CHAPTER

29 Pathophysiology of Ischemic Acute Kidney Injury

Charles L. Edelstein • Robert W. Schrier

INTRODUCTION

Acute kidney injury (AKI) is defined as a sudden decrease in the glomerular filtration rate (GFR) occurring over a period of hours to days. The Acute Dialysis Quality Initiative (ADQI) has developed the RIFLE classification of AKI that divides AKI into the following stages: (1) risk, (2) injury, (3) failure, (4) loss of function, and (5) end-stage kidney disease.[1–3] The RIFLE criteria have been validated in multiple studies (i.e., as the RIFLE class increases so does mortality).[1–3] The term AKI, in general, replaces the term acute renal failure (ARF) and ARF is restricted to patients that have AKI and need renal replacement therapy. AKI has also replaced the term acute tubular necrosis (ATN). The term ATN emerged from the early observation on renal biopsy that necrosis of some renal tubular epithelial cells may occur in humans with acute renal injury.[4] Tubular epithelial casts (muddy brown casts) are excreted in the urine of these patients. However, it is now known that the tubular necrosis is quite patchy and alone could not account for GFR less than 10 mL/min/1.73m^2, the functional hallmark of clinically significant AKI.[4] Moreover, a percentage, ranging from 30% to 70%, of the urinary tubular epithelial cells has been shown to be viable by culture and the exclusion of vital dyes.[5,6] This observation is somewhat surprising because, generally, cells, which are separated from their extracellular matrix, undergo apoptosis.[7–10] Emerging results suggest that adhesion molecules (e.g., cadherins and integrins) may allow a cell-to-cell or a cell-to-matrix adhesion that not only avoids apoptosis, but also may contribute to intratubular obstruction.[11–14] Intraluminal tubular casts on a renal biopsy are a hallmark of clinical AKI, and earlier nephron dissection studies by Jean Oliver demonstrated a preferential location of these casts in the medullary collecting duct.[15] This location is of particular relevance to the overall low GFR in AKI because thousands of nephrons drain into a single medullary collecting duct.

There is debate about whether the pathophysiology of clinical AKI is primarily tubular or vascular. In fact, initial rat micropuncture studies were unable to consistently detect an elevation in tubular pressures in experimental ARF and thus the term vasomotor nephropathy replaced the ATN term for the clinical syndrome.[16–18] Experimental results have emerged that both vascular and tubular factors are involved in the pathogenesis of clinical AKI. Most recently, the role of endothelial injury and dysfunction[19] in promoting an inflammatory response in AKI[20] has received prominence. Thus, this chapter will discuss the potential tubular and vascular factors, as well as inflammatory processes, involved in the pathogenesis of ischemic AKI (Fig. 29.1). Toxic AKI will be discussed in another chapter. However, it must be emphasized that AKI in humans is frequently multifactorial. Both ischemic and toxic insults combine in a synergistic fashion to cause clinical AKI.

The understanding of the pathogenesis of ischemic AKI is of considerable importance for several reasons. First, this clinical syndrome is quite frequent, occurring in 5% to 10% of hospitalized patients and in 30% to 40% of intensive care unit (ICU) patients.[21–23] The incidence is likely to increase in the future because of the use of newer nephrotoxic drugs and the performance of more complex procedures in older patients.[24] Second, ischemic AKI has a very high mortality particularly when requiring dialysis to treat the resultant uremic syndrome. Overall mortality averages from 40% to 50%; however, the patient in the ICU with ischemic AKI may have a mortality in excess of 80%, particularly if the patient has multiorgan failure.[25–30] It is widely quoted that the mortality of AKI has only improved slightly in the last 40 years.[31] However, a study[32] suggests there has been improvement in AKI mortality between the late 1970s and the early 1990s. Third, there is considerable evidence that a functional component of the renal failure exists. Specifically, a histologic examination of the kidney from patients with clinical AKI exhibits normal glomeruli, occasional tubular necrosis, some intraluminal casts, and modest interstitial edema.[4] There is virtually no evidence for irreversible tissue damage and the morphologic changes alone fail to support the presence of a GFR less than 10 mL/min/1.73m^2.

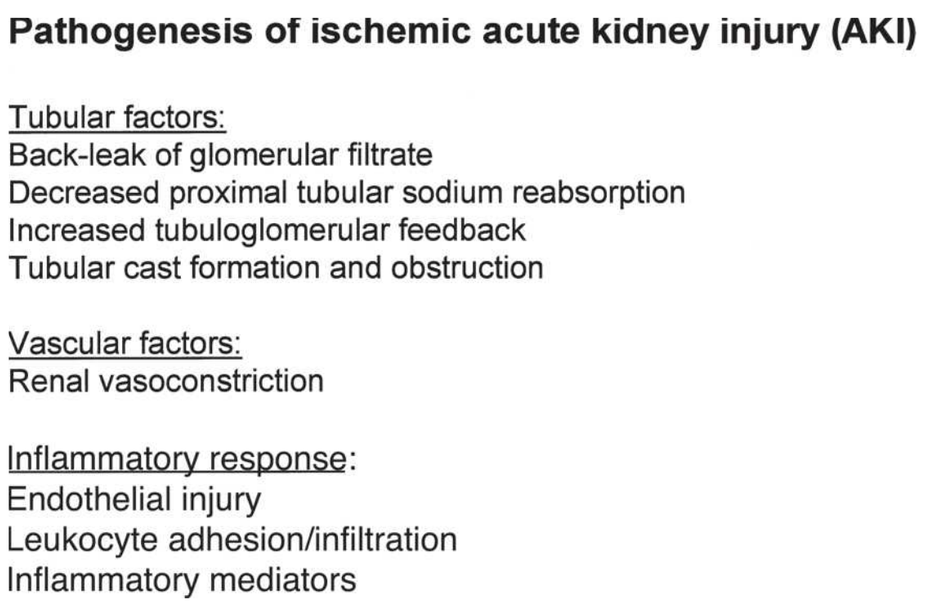

FIGURE 29.1 Vascular and tubular factors and inflammatory processes are involved in the pathophysiology of ischemic acute kidney injury.

