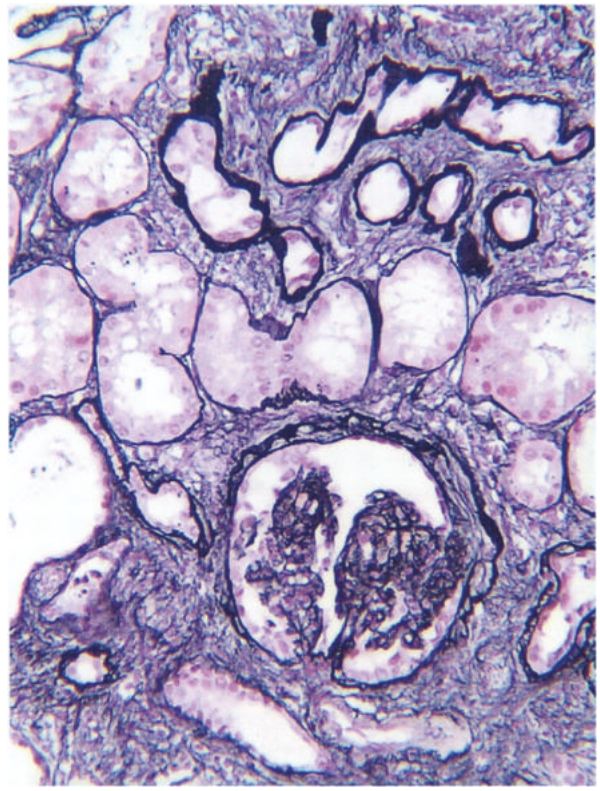

Fig. 82.2 Renal biopsy obtained from a 52-year-old patient with multiple myeloma and pamidronate treatment. The biopsy shows a glomerulus with global collapse of the glomerular tuft with surrounding tubules exhibiting atrophy. Interstitial fibrosis is also present. (Silver methenamine stain ×200.)
From Kunin et al. (2004), with permission.

In 2001, Markowitz and others first reported collapsing-type FSGS in seven patients who had received pamidronate (Markowitz et al., 2001). Of note, MCD has also been described in association with pamidronate (Markowitz et al., 2004). Recovery of nephrotic syndrome is possible after cessation of pamidronate treatment (Desikan et al., 2002) but reversal may be incomplete or even absent in some cases, particularly those with markedly impaired renal function (Izzedine et al., 2006). Progression to ESRD is also seen (Izzedine et al., 2006). It is not clear whether other bisphosphonates are also capable of causing FSGS. Deterioration of pre-existing FSGS in association with alendronate use has been reported (Miura et al., 2009), as well as worsening proteinuria and renal impairment associated with alendronate use in a liver transplant recipient with pre-existing chronic kidney disease (Pascual et al., 2007). Most cases reported so far have occurred in the context of high-dose pamidronate treatment for multiple myeloma (Perazella and Markowitz, 2008). Newer bisphosphonates such as pamidronate differ in mode of action in that they inhibit farnesyl synthase and thereby interfere with guanosine-5'-triphosphate (GTP)-binding proteins. It has been speculated that this difference accounts for the differential effects of pamidronate on the podocyte when compared to older substances from the same class (Markowitz et al., 2001). Of note, bisphosphonates are also capable of causing acute kidney injury through tubular damage (Markowitz et al., 2003). The spectrum of bisphosphonate nephrotoxicity is reviewed in detail elsewhere (Perazella and Markowitz, 2008). Finally, it has to be noted that the true incidence of podocyte injury, proteinuria, and FSGS in association with bisphosphonates remains unclear as long as good data from large trials are lacking (Perazella and Markowitz, 2008).

Lithium is another well-described cause of FSGS and the substance can also cause MCD as discussed above. Previously viewed almost exclusively as a tubulointerstitial disease, lithium nephropathy must now be regarded as a disease that often affects the glomerulus as well (Markowitz et al., 2000). FSGS due to lithium was considered truly rare until Markowitz and colleagues reported FSGS in 50% of 24 renal biopsies taken from patients on lithium treatment (Markowitz et al., 2000). It is difficult to exclude with certainty hyperfiltration due to nephron loss as the causative mechanisms (as opposed to a true effect of the podocyte per se). However, this study reported a high incidence of foot process fusion on electron microscopy, suggesting a direct podocytopathic effect of lithium treatment (Markowitz et al., 2000). Finally, it is worthwhile to note that FSGS and tubulointerstitial changes due to lithium often coexist (Markowitz et al., 2000).

In 2010, Herlitz and co-workers described FSGS in patients who had previously used anabolic steroids as body builders (Herlitz et al., 2010). In this cohort, collapsing variant FSGS was seen in three cases while the perihilar variant was seen in four patients. One patient progressed to ESRD. The authors speculated that secondary FSGS resulted from a combination of post-adaptive glomerular changes driven by increased lean body mass and a potential direct nephrotoxic effect of anabolic steroids (Herlitz et al., 2010). The causality must remain unclear, since FSGS in conjunction with obesity (Kambham et al., 2001) but also in patients with increased muscle mass is well described (Schwimmer et al., 2003).

Drug-induced membranous glomerulonephritis

Secondary membranous glomerulonephritis (MGN) is described in Chapter 63.

Overall, drug-induced MGN is rare: Rihova and colleagues reported 18 such cases in a series of 129 cases of MGN (Rihova et al., 2005). Furthermore, it must be emphasized that causality can be difficult to establish. A broad variety of drugs has been implicated (Table 82.1), particularly captopril (Hoorntje et al., 1979), D-penicillamine (Neild et al., 1979; Hall et al., 1988), gold (Hall et al., 1987), mercury, NSAIDs, and COX-2 inhibitors (Sennesael et al., 1986; Markowitz et al., 2003). Much of the literature relates to gold or penicillamine, neither of which is commonly used today.

Mechanisms remain ill-defined and derive from our current understanding of MGN in general. Here, the antigen may be the drug itself or its components or metabolites or the drug may act as a hapten (Nadasdy et al., 1998). A good example is the case of captopril where it has been suggested that the sulfhydryl moiety of the captopril molecule is involved (Izzedine et al., 2006), either directly or through an immune-mediated mechanism (Kallenberg et al., 1981, 1982). Accordingly, there is no convincing reports in conjunction with the use of other angiotensin-converting enzyme inhibitors.

It has been proposed that the drug-induced variant tends to be mild (Izzedine et al., 2006). Epi-membranous deposits can be small and widely spaced on electron microscopy (Izzedine et al., 2006).

MGN associated with gold (Hall et al., 1987) has now become exceedingly rare as the use of gold in the treatment of rheumatoid arthritis has been largely superseded. But the complication was uncommon. Katz and co-workers detected proteinuria in 3% of 1283 auranofin-treated patients, with four cases of GN (Katz et al., 1984).