EXPERIMENTAL MODELS OF ACUTE KIDNEY INJURY

Available models to study the pathophysiology of renal cell ischemia are listed in Table 29.1.[33] An understanding of these models will allow a better interpretation of the multiple studies discussed in this chapter.

Proximal and distal tubular cells in culture have been widely used to study tubular injury. These cells change from their normal dependence on oxidative mitochondrial metabolism to glycolysis under culture conditions.[34] As a result, these cultured tubules become less susceptible to oxygen deprivation. Thus, exposure to drugs like antimycin-A, ionomycin, or a combination to induce "chemical" ATP depletion and subsequent necrosis or apoptosis is used. Cultured cells also undergo considerable structural change, which includes simplification of both their apical and basolateral compartments.[35] The presence of necrosis rather than apoptosis in these cells may be related to the level of ATP depletion. In cultured mouse proximal tubules subjected to ATP depletion below 15% of control values, the cells died of necrosis; whereas in ATP depletion to 25% to 70% of control values all the cells died of apoptosis.[36] Therefore, although cultured tubule cells are the least complex model and allow for an understanding of the mechanisms involved, the therapeutic implications for in vivo AKI are limited.

Freshly isolated rat or rabbit proximal tubules in suspension are also widely used to study proximal tubular injury.[37–45] The method of isolation of tubules is by collagenase digestion and Percoll centrifugation. Hypoxia is achieved by gassing the suspension with 95%N_2/5%CO_2 for up to 15 minutes, thereby reducing the pO_2 to approximately 30 mm Hg. Lactic dehydrogenase (LDH) release into the suspension medium is measured as an index of lethal membrane injury.[46,47] The tubules are preincubated with cytoprotective agents and enzyme inhibitors before the induction of hypoxia, and the effect of these agents on cell membrane injury can be determined. The presence of necrosis rather than apoptosis during short periods of hypoxia (15 to 30 minutes) in this model was demonstrated using DNA-specific dyes, such as Hoechst 33342 and propidium iodide.[48] Another study has also demonstrated that during hypoxia there is endonuclease activation without morphologic features of apoptosis in the same model of rat proximal tubules.[49] Freshly isolated tubules are valuable for both structural and metabolic investigations because they retain the biochemical properties of the in vivo state and a high degree of structural integrity, and are highly polarized and fully differentiated.[33] However, the tubules are highly sensitive to ATP depletion, and severe hypoxia or anoxia results in necrosis of more than 50% of the cells after 30 minutes. Isolation methods also expose the tubules to repeated 4°C exposure and collagenase.

In whole animal studies—usually rats, rabbits, or mice—a clamp model of ischemic AKI is used.[50–52] Ischemic AKI is generally induced by (1) clamping of both the right and left renal pedicles or renal arteries or (2) unilateral renal pedicle or artery clamp preceded by contralateral nephrectomy. The renal vessels are clamped for varying periods of time, generally from 30 to 60 minutes, followed by varying periods of reperfusion. This results in a reversible model of AKI in which the blood urea nitrogen (BUN) and serum creatinine reach a peak at 24 to 48 hours reperfusion and

29.1 Available Models to Study the Pathophysiology of Renal Cell Ischemia (Increasing Order of Complexity)

Model	Origin
Cultured tubular cells	Primary culture of human, rat, and mouse; Madin-Darby canine kidney (MDCK) cells (distal); porcine renal epithelial (LLC-PK1) cells (proximal); opossum kidney (OK) cells; human kidney (HK) cells
Freshly isolated proximal tubules in suspension	Rabbits, rats, mice
Isolated perfused kidney	Rats
Whole animals	Rabbits, rats, mice, dogs (not much used anymore)
Human patients	Renal biopsy studies; urine and serum biomarkers of acute kidney injury

Adapted from Reference 33.

then gradually normalize over the next 7 days.[50–53] However, renal vessel clamping in rats results in extensive necrosis of proximal tubules. This necrosis is much more extensive than is seen in humans with ischemic AKI. Nevertheless, although animal models of ischemic AKI are complex with many experimental limitations, they provide important leads for future therapeutic clinical interventions.

In the isolated perfused kidney model, the kidney is removed from the animal. The perfusate usually consists of a Krebs-Henseleit buffer with albumin. Urine is collected by cannulation of the ureter. This model allows for the study of factors independent of changes in systemic hemodynamics and neural activity. Further advantages include the study of specific circulatory factors or pharmacologic agents that are added to the perfusate. These agents are thus delivered directly to the kidney. The disadvantages of the model are (1) the absence of red blood cells in the perfusate impairs oxygen delivery to the medullary thick ascending limb (mTAL) and (2) perfusate flow greatly exceeds normal in vivo values. The isolated perfused kidney is regarded as a model of selective hypoxia to the medullary thick ascending limb.[54]

Studies in patients with AKI, although having important experimental limitations, have the most direct therapeutic value. An analysis of urine cytology represents a noninvasive method for potentially defining the cause of AKI.[4] Meyers and colleagues[55–57] have examined patients with ischemic AKI postrenal transplantation by obtaining biopsies of these allografts at the time of transplantation. Biomarkers of kidney injury would greatly facilitate the early detection and the precise diagnosis of AKI.

BIOMARKERS OF ACUTE KIDNEY INJURY

AKI is usually diagnosed by recording increases in serum creatinine and decreased urine output over several days. However, serum creatinine is not a good marker of renal function in AKI because its concentration can be affected by factors not related to renal function such as the volume of distribution, muscle mass, and creatinine secretion.[28] When the kidney is injured and the true GFR suddenly drops, but there is a slow increase in serum creatinine over days. This new steady state, which may take up to 7 days, is reached when creatinine generation equals creatinine excretion. In contrast to serum troponin in myocardial infarction, an increase in serum creatinine lags and may not be directly related to tubular injury in AKI. Recent studies have examined urine and serum biomarkers of kidney injury that have the potential to facilitate the diagnosis of AKI.