Nephropathy due to mercury was described in 1811 by Scottish-American physician William Charles Wells (George, 2011). Exposure in contemporary cases is usually from mercury-containing skin cream (Soo et al., 2003), hair-dying agents, or inhalation of mercury vapour at the workplace. Li and colleagues reported on a series of 11 patients with proteinuria and normal renal function and described MN with a particular pattern of IgG subclass deposition (Li et al., 2010). MCD due to mercury has also been reported (Tang et al., 2006). Withdrawal of the offending substance led to complete recovery in the majority of cases (Li et al., 2010). Generally, it has been suggested that the course of drug-induced MGN is often favourable once the offending agent has been stopped (Izzedine et al., 2006).

Drug-induced crescentic glomerulonephritis and lupus/lupus-like nephritis

Drug-induced crescentic glomerulonephritis (see Chapter 70) is rare. The causality may be difficult to establish, but in general the mechanism is usually via causing a small vessel vasculitis (see Chapter 157). ANCA may be either positive or negative (Table 82.1). Some cases of drug-induced crescentic GN occur in the context of drug-associated systemic vasculitis, which is often associated with ANCA, overwhelmingly with a perinuclear pattern and specificity for myeloperoxidase (MPO) (Table 82.1). The only exception to this rule seems to be minocycline, which is capable of inducing a whole variety of ANCA specificities, such as cathepsin G (Elkayam et al., 1998), elastase (Elkayam et al., 1998), and bactericidal permeability increasing protein (Elkayam et al., 1998). Other cases occur as crescentic glomerulonephritis without systemic involvement. The topic of drug-induced ANCA-associated vasculitis is discussed in detail by Choi and colleagues (Choi et al., 2000). A vigorous accompanying interstitial nephritis is sometimes seen (Abt and Gordon, 1985). The underlying mechanisms remain essentially unknown.

Penicillamine also deserves special attention in that the substance is capable of causing a surprising variety of different renal lesions, including membranous glomerulonephritis, ANCA-negative crescentic GN and, finally, an ANCA-positive systemic vasculitis (Table 82.1). Remission after cessation of the offending drug has been described, as has been successful immunosuppressive treatment in more severe cases (Ntoso et al., 1986). At present, it is therefore difficult to provide any valid guidance for treatment, chiefly because the number of cases overall is so small.

Drug-induced lupus or lupus-like nephritis is an equally uncommon condition and differs in its manifestation from drug-induced vasculitis. The topic of drug-induced lupus is reviewed in detail elsewhere (Chang and Gershwin, 2011). Even among cases with drug-induced lupus, renal involvement is believed to be rare, although good data are lacking and subtle renal involvement may be under-diagnosed.

Drug-induced lupus nephritis is typically associated with only a handful of drugs (Table 82.1). Sheikh and co-workers documented a case of crescentic lupus nephritis in drug-induced lupus due to procainamide (Sheikh et al., 1981). McLaughlin and others reported a similar case, again in association with procainamide. Nephrotic syndrome has been reported as well (Zech et al., 1979). Several cases of lupus nephritis have been described in conjunction with the use of tumour necrosis factor inhibitors in rheumatoid arthritis (Stokes et al., 2005). These agents are capable of inducing vasculitis as well. The clinical spectrum of autoimmune phenomena cause by these agents is reviewed in detail elsewhere (Ramos-Casals et al., 2007).

Other forms of drug-induced glomerular damage associated with individual substances or classes of substances

Chloroquine/hydroxychloroquine-induced storage disorder

Both chloroquine and hydroxychloroquine can cause a rare pseudo-Fabry (see Chapter 335) storage disorder with glomerular involvement. Findings include 'zebra bodies' on electron

microscopy, which can be indistinguishable from those seen in Fabry disease (Fig. 82.3). The lack of extrarenal manifestations of Fabry's or family history should lead to questioning the diagnosis of Fabry Syndrome and scrutiny of the medication (Woywodt et al., 2007). Albay and co-workers suggested criteria to distinguish chloroquine-induced storage disorder from true Fabry disease (Albay et al., 2005). Bracamonte and co-workers review this topic in great detail (Bracamonte et al., 2006).

Glomerulopathy due to foscarnet

Foscarnet is a second-line antiviral drug used for ganciclovir-resistant cytomegalovirus (CMV) infection. Nephrotoxicity through widespread intrarenal deposition of tricalcium or mixed sodium/calcium foscarnet crystals is well described (Goldfarb and Coe, 1998; Deray et al., 1989; Zanetta et al., 1999) and occurs in as many as 20–30% of patients (Wagstaff and Bryson, 1994). Good supportive care, hydration, and dose adjustment are effective for prevention and nephrotoxicity is often reversible (Wagstaff and Bryson, 1994). Tubular toxicity appears to be the predominant mechanisms causing but there is unequivocal evidence of glomerular involvement (Goldfarb and Coe, 1998; Maurice-Estepa et al., 1998). Proteinuria around 1 g/day is therefore common in foscarnet-induced nephrotoxicity. The urinary sediment can be unremarkable but microscopic haematuria has been described as well (Maurice-Estepa et al., 1998). Glomerular capillaries are also involved in the disease process (Beaufils et al., 1990) and crescentic glomerulonephritis has been reported (Trolliet et al., 1995).

Glomerulopathy caused by heavy metals

Several heavy metals, such as cadmium, lead, mercury, and uranium, are well-described nephrotoxins. However, their nephrotoxicity is determined chiefly by their ability to cause tubulointerstitial damage with varying degrees of chronicity. There are two exceptions, membranous glomerulonephritis due to mercury (discussed above) and the glomerulopathy caused by uranium. The nephrotoxicity of uranium is described in detail elsewhere (Arzuaga et al., 2010; Vicente-Vicente et al., 2010; see Chapter 362) but very few cases have been reported. Uranium causes both tubulointerstitial and glomerular damage (Bentley et al., 1985) and a variety of reversible changes of the podocyte foot processes is seen in rats (Kobayashi et al., 1984). Others have suggested that glomerular endothelial cells, rather than podocytes, are the prime target of uranium-induced nephrotoxicity (Avasthi et al., 1980).

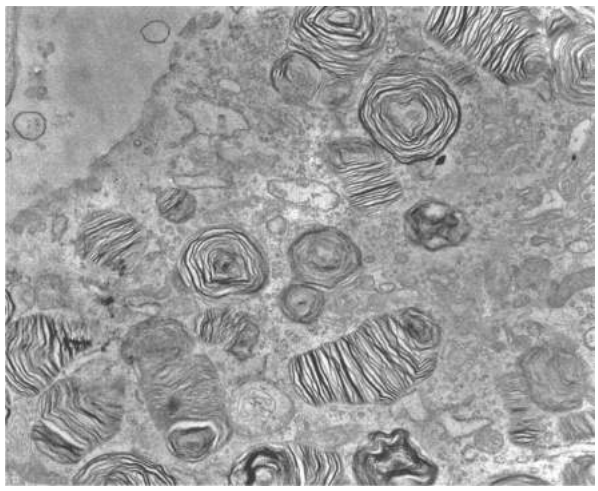

Fig. 82.3 Laminated intra-cytoplasmic inclusions ('zebra bodies') and myelin figures within podocytes in chloroquine-induced storage disorder. (Renal biopsy, transmission electron microscopy, 8000× magnification.)
From Woywodt et al. (2007), with permission.

Glomerulopathy due to opiates and cocaine

Evidence that use of street heroin may be associated with proteinuria and progressive renal failure first emerged in small case series in the 1970s (Rao et al., 1974). Renal biopsy showed FSGS in the majority of cases (Rao et al., 1974). Subsequent reports have emphasized rapid progression to ESRD (Hill, 1986). Other histological lesions have been described as well, particularly immune-complex glomerulonephritis and membranoproliferative glomerulonephritis (MPGN). Renal amyloidosis has been reported in association with subcutaneous injection of heroin ('skin popping') (Hill, 1986).

Some authors have questioned the existence of heroin nephropathy as an entity in its own right (Jaffe and Kimmel, 2006) while others have speculated that additives, rather than heroin itself, may be responsible (Friedman and Tao, 1995). This is supported by the observation that despite continuing use of the drug the incidence of heroin-associated renal failure has decreased markedly since the end of the 1980s (Friedman and Tao, 1995). Opiate use is still associated with ESRD (Perneger et al., 2001), but immune complex glomerulonephritis due to endocarditis and hepatitis C as well as HIV nephropathy and renal amyloidosis are also prevalent in this population.

The use of cocaine, crack and other drugs also shows a weak association with ESRD although robust data are lacking (Jaffe and Kimmel, 2006). A variety of histological lesions has been described in cocaine users, including accelerated vascular nephropathy, glomerulosclerosis and tubulointerstitial damage (Jaffe and Kimmel, 2006). Cocaine is also capable of causing renal infarction through vasospasm (Madhrira et al., 2009) as well as interstitial nephritis (Wojciechowski et al., 2008).

Glomerulopathy due to mTOR inhibitors

mTOR is a highly conserved serine/threonine kinase, which controls cell growth and metabolism in response to nutrients, growth factors, cellular energy, and stress. mTOR inhibitors are sometimes used in renal transplantation, either in transplant recipients with malignancy or in those with chronic allograft nephropathy (see Chapter 286). The fact that these substances cause proteinuria is now well appreciated (Rangan, 2006) although both the clinical course of this disorder and its mechanisms remain ill-defined. Studies by Letavernier and co-workers (Letavernier et al., 2005) and by Ruiz and colleagues (Ruiz et al., 2006) provided first robust evidence of proteinuria in renal transplant recipients converted to sirolimus. Proteinuria can be substantial and reach the subnephrotic and even nephrotic range in some cases (Perlman et al., 2007). Some authors have proposed predictive factors, such as pre-conversion proteinuria and blood pressure (Padiyar et al., 2010). The effect is usually reversible after withdrawal of the offending drug (Perlman et al., 2007). Histological correlates of this entity remain particularly ill-defined although FSGS (Straathof-Galema et al., 2006; Franco et al., 2007; Letavernier et al., 2007) and its collapsing variant (Dogan et al., 2011) have been described. Others have proposed a

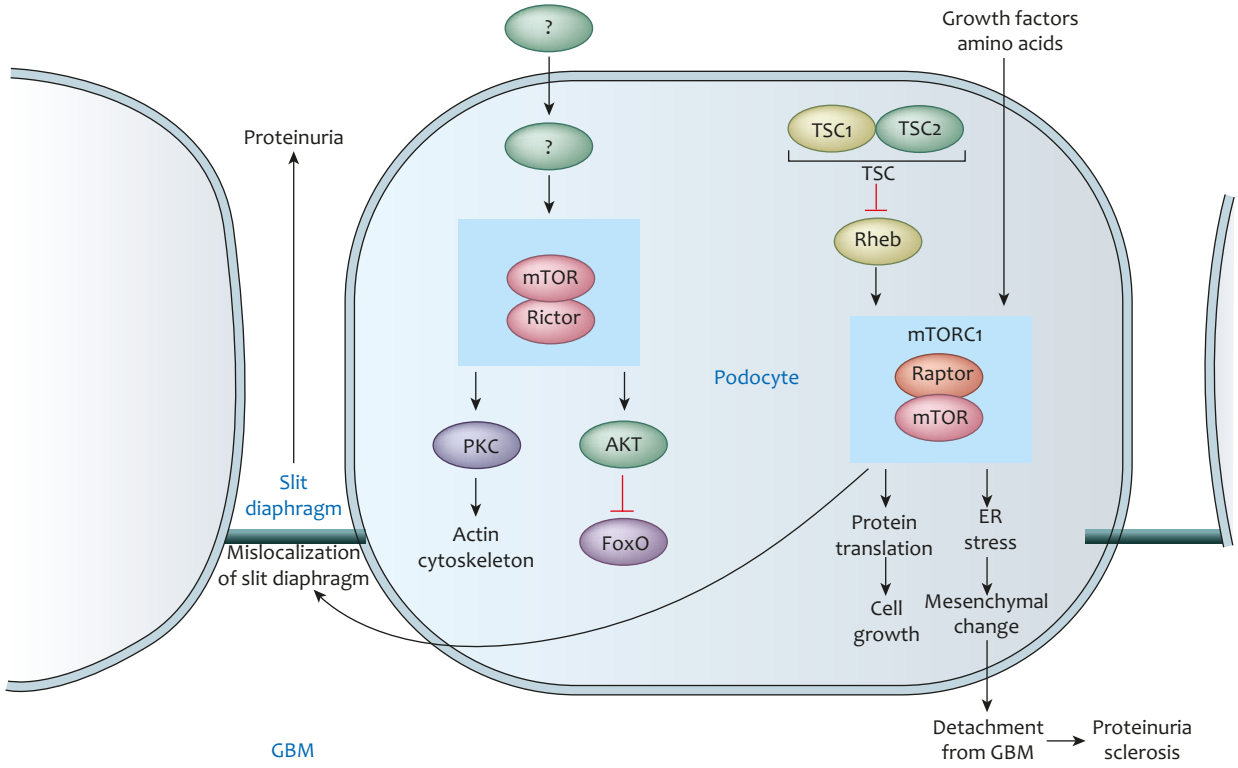

Fig. 82.4 Putative mechanisms of glomerulopathy due to mTOR inhibitors. Podocyte integrity depends on careful balance of the two mTOR complexes, mTORC1 and 2. Raptor and rictor are the co-factors complexed in mTORC1 and 2, respectively. Growth factors and amino acids induce mTORC1 activity, their counterparts for mTORC2 activation are unknown. Activation of mTORC1, as seen in diabetic nephropathy, can lead to a variety of changes, including a more mesenchymal phenotype, detachment from the basement membrane, proteinuria, and sclerosis. Conversely, inhibition of mTORC1, can also cause podocyte injury. Added mTORC2 inhibition causes more severe podocyte injury. Sirolimus is capable of inhibiting both mTORC1 and 2, and of disturbing the mTORC1/2 balance.
From Fogo (2011), with permission.