Recently described molecules such as the cytokine interleukin (IL)-18,[58] kidney injury molecule-1 (KIM-1),[59] cysteine-rich protein 61 (Cry61),[60] neutrophil gelatinase–associated lipocalin (NGAL),[61] and sodium/hydrogen exchanger isoform 3 (NHE3)[62] have demonstrated compelling results as markers of AKI at the preclinical level. Studies have been initiated to explore these molecules in human AKI.

Because of the crucial importance of early therapy in the management of AKI, markers are being explored for early diagnosis. NGAL was investigated as an early biomarker for AKI following cardiopulmonary bypass in 45 patients.[63] Urine and serum were collected at baseline and at frequent intervals for 5 days following a cardiopulmonary bypass. All patients who developed AKI (defined as a 50% increase in serum creatinine) displayed a significant increase in serum and urine NGAL very early after the cardiopulmonary bypass compared to patients without AKI. These results show that NGAL may be a sensitive, early urinary, and serum biomarker for AKI.

In a nested case-control study within the adult respiratory distress syndrome (ARDS) network trial, urinary IL-18 was investigated as an early marker of AKI.[64] Median urine IL-18 levels were significantly higher in AKI cases (defined as a 50% increase in serum creatinine) as compared to controls. On multivariable analysis, urine IL-18 values predicted the development of AKI 24 and 48 hours later after adjusting for demographics, sepsis, acute physiology and chronic health evaluation (APACHE) III score, serum creatinine, and urine output. After controlling for other parameters, a rise in urine IL-18 by 25 pg per milliliter was associated with an increased odds ratio of AKI by 19% for the next 24 hours. Urine IL-18 performed as a diagnostic test with an area under the receiver operator characteristic curve of 73%. The conclusion of this study is that urinary IL-18 levels can be used for the early diagnosis of AKI.

A recent study demonstrated that serum cystatin C appears to increase 24 to 48 hours before serum creatinine in patients with AKI.[65] However, cystatin C is a marker of GFR, or a functional marker, and is not a structural or biochemical marker of renal tubular injury.

There are multiple promising serum and urinary biomarkers (eg, IL-18, neutrophil-gelatinase-associated lipocalin [NGAL], kidney injury molecule-1 [KIM-1], cystatin C, liver fatty acid-binding protein [L-FABP]), which detect AKI before the rise in serum creatinine and predict outcomes in patients with AKI.[66] Prospective studies to determine the use of these biomarkers in larger populations have been initiated. In this regard, a National Institutes of Health (NIH)-funded clinical consortium consisting of investigators from nine academic centers called TRIBE-AKI (Translational Research Investigating Biomarkers in Early Acute Kidney Injury) has been established. Currently, the consortium is performing a prospective multicenter observational cohort study of 1,800 patients receiving cardiac surgery to determine whether urine IL-18, urine NGAL, and serum cystatin C are biomarkers for the early diagnosis and long-term outcomes of AKI. Ultimately, disease control studies to determine the impact of a biomarker screening on AKI morbidity and mortality are desirable. In this regard, a prospective study in over 500 ICU patients in New Zealand determined whether erythropoietin therapy decreases the incidence of AKI, as determined by serum creatinine and serum cystatin C, and lowers levels of urinary IL-18, NGAL, and KIM-1.[67] In this

study, early intervention with erythropoietin based on urine biomarker levels did not affect the outcome of AKI.[67]

BACK-LEAK OF GLOMERULAR FILTRATE POSTRENAL ISCHEMIA

It has been proposed that GFR in ischemic AKI is really not as low as measured because glomerular filtrate leaks across the damaged epithelial monolayer and/or the tubular basement membranes. In some toxic experimental models of ischemic AKI, diffuse tubular necrosis and basement membrane damage has been associated with evidence for back-leak of glomerular filtrate. The term "back-leak" of glomerular filtrate refers to the unregulated passage of salt and water from the tubular lumen into the interstitium and later back into the renal venous capillaries and renal veins.[68] However, the level of epithelial and basement membrane damage with these experimental toxic models (e.g., cisplatin, mercuric chloride) is virtually never observed in human AKI. Myers et al.[69] have performed human studies using solute sieving curves in search of tubular back-leak of glomerular filtrate. They found dextran sieving curves could sometimes exceed inulin sieving curves, thus providing evidence in support of the back-leak of solutes (i.e., dextran), which are normally unable to cross intact tubular epithelial basement membranes. However, even when accepting the validity of this method for documenting tubular back-leak of filtrate, the calculated amount would only account for a decrease in renal function of 8% to 10%. Thus, although tubular back-leak of glomerular filtrate might occasionally occur in patients with severe ischemic AKI, it is unlikely to be a dominant pathogenic factor.