tubular mechanism (Lieberthal et al., 2001; Straathof-Galema et al., 2006) although it is difficult to understand how a tubular mechanism should cause nephrotic-range proteinuria (Letavernier et al., 2005). Sirolimus is capable of inducing proteinuria not only in renal transplant recipients but also in native kidneys as described in the context of bone marrow (Jhaveri et al., 2008) and cardiac transplantation (Aliabadi et al., 2008).

Proteinuria appears to be a class effect of all mTOR inhibitors and is also seen with everolimus, both in animal models (Vogelbacher et al., 2007), and in humans (Bertoni et al., 2009) and with temsirolimus (Izzedine et al., 2009). Some authors report a decrease in proteinuria after conversion from sirolimus to everolimus (Neau-Cransac et al., 2009), but this is difficult to assess.

Possible mechanisms include interference with VEGF synthesis in podocytes. But they also decrease *Akt* phosphorylation *in vivo*, in parallel with changes of cytoskeleton and cell phenotype (Letavernier et al., 2009). More recently, Stallone and others confirmed a dose-dependent effect of mTOR inhibitors on key podocyte structures (Stallone et al., 2011). Recent studies by Inoki et al. (2011) and Goedel et al. (2011) have also underscored the crucial role of the mTOR pathway in podocytes. It is now believed that podocyte maintenance is dependent on a fine-tuned balance of two mTOR complexes, mTORC1 and mTORC2 (Fogo, 2011) and that this balance can be disturbed by mTOR inhibitors (Fig. 82.4). There may also be a genetic basis for the susceptibility to the effects of mTOR inhibitors on podocytes (Fogo, 2011).

Glomerulopathy due to VEGF inhibition

Glomerulopathy has been repeatedly described in association with inhibitors of vascular endothelial growth factor (VEGF) (Izzedine et al., 2010). George and colleagues, in 2007, first reported nephrotic syndrome in a patient treated with Bevacizumab for pancreatic adenocarcinoma (George et al., 2007). Renal biopsy showed focal glomerulonephritis with immune complex deposition (George et al., 2007). In a meta-analysis of published cancer trials with Bevacizumab, Wu and co-workers reported that treatment increases the risk of severe proteinuria (Wu et al., 2010). The renal histology in published cases appears to be heterogenous and includes cryoglobulinaemic glomerulonephritis (Johnson et al., 2004) collapsing glomerulopathy (Miller et al., 2005), and immune complex-associated focal proliferative glomerulonephritis (George et al., 2007). VEGF inhibition has also been associated with thrombotic microangiopathy (Eremina et al., 2008). Electron microscopy showed endothelial injury in these cases (Eremina et al., 2008). Other VEGF-signalling blockers (axitinib, sorafenib, sunitinib, VEGF-Trap) have also been associated with severe proteinuria (Wu et al., 2010). An algorithm for management of proteinuria due to VEGF inhibition has been suggested elsewhere (Izzedine

et al., 2010). Manjunath and colleagues very recently reported proteinuria in a patient treated with Vargatef®, an investigational agent that blocks not only VEGF but also platelet-derived growth factor receptor (PDGF) and fibroblast growth factor (FGF) receptor (Manjunath et al., 2011). Renal biopsy showed cytoplasmic vacuoles with osmiophilic material in the podocytes, mesangial and endothelial cells (Manjunath et al., 2011). It is possible that concomitant interference with the PDGF and FGF pathway accounted for the distinct histological phenotype in this case.

The putative mechanisms of this peculiar disorder have been the focus of considerable scientific interest. It is well known that VEGF is produced by podocytes and that glomerular endothelial cells possess VEGF receptors (Eremina et al., 2003). More recent evidence has further emphasized the crucial role of VEGF in crosstalk between glomerular endothelial cells and podocytes as reviewed in great detail elsewhere (Fogo and Kon, 2010). In animal models, VEGF inhibition causes glomerular endothelial cell detachment and hypertrophy, in association with downregulation of nephrin (vet al., 2003). Some have compared renal injury due to VEGF inhibition with pre-eclampsia (see Chapter 298), with which it has multiple similarities (Muller-Deile and Schiffer, 2011) where increased sFLT-1 receptor is believed to lead to low levels of free VEGF, with consecutive glomerular damage (Maynard et al., 2003).

References

Abt, A. B. and Gordon, J. A. (1985). Drug-induced interstitial nephritis. Coexistence with glomerular disease. *Arch Intern Med*, 145, 1063–7.

Ackoundou-N'guessan, C., Canaud, B., Leray-Moragues, H., *et al*. (2007). Collapsing focal segmental glomerulosclerosis as a possible complication of valproic acid. *S Afr Med J*, 97, 388, 90.

Alavi, N., Lianos, E. A., Venuto, R. C., *et al*. (1986). Reduction of proteinuria by indomethacin in patients with nephrotic syndrome. *Am J Kidney Dis*, 8, 397–403.

Albay, D., Adler, S. G., Philipose, J., *et al*. (2005). Chloroquine-induced lipidosis mimicking Fabry disease. *Mod Pathol*, 18, 733–8.

Aliabadi, A. Z., Pohanka, E., Seebacher, G., *et al*. (2008). Development of proteinuria after switch to sirolimus-based immunosuppression in long-term cardiac transplant patients. *Am J Transplant*, 8, 854–61.

Alloway, J. A. and Salata, M. P. (1995). Quinidine-induced rheumatic syndromes. *Semin Arthritis Rheum*, 24, 315–22.

Alper, A. B., Jr., Meleg-Smith, S., and Krane, N. K. (2002). Nephrotic syndrome and interstitial nephritis associated with celecoxib. *Am J Kidney Dis*, 40, 1086–90.

Arisz, L., Donker, A. J., Brentjens, J. R., *et al*. (1976). The effect of indomethacin on proteinuria and kidney function in the nephrotic syndrome. *Acta Med Scand*, 199, 121–5.

Arzuaga, X., Rieth, S. H., Bathija, A., *et al*. (2010). Renal effects of exposure to natural and depleted uranium: a review of the epidemiologic and experimental data. *J Toxicol Environ Health B Crit Rev*, 13, 527–45.