The tight junction of polarized tubular epithelial cells is the most apical component of the junctional complex and serves as an important permeability barrier.[70] Tight junctions also control cell polarity.[71] However, the tight junction in the proximal tubule is relatively "leaky" with as much as one-third of proximal sodium reabsorption occurring via the paracellular route. The tight junctional complex is a dynamic and regulated structure. Some of its protein components have been identified and include the transmembrane protein occludin. Nontransmembrane proteins on the cytosolic leaflet include zona occludens (ZO)-1, ZO-2, cingulin, 7H6, and several unidentified phosphoproteins. Interactions of some of these proteins with the actin cytoskeleton is a major determinant of the tight junction structure and may also play a role in the regulation of tight junction assembly.[70] The integrity of the tight junction is disrupted during ischemic injury and must be reestablished for recovery.[72] There is in vitro experimental evidence in cell culture studies for an impaired tight junction between tubular epithelial cells undergoing chemical anoxia.[73] Ruthenium red, which normally is impermeable to tight junctions, has been shown to enter the ZO after chemical hypoxia and renal ischemia.[73,74] Energy depletion abolishes the gate function of the tight junction, as determined by the dramatic decrease in transepithelial resistance, but it leaves the fence function intact, as determined by the maintenance of lipid polarity.[75] In an ATP depletion–repletion model in Madin-Darby canine kidney cells, tight junction proteins such as ZO-1 reversibly form large complexes and associate with cytoskeletal proteins.[76] A model has been proposed in which a key, potentially regulated step in the generation of the ischemic epithelial cell phenotype is the interaction between tight junction proteins and fodrin and/or other cytoskeletal proteins.[76] Intracellular calcium plays a role in tight junction reassembly after ATP depletion.[72] In this study, the role of intracellular calcium in tight junction reassembly after ATP depletion–repletion was studied using the cell-permeable calcium chelator 1,2-bis(2-aminophenoxy)ethane-N,N,N',N'-tetra-acetic acid-AM (BAPTA-AM). Lowering intracellular calcium during ATP depletion was associated with a significant inhibition of the reestablishment of the permeability barrier following ATP repletion as measured by transepithelial electrical resistance and mannitol flux, marked alterations in the subcellular localization of occludin by immunofluorescent analysis, and decreased solubility of ZO-1, and other tight junction proteins by the Triton X-100 extraction assay. This suggested that lowering intracellular calcium potentiates the interaction of tight junction proteins with the cytoskeleton.

Studies have also shown the importance of small GTPases (Rho, Rac, Cdc-42) in the integrity of the ZO.[77] ATP depletion with chemical hypoxia has been demonstrated to inactivate these GTPases and thereby contribute to increased paracellular back-leak of filtrate.[71,78] Expression of constitutively active ras homolog gene family, member A (RhoA) GTPase in ATP-depleted Madin Darby canine kidney (MDCK) cells prevents tight junction disassembly.[71] Cyanide-induced chemical hypoxia increases the kinase activity of c-Src and causes its translocation to cell–cell junctions where it binds to and phosphorylates β-catenin and p120, suggesting that this may contribute to the loss of epithelial barrier function.[79] It is likely that the effects on tight junction integrity seen in ATP depletion are due, at least in part, to the inhibition of Na-K-ATPase.[80] The relevance of these cell culture observations to clinical ischemic AKI in patients remains to be proven.

DECREASED TUBULAR SODIUM REABSORPTION

Proximal tubular injury, whether it be sublethal reversible dysfunction, necrosis, or apoptosis, has been extensively studied. Mechanisms of proximal tubular injury that will be discussed in this section are outlined in Table 29.2. The study of proximal tubular injury is of special relevance in order to explain the decreased tubular sodium reabsorption that occurs with a postrenal ischemia.

In the normal kidney, Na^+ is vectorially transported from the proximal tubule lumen across the apical membrane microvilli into the tubular epithelial cells and then across the basolateral membrane into the interstitium and the peritubular circulation.[81] Na^+ influx into the polarized proximal tubular epithelial cells across the apical membrane is passive

29.2 Mechanisms of Hypoxic/Ischemic Proximal Tubular Injury

Sublethal Reversible Injury
- Cytoskeletal disruption and loss of polarity
- Loss of tight junction function
- Loss of cell-matrix adhesion
- Abnormal gene expression

Necrosis
- Severe ATP depletion (15% of normal)
- Calcium influx
- Calcium-dependent phospholipase A_2 ($cPLA_2$)
- Calcium-dependent cysteine proteases (e.g., calpain)
- Calcium-independent PLA_2
- Caspase-1
- Interleukin-18 (IL-18)
- Metalloproteases
- Oxygen radicals
- Lipid peroxidation
- Deficiency of glycine
- Nitric oxide (generated by iNOS)
- Endonuclease activation
- Deficient heat stress response
- Potassium efflux
- Klotho deficiency

Apoptosis
- Mild ATP depletion (25%–50% of normal)
- Caspase-3
- Caspase-1
- Caspase-6
- Endonuclease activation
- Serine proteases
- Insulinlike growth factor I receptor deficiency
- Deficient heat stress response
- Erythropoietin
- NGAL
- Ghrelin
- Bcl-2 proteins
- Mitochondrial fragmentation

iNOS, inducible nitric oxide; NGAL, neutrophil gelatinase–associated lipocalin.

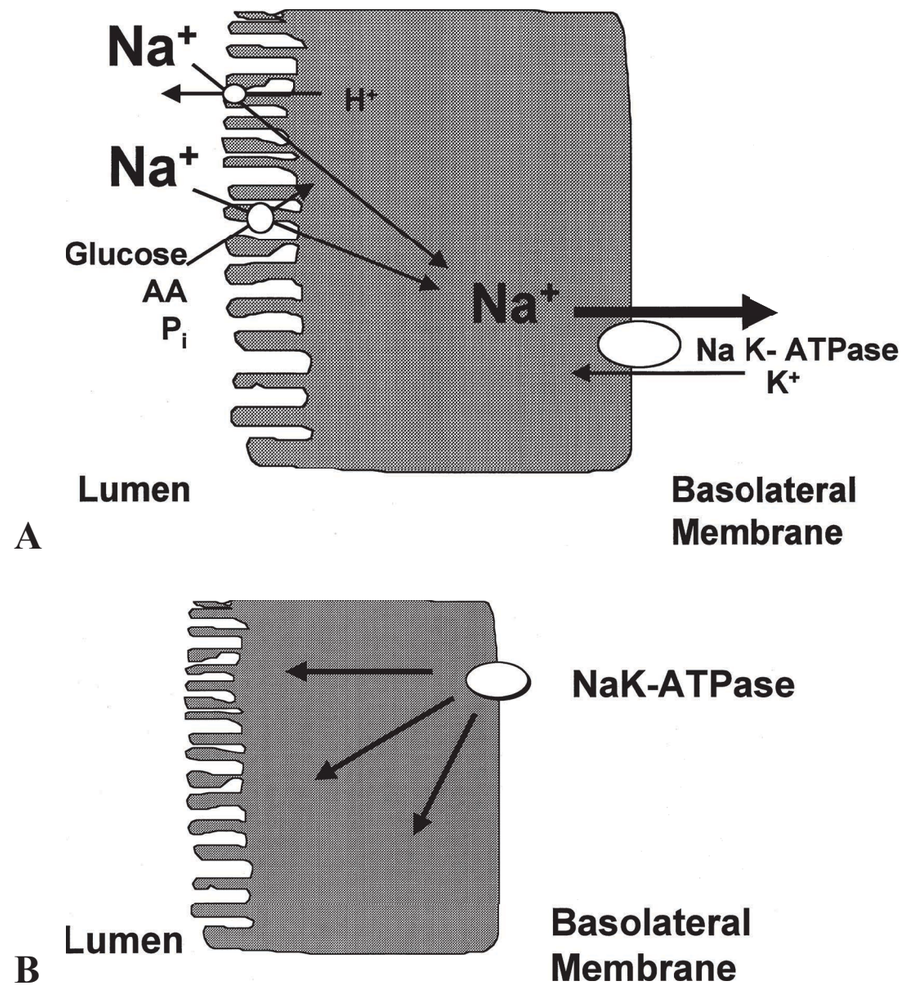

FIGURE 29.2 **A:** Normal reabsorption of sodium in the proximal tubule. The NaK-ATPase pump is located on the basolateral surface of the proximal tubule. **B:** Translocation of the Na-K-ATPase pump away from the basolateral proximal tubule membrane during hypoxia/ischemia. The loss of polarity of proximal tubule cells during chemical anoxia and ischemia results in a translocation of the Na-K-ATPase. During ATP depletion in cultured cells, the Na-K-ATPase translocates to the apical membrane. In human kidney allografts with delayed function, the Na-K-ATPase translocates to the cytoplasm. The translocated Na-K-ATPase remains functional.

down the Na^+ gradient via the H^+/Na^+ exchanger and various Na^+ cotransporters. The Na^+ gradient is maintained by an active transport via the Na^+/K^+-ATPase at the basolateral membrane of the proximal tubular cells (Fig. 29.2A).

The earliest signs of clinical AKI are urinary muddy brown casts and an increased fractional excretion of sodium (FE_{Na}).[82,83] The proximal tubule is the most frequent morphologic site of injury in ischemic AKI in both humans and animals.[84] The S_3 segment of the proximal tubule is particularly prone to ischemic injury, perhaps because of its location in the outer medulla, which is relatively hypoxic compared to the renal cortex.[84] The proximal tubule nephron site is also associated with impaired vectorial sodium transport. The earliest morphologic changes with ischemic injury include invagination and sloughing of the brush border membrane into the lumen, an abnormality that is compatible with impairment and a loss of apical sodium antiporters and cotransporters responsible for sodium entry into the proximal tubular epithelium.[85–87] The tubules lose their polarity.[83] In vitro studies have shown that ATP depletion leads to dephosphorylation and inactivation of the actin binding protein, ezrin, and activation of the actin depolarizing protein in the proximal tubule membrane.[88,89] This leads to a disruption of the microvillar actin and a loss of the brush border membrane.[90] A loss of polarity of the proximal tubule cells during chemical anoxia and ischemia has also been shown with the translocation of the Na-K-ATPase to the apical membrane (Fig. 29.2B).[91–93] The translocated Na-K-ATPase remains functional.[94] In MDCK cells exposed to ATP depletion, there is a loss of polarity of Na-K-ATPase and a dissociation of the membrane–cytoskeleton complex at the spectrin–ankyrin interface.[95] Thus, sodium transport back

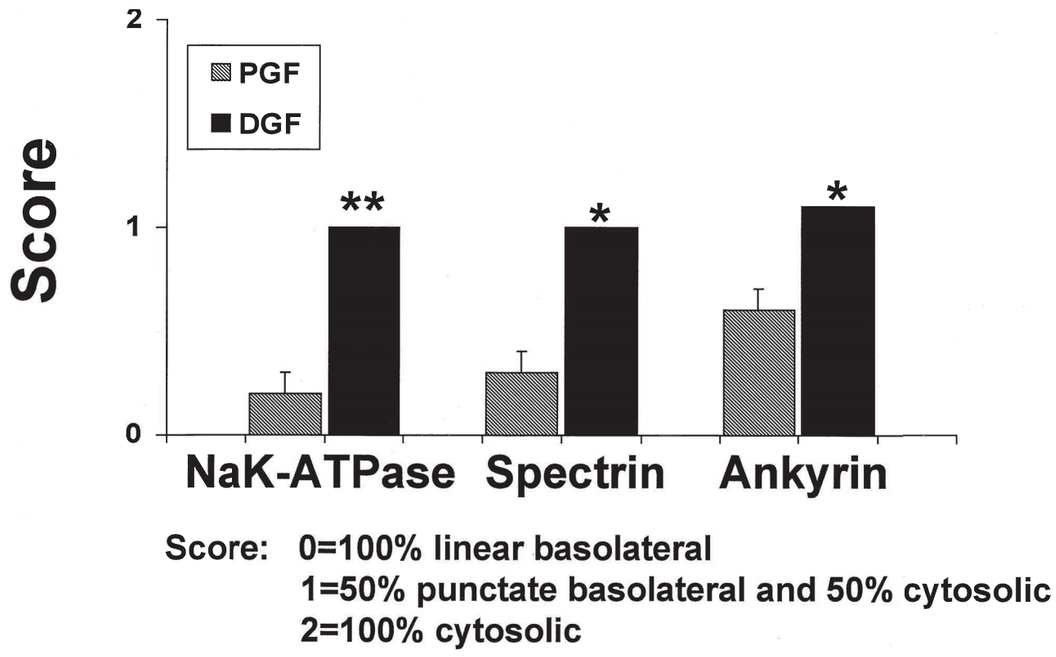

FIGURE 29.3 The cellular location on immunohistochemistry of the actin binding proteins, ankyrin and spectrin, and Na-K-ATPase in cadaveric transplanted kidneys with prompt graft function (PGF) and delayed graft function (DGF) was compared. In those kidneys with DGF, approximately 50% of the ankyrin, spectrin, and Na-K-ATPase was translocated from the basolateral membrane to the cytoplasm. Whereas those kidneys with a PGF had only minimal translocation of these proteins from the basolateral membrane. * $P < .01$ versus PGF; ** $P < .05$ versus PGF. Adapted from Alejandro et al.[55]