Avasthi, P. S., Evan, A. P., and Hay, D. (1980). Glomerular endothelial cells in uranyl nitrate-induced acute renal failure in rats. *J Clin Invest*, 65, 121–7.

Barri, Y. M., Munshi, N. C., Sukumalchantra, S., *et al*. (2004). Podocyte injury associated glomerulopathies induced by pamidronate. *Kidney Int*, 65, 634–41.

Beaufils, H., Deray, G., Katlama, C., *et al*. (1990). Foscarnet and crystals in glomerular capillary lumens. *Lancet*, 336, 755.

Bentley, K. W., Stockwell, D. R., Britt, K. A., *et al*. (1985). Transient proteinuria and aminoaciduria in rodents following uranium intoxication. *Bull Environ Contam Toxicol*, 34, 407–16.

Bertoni, E., Bruschi, M., Candiano, G., *et al*. (2009). Posttransplant proteinuria associated with everolimus. *Transplant Proc*, 41, 1216–17.

Bienaime, F., Clerbaux, G., Plaisier, E., *et al*. (2007). D-Penicillamine-induced ANCA-associated crescentic glomerulonephritis in Wilson disease. *Am J Kidney Dis*, 50, 821–5.

Blowey, D. L. (2005). Nephrotoxicity of over-the-counter analgesics, natural medicines, and illicit drugs. *Adolesc Med Clin*, 16, 31–43, x.

Boag, F., Chappell, M., Beckett, A. G., *et al*. (1985). Lipohypertrophy and glomerulonephritis after the use of aprotinin in an insulin-dependent diabetic. *N Engl J Med*, 312, 245–6.

Bosch, X., Campistol, J. M., Montoliu, J., *et al*. (1988). Myelofibrosis and focal segmental glomerulosclerosis associated with toluene poisoning. *Hum Toxicol*, 7, 357–61.

Bracamonte, E. R., Kowalewska, J., Starr, J., *et al*. (2006). Iatrogenic phospholipidosis mimicking Fabry disease. *Am J Kidney Dis*, 48, 844–50.

Brik, R., Magen, D., Ben-Yzhak, O., *et al*. (1998). Isoniazid-induced crescentic glomerulonephritis in a child with a positive tuberculin skin test. *Am J Nephrol*, 18, 430–2.

Chang, C. and Gershwin, M. E. (2011). Drug-induced lupus erythematosus: incidence, management and prevention. *Drug Saf*, 34, 357–74.

Choi, H. K., Merkel, P. A., and Niles, J. L. (1998). ANCA-positive vasculitis associated with allopurinol therapy. *Clin Exp Rheumatol*, 16, 743–4.

Choi, H. K., Merkel, P. A., Walker, A. M., *et al*. (2000). Drug-associated antineutrophil cytoplasmic antibody-positive vasculitis: prevalence among patients with high titers of antimyeloperoxidase antibodies. *Arthritis Rheum*, 43, 405–13.

Clive, D. M. and Stoff, J. S. (1984). Renal syndromes associated with nonsteroidal antiinflammatory drugs. *N Engl J Med*, 310, 563–72.

Dai, C., Stolz, D. B., Kiss, L. P., *et al*. (2009). Wnt/beta-catenin signaling promotes podocyte dysfunction and albuminuria. *J Am Soc Nephrol*, 20, 1997–2008.

Den Broeder, A. A., Assmann, K. J., van Riel, P. L., *et al*. (2003). Nephrotic syndrome as a complication of anti-TNFalpha in a patient with rheumatoid arthritis. *Neth J Med*, 61, 137–41.

Deray, G., Martinez, F., Katlama, C., *et al*. (1989). Foscarnet nephrotoxicity: mechanism, incidence and prevention. *Am J Nephrol*, 9, 316–21.

Desikan, R., Veksler, Y., Raza, S., *et al*. (2002). Nephrotic proteinuria associated with high-dose pamidronate in multiple myeloma. *Br J Haematol*, 119, 496–9.

Dizer, U., Beker, C. M., Yavuz, I., *et al*. (2003). Minimal change disease in a patient receiving IFN-alpha therapy for chronic hepatitis C virus infection. *J Interferon Cytokine Res*, 23, 51–4.

Dobre, M., Wish, J., and Negrea, L. (2009). Hydralazine-induced ANCA-positive pauci-immune glomerulonephritis: a case report and literature review. *Ren Fail*, 31, 745–8.

Dogan, E., Ghanta, M., and Tanriover, B. (2011). Collapsing glomerulopathy in a renal transplant recipient: potential molecular mechanisms. *Ann Transplant*, 16, 113–16.

Elkayam, O., Levartovsky, D., Brautbar, C., *et al*. (1998). Clinical and immunological study of 7 patients with minocycline-induced autoimmune phenomena. *Am J Med*, 105, 484–7.

Eremina, V., Jefferson, J. A., Kowalewska, J., *et al*. (2008). VEGF inhibition and renal thrombotic microangiopathy. *N Engl J Med*, 358, 1129–36.

Eremina, V., Sood, M., Haigh, J., *et al*. (2003). Glomerular-specific alterations of VEGF-A expression lead to distinct congenital and acquired renal diseases. *J Clin Invest*, 111, 707–16.

Fellner, S. K. (1985). Piroxicam-induced acute interstitial nephritis and minimal-change nephrotic syndrome. *Am J Nephrol*, 5, 142–3.

Fogo, A. B. and Kon, V. (2010). The glomerulus—a view from the inside—the endothelial cell. *Int J Biochem Cell Biol*, 42, 1388–97.

Fogo, A. B. (2011). The targeted podocyte. *J Clin Invest*, 121, 2142–5.

Fournier, A., Nony, A., and Rifard, K. (2009). [Antineutrophil cytoplasmic antibody associated vasculitis in a patient treated with adalimumab for a rheumatoid arthritis]. *Nephrol Ther*, 5, 652–7.

Francis, K. L., Jenis, E. H., Jensen, G. E., *et al*. (1984). Gold-associated nephropathy. *Arch Pathol Lab Med*, 108, 234–8.

Franco, A. F., Martini, D., Abensur, H., *et al*. (2007). Proteinuria in transplant patients associated with sirolimus. *Transplant Proc*, 39, 449–52.

Friedman, E. A. and Tao, T. K. (1995). Disappearance of uremia due to heroin-associated nephropathy. *Am J Kidney Dis*, 25, 689–93.

George, B. A., Zhou, X. J., and Toto, R. (2007). Nephrotic syndrome after bevacizumab: case report and literature review. *Am J Kidney Dis*, 49, e23–9.

George, C. R. (2011). Mercury and the kidney. *J Nephrol*, 24 Suppl 17, S126–32.

Gödel, M., Hartleben, B., Herbach, N., *et al.* (2011). Role of mTOR in podocyte function and diabetic nephropathy in humans and mice. *J Clin Invest*, 121, 2197–209.