across the apical membrane and into the proximal lumen, as well as decreased proximal tubular sodium reabsorption, has been proposed in response to hypoxia and ischemia.

Studies in cadaveric transplanted kidneys with both prompt and delayed graft functions have been compared relative to the cellular location of the actin binding proteins, ankyrin and spectrin, and Na-K-ATPase using selective antibodies. In kidneys with delayed graft function, approximately 50% of the ankyrin, spectrin, and Na-K-ATPase were translocated from the basolateral membrane to the cytoplasm (Fig. 29.3). Those kidneys with prompt graft function had only minimal translocation of these proteins from the basolateral membrane (Fig. 29.3).[55] These observations therefore have contributed to our understanding of reversible and sublethal tubular dysfunction in ischemic kidneys in vivo (Fig. 29.3).

Because proximal tubular cells in a culture convert from primarily oxidative to glycolytic metabolism and alter their phenotype,[34,35] confirmation of experimental results in other systems is advisable. The use of freshly isolated proximal tubules to study the response to hypoxia has also been enlightening. These tubules, in general, maintain their phenotype and oxidative metabolism but are more sensitive to hypoxia, as assessed by LDH release, than in vivo tubules.[96–99] Hypoxia for 15 to 30 minutes causes a reproducible release of LDH, and the cells die by necrosis.[48,49] The central role of intracellular calcium and various protective maneuvers against hypoxic injury have been demonstrated in these isolated proximal tubules. However, before discussing the role of intracellular calcium in proximal tubular injury, we shall briefly consider adenine nucleotides. It is unquestioned that the first effect of ischemia, hypoxia, or mitochondrial inhibition in most in vitro and in vivo models is to compromise adenine nucleotide metabolism. A decreased production of ATP precedes the increase in intracellular calcium.

Adenine Nucleotides

The removal of oxygen from renal cells or whole kidneys results in prompt decreases in the cellular ATP pool. Initially, adenosine diphosphate (ADP) and adenosine monophosphate (AMP) concentrations increase,[52] and further catabolism of AMP to adenosine and then to hypoxanthine and, in some species to xanthine, occurs as the ischemic period is prolonged.[100,101] The provision of exogenous ATP-$MgCl_2$ to ischemic rat kidneys protects against ischemic injury.[102] Mechanisms whereby a loss of ATP results in cellular injury include the loss of purine nucleosides themselves (in some species, the generation of oxygen free radicals during reperfusion), and the loss of many metabolic functions (e.g., phosphorylation of important enzymes, ion channels, and the functions of ion transporters that are dependent on adequate ATP levels).

Ischemic preconditioning protects the heart, and in some studies the kidneys, from subsequent ischemia–reperfusion injury. Ischemic preconditioning appears to be mediated via the activation of adenosine receptors, specifically the A_1 adenosine receptors. In support of this are studies that the exogenous administration of adenosine or A_1 adenosine agonists mimic ischemic preconditioning in cardiac muscle.[103] It was recently demonstrated that rat kidneys can be preconditioned to attenuate ischemic–reperfusion injury. In this study, adenosine infusion before the ischemic insult protects renal function via A_1 adenosine receptor activation, and adenosine A_1 antagonism blocks adenosine-induced protection. In a more recent study, acute and delayed protection against renal ischemia was seen with an A_1 adenosine receptor agonist.[104] In addition, adenosine A_3 receptor activation before the ischemia worsens the renal ischemia–reperfusion injury and A_3 receptor antagonism protects renal function.[103] A_2A adenosine receptors mediate the inhibition of ischemic AKI in rats due to an inhibitory effect on neutrophil adhesion.[105,106] A combined infusion of an A_{2A} adenosine receptor agonist and a type IV phosphodiesterase (PDE 4) inhibitor leads to enhanced protection against ischemia–reperfusion injury in mice.[107] Protection against renal ischemia–reperfusion injury by A_2A receptor agonists or endogenous adenosine requires the activation of receptors expressed on bone marrow–derived cells.[108] The A_2A adenosine receptor may be a novel therapeutic target in renal ischemia–reperfusion injury.[109,110]

Intracellular Calcium

The normal regulation of epithelial cell calcium is demonstrated in Figure 29.4A. Calcium exists in the cell as cytosolic free calcium, which is the smallest pool, but the most critical for regulation of intracellular events. Calcium is also bound to proteins and anions in the cytosol and to membrane

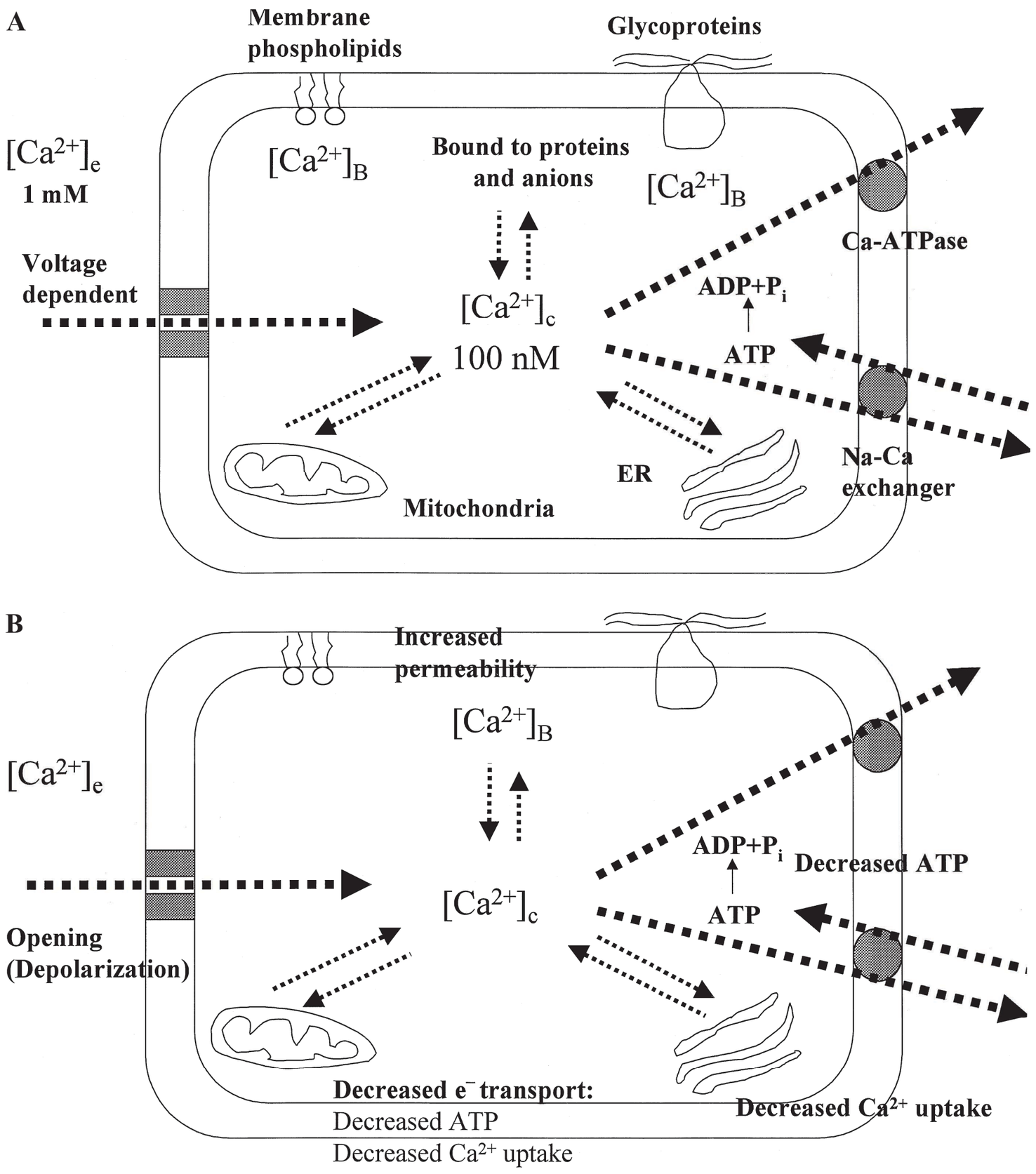

FIGURE 29.4 **A:** The normal regulation of epithelial cell calcium. The cytosolic free calcium $[Ca^{2+}]_c$ is the smallest amount, but the most critical for the regulation of intracellular events. The concentration of calcium in the cytosol is about 100 nM, which is 1/10,000 of the extracellular calcium, which is in a micromolar concentration of $[Ca^{2+}]_e$. Calcium is also bound to proteins and anions in the cytosol and to membrane phospholipids and glycoproteins $[Ca^{2+}]_b$. The largest pool of intracellular calcium is in the mitochondria and the endoplasmic reticulum. The large electrochemical gradient is maintained by binding calcium to intracellular components and by apical and basolateral transport systems. Transport systems, which maintain the large electrochemical gradient between intracellular and extracellular Ca^{2+}, may be voltage dependent or ATP dependent like the $Ca2^{+}$ ATPase pump and the $Na^{+}/Ca2^{+}$ exchanger. During cell injury, active mitochondrial sequestration appears to be quantitatively the most important process for buffering elevations in cytosolic calcium. **B:** During epithelial cell injury, several factors favor increases in cytosolic free calcium. There is (1) depolarization or an opening of voltage-dependent channels; (2) increased membrane permeability; and (3) decreased mitochondrial electron transport leading to decreased ATP levels. The decreased ATP leads to decreased calcium uptake by mitochondria and the endoplasmic reticulum, and a decreased ability to pump calcium out of the cell.

phospholipids and glycoproteins. The largest pools of intracellular calcium are in mitochondria and the endoplasmic reticulum.[111,112] The concentration of calcium in the cytosol is about 100 nM, which is 1/10,000 of extracellular calcium concentrations.[111] The large electrochemical gradient between intracellular and extracellular calcium is maintained by the binding of calcium to intracellular components and by apical and basolateral transport systems. Transport systems may be voltage dependent or ATP dependent. Calcium efflux is mediated in basolateral membranes by both calcium ATPase, which is ATP dependent, and by a Na^{+}/Ca^{2+} exchanger on the basolateral membrane, which is ATP independent.[113] Normally, the cell membrane is impermeable to calcium and maintains the steep calcium gradient between cytosolic free calcium and the extracellular space.[112] However, when cytosolic calcium increases in response to either increased cellular membrane permeability or decreased calcium efflux, or both, the mitochondria and the endoplasmic reticulum (ER)

actively increase their calcium uptake. Mitochondrial uptake and retention of calcium becomes substantial only when cytosolic levels exceed 400 to 500 nM, as occurs with cell injury.[111] Mitochondrial uptake is regulated by a calcium uniporter in the mitochondrial inner membrane. Thus, during cell injury, active mitochondrial sequestration appears to be quantitatively the most important process for buffering elevations in cytosolic calcium.