Goldfarb, D. S. and Coe, F. L. (1998). Foscarnet crystal deposition and renal failure. *Am J Kidney Dis*, 32, 519–20.

Hall, C. L., Fothergill, N. J., Blackwell, M. M., *et al.* (1987). The natural course of gold nephropathy: long term study of 21 patients. *Br Med J (Clin Res Ed)*, 295, 745–8.

Hall, C. L., Jawad, S., Harrison, P. R., *et al*. (1988). Natural course of penicillamine nephropathy: a long term study of 33 patients. *Br Med J (Clin Res Ed)*, 296, 1083–6.

Hanson, B., D'Hondt, A., Depierreux, M., *et al.* (1996). Nephrotic Syndrome after Norfloxacin. *Nephron*, 74, 446.

Herlitz, L. C., Markowitz, G. S., Farris, A. B., *et al*. (2010). Development of focal segmental glomerulosclerosis after anabolic steroid abuse. *J Am Soc Nephrol*, 21, 163–72.

Herve, J. P., Leguy, P., Cledes, J., *et al.* (1980). [Nephrotic syndrome with minimal glomerular lesions during treatment with D-penicillamine]. *Nouv Presse Med*, 9, 2847.

Hill, G. S. (1986). Drug-associated glomerulopathies. *Toxicol Pathol*, 14, 37–44.

Hoorntje, S. J., Weening, J. J., Kallenberg, C. G., *et al*. (1979). Serum-sickness-like syndrome with membranous glomerulopathy in patient on captopril. *Lancet*, 2, 1297.

Inoki, K., Mori, H., Wang, J., *et al*. (2011). mTORC1 activation in podocytes is a critical step in the development of diabetic nephropathy in mice. *J Clin Invest*, 121, 2181–96.

Izzedine, H., Boostandoot, E., Spano, J. P., *et al.* (2009). Temsirolimus-induced glomerulopathy. *Oncology*, 76, 170–2.

Izzedine, H., Brocheriou, I., Becart, J., *et al*. (2007). Probenecid-induced membranous nephropathy. *Nephrol Dial Transplant*, 22, 2405–6.

Izzedine, H., Launay-Vacher, V., Bourry, E., *et al*. (2006). Drug-induced glomerulopathies. *Exp Opin Drug Saf*, 5, 95–106.

Izzedine, H., Massard, C., Spano, J. P., *et al*. (2010). VEGF signalling inhibition-induced proteinuria: Mechanisms, significance and management. *Eur J Cancer*, 46, 439–48.

Jaffe, J. A. and Kimmel, P. L. (2006). Chronic nephropathies of cocaine and heroin abuse: a critical review. Clin *J Am Soc Nephrol*, 1, 655–67.

Jhaveri, K. D., Schatz, J. H., Young, J. W., *et al.* (2008). Sirolimus (rapamycin) induced proteinuria in a patient undergoing allogeneic hematopoietic stem cell transplant. *Transplantation*, 86, 180–1.

Johnson, D. H., Fehrenbacher, L., Novotny, W. F., *et al.* (2004). Randomized phase II trial comparing bevacizumab plus carboplatin and paclitaxel with carboplatin and paclitaxel alone in previously untreated locally advanced or metastatic non-small-cell lung cancer. *J Clin Oncol*, 22, 2184–91.

Kallenberg, C. G., Hoorntje, S. J., Smit, A. J., *et al.* (1982). Antinuclear and antinative DNA antibodies during captopril treatment. *Acta Med Scand*, 211, 297–300.

Kallenberg, C. G., van der Laan, S., and de Zeeuw, D. (1981). Captopril and the immune system. *Lancet*, 2, 92.

Kalra, A., Yokogawa, N., Raja, H., *et al.* (2010). Hydralazine-induced pulmonary-renal syndrome: a case report. *Am J Ther*, 19(4), e136–8.

Kambham, N., Markowitz, G. S., Valeri, A. M., *et al*. (2001). Obesity-related glomerulopathy: an emerging epidemic. *Kidney Int*, 59, 1498–509.

Katz, W. A., Blodgett, R. C., Jr., and Pietrusko, R. G. (1984). Proteinuria in gold-treated rheumatoid arthritis. *Ann Intern Med*, 101, 176–9.

Kitiyakara, C., Eggers, P., and Kopp, J. B. (2004). Twenty-one-year trend in ESRD due to focal segmental glomerulosclerosis in the United States. *Am J Kidney Dis*, 44, 815–25.

Kobayashi, S., Nagase, M., Honda, N., *et al*. (1984). Glomerular alterations in uranyl acetate-induced acute renal failure in rabbits. *Kidney Int*, 26, 808–15.

Kunin, M., Kopolovic, J., Avigdor, A., *et al*. (2004). Collapsing glomerulopathy induced by long-term treatment with standard-dose pamidronate in a myeloma patient. *Nephrol Dial Transplant*, 19, 723–6.

Kunin, M., Kopolovic, J., Avigdor, A., *et al*. (2004). Collapsing glomerulopathy induced by long-term treatment with standard-dose pamidronate in a myeloma patient. *Nephrol Dial Transplant*, 19, 723–6.

Lee, V. W. and Harris, D. C. (2011). Adriamycin nephropathy: a model of focal segmental glomerulosclerosis. *Nephrology (Carlton)*, 16, 30–8.

Letavernier, E., Bruneval, P., Mandet, C., *et al*. (2007). High sirolimus levels may induce focal segmental glomerulosclerosis de novo. *Clin J Am Soc Nephrol*, 2, 326–33.

Letavernier, E., Bruneval, P., Vandermeersch, S., *et al*. (2009). Sirolimus interacts with pathways essential for podocyte integrity. *Nephrol Dial Transplant*, 24, 630–8.

Letavernier, E., Pe'raldi, M. N., Pariente, A., *et al*. (2005). Proteinuria following a switch from calcineurin inhibitors to sirolimus. *Transplantation*, 80, 1198–203.

Leung, A. C., McLay, A., Dobbie, J. W., *et al.* (1985). Phenylbutazone-induced systemic vasculitis with crescentic glomerulonephritis. *Arch Intern Med*, 1985; 145, 685–7.

Li, S. J., Zhang, S. H., Chen, H. P., *et al*. (2010). Mercury-induced membranous nephropathy: clinical and pathological features. Clin *J Am Soc Nephrol*, 5, 439–44.

Lieberthal, W., Fuhro, R., Andry, C. C., *et al.* (2001). Rapamycin impairs recovery from acute renal failure: role of cell-cycle arrest and apoptosis of tubular cells. *Am J Physiol Renal Physiol*, 281, F693–706.

Madhrira, M. M., Mohan, S., Markowitz, G. S., *et al.* (2009). Acute bilateral renal infarction secondary to cocaine-induced vasospasm. *Kidney Int*, 76, 576–80.

Manjunath, V., Moeckel, G. W., Mahnensmith, R., *et al.* (2011). Proteinuria and glomerular injury associated with the anti-angiogenesis drug Vargatef™. *NDT Plus*, 4(6), 430–3.

Markowitz, G. S., Appel, G. B., Fine, P. L., *et al.* (2001). Collapsing focal segmental glomerulosclerosis following treatment with high-dose pamidronate. *J Am Soc Nephrol*, 12, 1164–72.

Markowitz, G. S., Falkowitz, D. C., Isom, R., *et al.* (2003b). Membranous glomerulopathy and acute interstitial nephritis following treatment with celecoxib. *Clin Nephrol*, 59, 137–42.

Markowitz, G. S., Fine, P. L., Stack, J. I., *et al.* (2003a). Toxic acute tubular necrosis following treatment with zoledronate (Zometa). *Kidney Int*, 64, 281–9.

Markowitz, G. S., Nasr, S. H., Stokes, M. B., *et al.* (2010). Treatment with IFN-{alpha}, -{beta}, or -{gamma} is associated with collapsing focal segmental glomerulosclerosis. Clin *J Am Soc Nephrol*, 5, 607–15.

Markowitz, G. S., Radhakrishnan, J., Kambham, N., *et al.* (2000). Lithium nephrotoxicity: a progressive combined glomerular and tubulointerstitial nephropathy. *J Am Soc Nephrol*, 11, 1439–48.

Maurice-Estepa, L., Daudon, M., Katlama, C., *et al.* (1998). Identification of crystals in kidneys of AIDS patients treated with foscarnet. *Am J Kidney Dis*, 32, 392–400.

Maynard, S. E., Min, J. Y., Merchan, J., *et al*. (2003). Excess placental soluble fms-like tyrosine kinase 1 (sFlt1) may contribute to endothelial dysfunction, hypertension, and proteinuria in preeclampsia. *J Clin Invest*, 111, 649–58.

McLaughlin, K., Gholoum, B., Guiraudon, C., *et al.* (1998). Rapid development of drug-induced lupus nephritis in the absence of extrarenal disease in a patient receiving procainamide. *Am J Kidney Dis*, 32, 698–702.

Miller, K. D., Chap, L. I., Holmes, F. A., *et al.* (2005). Randomized phase III trial of capecitabine compared with bevacizumab plus capecitabine in

patients with previously treated metastatic breast cancer. *J Clin Oncol*, 23, 792–9.
Miura, N., Mizuno, N., Aoyama, R., *et al.* (2009). Massive proteinuria and acute renal failure after oral bisphosphonate (alendronate) administration in a patient with focal segmental glomerulosclerosis. *Clin Exp Nephrol*, 13, 85–8.
Molnar, T., Farkas, K., Nagy, F., *et al.* (2010). Sulfasalazine-induced nephrotic syndrome in a patient with ulcerative colitis. *Inflamm Bowel Dis*, 16, 552–3.
Muller-Deile, J. and Schiffer, M. (2011). Renal involvement in preeclampsia: similarities to VEGF ablation therapy. *J Pregnancy*, 2011, 176973.
Nadasdy, T. and Racusen, L. (1998). Renal injury caused by therapuetic and diagnostic agents and abuse of analgesics and narcotics. In J. C. Jennette, J. L. Olson, M. M. Schwartz, *et al.* (eds.) *Heptinstall's Pathology of the Kidney*, pp. 811–61. Philadelphia: Lippincott-Raven.
Nakao, K., Sugiyama, H., Makino, E., *et al.* (2002). Minimal change nephrotic syndrome developing during postoperative interferon-beta therapy for malignant melanoma. *Nephron*, 90, 498–500.
Nanke, Y., Akama, H., Terai, C., *et al.* (2000). Rapidly progressive glomerulonephritis with D-penicillamine. *Am J Med Sci*, 320, 398–402.
Neau-Cransac, M., Moreau, K., Deminiere, C., *et al.* (2009). Decrease in sirolimus-induced proteinuria after switch to everolimus in a liver transplant recipient with diabetic nephropathy. *Transpl Int*, 22, 586–7.
Neild, G. H., Gartner, H. V., and Bohle, A. (1979). Penicillamine induced membranous glomerulonephritis. *Scand J Rheumatol Suppl*, 1979, 79–90.
Neradova, A., Stam, F., van den Berg, J. G., *et al.* (2009). Etanercept-associated SLE with lupus nephritis. *Lupus*, 18, 667–8.
Ntoso, K. A., Tomaszewski, J. E., Jimenez, S. A., *et al.* (1986). 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*, 8, 159–63.
Ogata, H., Kubo, M., Tamaki, K., *et al.* (1998). Crescentic glomerulonephritis due to rifampin treatment in a patient with pulmonary atypical mycobacteriosis. *Nephron*, 78, 319–22.
Padiyar, A., Bodziak, K. A., Hricik, D. E., *et al.* (2010). Clinical predictors of proteinuria after conversion to sirolimus in kidney transplant recipients. *Am J Transplant*, 10, 310–4.
Pascual, J., Torrealba, J., Myers, J., *et al.* (2007). Collapsing focal segmental glomerulosclerosis in a liver transplant recipient on alendronate. *Osteoporos Int*, 18, 1435–8.
Perazella, M. A. and Markowitz, G. S. (2008). Bisphosphonate nephrotoxicity. *Kidney Int*, 74, 1385–93.
Perlman, A. S., Kim, E. H., Kallakury, B., *et al.* (2007). Clinically significant proteinuria following the administration of sirolimus to renal transplant recipients. *Drug Metab Lett*, 1, 267–71.
Perneger, T. V., Klag, M. J., and Whelton, P. K. (2001). Recreational drug use: a neglected risk factor for end-stage renal disease. *Am J Kidney Dis*, 38, 49–56.
Phan, L., Coulomb, F., Boudon, M., *et al.* (1991). [Extramembranous glomerulonephritis induced by lithium]. *Nephrologie*, 12, 185–7.
Ramos-Casals, M., Brito-Zeron, P., Munoz, S., *et al.* (2007). Autoimmune diseases induced by TNF-targeted therapies: analysis of 233 cases. *Medicine (Baltimore)*, 86, 242–51.
Rangan, G. K. (2006). Sirolimus-associated proteinuria and renal dysfunction. *Drug Saf*, 29, 1153–61.
Rao, T. K., Nicastri, A. D., and Friedman, E. A. (1974). Natural history of heroin-associated nephropathy. *N Engl J Med*, 290, 19–23.
Reynolds, L. R. and Bhathena, D. (1979). Nephrotic syndrome associated with methimazole therapy. *Arch Intern Med*, 139, 236–7.
Rihova, Z., Honsova, E., Merta, M., *et al.* (2005). Secondary membranous nephropathy—one center experience. *Ren Fail*, 27, 397–402.
Ruiz, J. C., Campistol, J. M., Sanchez-Fructuoso, A., *et al.* (2006). Increase of proteinuria after conversion from calcineurin inhibitor to sirolimus-based treatment in kidney transplant patients with chronic allograft dysfunction. *Nephrol Dial Transplant*, 21, 3252–7.
Schwimmer, J. A., Markowitz, G. S., Valeri, A. M., *et al.* (2003). Secondary focal segmental glomerulosclerosis in non-obese patients with increased muscle mass. *Clin Nephrol*, 60, 233–41.
Sekhon, I., Munjal, S., Croker, B., *et al.* (2005). Glomerular tip lesion associated with nonsteroidal anti-inflammatory drug-induced nephrotic syndrome. *Am J Kidney Dis*, 46, e55–8.
Sennesael, J., Van den Houte, K., and Verbeelen, D. (1986). Reversible membranous glomerulonephritis associated with ketoprofen. *Clin Nephrol*, 26, 213–5.
Sethi, S., Sahani, M., and Oei, L. S. (2003). ANCA-positive crescentic glomerulonephritis associated with minocycline therapy. *Am J Kidney Dis*, 42, E27–31.
Shapiro, K. S., Pinn, V. W., Harrington, J. T., *et al.* (1984). Immune complex glomerulonephritis in hydralazine-induced SLE. *Am J Kidney Dis*, 3, 270–4.
Sheikh, T. K., Charron, R. C., and Katz, A. (1981). Renal manifestations of drug-induced systemic lupus erythematosus. *Am J Clin Pathol*, 75, 755–62.
Simms, R., Kipgen, D., Dahill, S., *et al.* (2008). ANCA-associated renal vasculitis following anti-tumor necrosis factor alpha therapy. *Am J Kidney Dis*, 51, e11–4.
Soo, Y. O., Chow, K. M., Lam, C. W., *et al.* (2003). A whitened face woman with nephrotic syndrome. *Am J Kidney Dis*, 41, 250–3.
Stallone, G., Infante, B., Pontrelli, P., *et al.* (2011). Sirolimus and proteinuria in renal transplant patients: evidence for a dose-dependent effect on slit diaphragm-associated proteins. *Transplantation*, 91, 997–1004.
Stokes, M. B., Erazo, M. C., and D'Agati, V. D. (2008). Glomerular disease related to anti-VEGF therapy. *Kidney Int*, 74, 1487–91.
Stokes, M. B., Foster, K., Markowitz, G. S., *et al.* (2005). Development of glomerulonephritis during anti-TNF-alpha therapy for rheumatoid arthritis. *Nephrol Dial Transplant*, 20, 1400–6.
Straathof-Galema, L., Wetzels, J. F., Dijkman, H. B., *et al.* (2006). Sirolimus-associated heavy proteinuria in a renal transplant recipient: evidence for a tubular mechanism. *Am J Transplant*, 6, 429–33.
Sugimoto, H., Hamano, Y., Charytan, D., *et al.* (2003). Neutralization of circulating vascular endothelial growth factor (VEGF) by anti-VEGF antibodies and soluble VEGF receptor 1 (sFlt-1) induces proteinuria. *J Biol Chem*, 278, 12605–8.
Tam, V. K., Green, J., Schwieger, J., *et al.* (1996). Nephrotic syndrome and renal insufficiency associated with lithium therapy. *Am J Kidney Dis*, 27, 715–20.
Tang, H. L., Chu, K. H., Mak, Y. F., *et al.* (2006). Minimal change disease following exposure to mercury-containing skin lightening cream. *Hong Kong Med J*, 12, 316–8.
Traynor, A., Kuzel, T., Samuelson, E., *et al.* (1994). Minimal-change glomerulopathy and glomerular visceral epithelial hyperplasia associated with alpha-interferon therapy for cutaneous T-cell lymphoma. *Nephron*, 67, 94–100.
Trolliet, P., Dijoud, F., Cotte, L., *et al.* (1995). Crescentic glomerulonephritis and crystals within glomerular capillaries in an AIDS patient treated with foscarnet. *Am J Nephrol*, 15, 256–9.
Vicente-Vicente, L., Quiros, Y., Perez-Barriocanal, F., *et al.* (2010). Nephrotoxicity of uranium: pathophysiological, diagnostic and therapeutic perspectives. *Toxicol Sci*, 118, 324–47.
Vogelbacher, R., Wittmann, S., Braun, A., *et al.* (2007). The mTOR inhibitor everolimus induces proteinuria and renal deterioration in the remnant kidney model in the rat. *Transplantation*, 84, 1492–9.
Wagstaff, A. J. and Bryson, H. M. (1994). Foscarnet. A reappraisal of its antiviral activity, pharmacokinetic properties and therapeutic use in immunocompromised patients with viral infections. *Drugs*, 48, 199–226.
Warren, G. V., Korbet, S. M., Schwartz, M. M., *et al.* (1989). Minimal change glomerulopathy associated with nonsteroidal antiinflammatory drugs. *Am J Kidney Dis*, 13, 127–30.
Wojciechowski, D., Kallakury, B., and Nouri, P. (2008). A case of cocaine-induced acute interstitial nephritis. *Am J Kidney Dis*, 52, 792–5.

Wolters, J., Frederik, P., van Rie, H., *et al.* (1987). Minimal change nephropathy during gold treatment. A case with unusual histopathological and immunopathological features. *Neth J Med*, 31, 234–40.

Wood, I. K., Parmelee, D. X., and Foreman, J. W. (1989). Lithium-induced nephrotic syndrome. *Am J Psychiatry*, 146, 84–7.

Woywodt, A., Hellweg, S., Schwarz, A., *et al.* (2007). A wild zebra chase. *Nephrol Dial Transplant*, 22, 3074–7.

Wu, S., Kim, C., Baer, L., *et al.* (2010). Bevacizumab increases risk for severe proteinuria in cancer patients. *J Am Soc Nephrol*, 21, 1381–9.

Yu, F., Chen, M., Gao, Y., *et al.* (2007). Clinical and pathological features of renal involvement in propylthiouracil-associated ANCA-positive vasculitis. *Am J Kidney Dis*, 49, 607–14.

Zanetta, G., Maurice-Estepa, L., Mousson, C., *et al.* (1999). Foscarnet-induced crystalline glomerulonephritis with nephrotic syndrome and acute renal failure after kidney transplantation. *Transplantation*, 67, 1376–8.

Zech, P., Colon, S., Labeeuw, M., *et al.* (1979). Nephrotic syndrome in procainamide induced lupus nephritis. *Clin Nephrol*, 11, 218–21.