During epithelial cell injury, several factors favor increases in cytosolic free calcium (Fig. 29.4B). This includes (1) decreased mitochondrial electron transport leading to decreased ATP levels, (2) increased membrane permeability, and (3) depolarization or opening of voltage-dependent channels. The decreased ATP leads to a decreased calcium uptake by mitochondria and ER and a decreased ability to pump calcium out of the cell.

With this background on the normal regulation of cell calcium, we shall now consider the role of intracellular calcium in tubular injury. In 1981, it was proposed that calcium ions were important participants in the functional, biochemical, and morphologic disturbances that characterize AKI.[114,115] Numerous studies over the past 15 years in different injury models and cell types have demonstrated an increase in cytosolic calcium in renal epithelial cell injury. These studies are summarized in Table 29.3.

The crucial questions to implicate calcium as a primary factor in cell injury are (1) whether the increase in cytosolic calcium precedes the injury and (2) whether preventing the rise in cytosolic calcium attenuates the injury.[116,117] To investigate whether hypoxia is associated with an increase in free cytosolic calcium in proximal tubular cells, which precedes any evidence of membrane damage, a video imaging technique was developed in which free intracellular calcium could be measured simultaneously with staining of nuclei with the membrane impermeable indicator, propidium iodide, as an index of hypoxia-induced membrane damage.[118] Propidium iodide enters the cell through the damaged plasma membrane and stains the cell nucleus. The percent of nuclei that stain with propidium iodide is quantitated and is an index of plasma membrane damage. Hypoxia in rat proximal tubules is associated with a significant rise in cytosolic calcium, which antecedes evidence of membrane damage as assessed by propidium iodide staining.[99] Cytosolic calcium increased from 170 to 390 nM during 5 minutes of hypoxia. The increase in cytosolic calcium preceded propidium iodide–detectable cell injury (Fig. 29.5). The increase in cytosolic calcium that preceded the hypoxic membrane damage was promptly reversible with reoxygenation after 8 minutes of hypoxia. This is important because if cytosolic calcium is increased only after lethal cell membrane damage, reoxygenation should not have

29.3 Increases in Cytosolic Calcium in Renal Epithelial Cell Injury

Injury Model	Cell Type	Reference
Calcium ionophore	Rabbit proximal tubules	Mandel and Murphy, 1984[467]
Anoxia	LLCMK2 cells	Snowdowne et al., 1985[468]
Chemical ATP depletion	MDCK cells	McCoy et al., 1988[469]
Calcium ionophore, chemical ATP depletion	Cultured rabbit proximal tubules	Phelps et al., 1989[470]
Chemical anoxia	Rabbit proximal tubules	Weinberg et al., 1991[471]
Hypoxia	Rabbit proximal tubules	Jacobs et al., 1991[472]
Hydrogen peroxide	LLCPK1 cells	Ueda and Shah, 1992[473]
Anoxia and hypoxia	Rat proximal tubules	Almeida et al., 1992[474]
Chemical anoxia	Opossum kidney cells	Li et al., 1993[475]
Hypoxia-reoxygenation	Primary culture rat proximal tubules	Greene and Paller, 1994[476]
Hypoxia	Rat proximal tubules	Kribben et al., 1994[99]
Anoxia	Rabbit proximal tubules	Rose et al., 1994[477]

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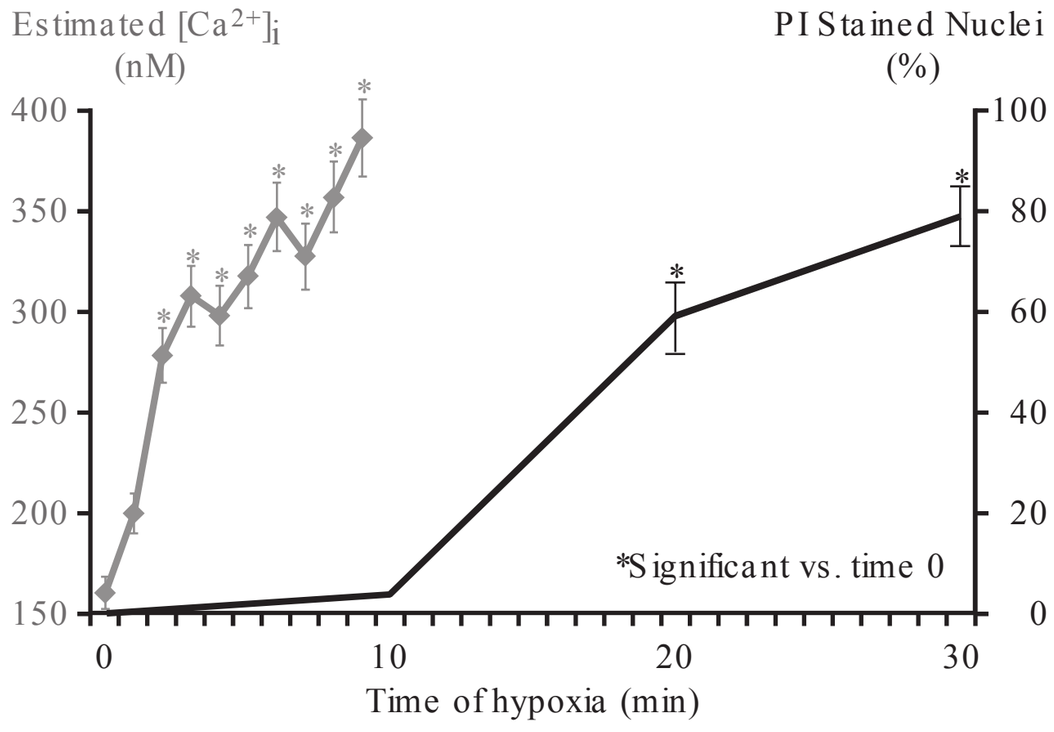


FIGURE 29.5 In isolated proximal tubules, the increase in free cytosolic calcium as measured by Fura-2 precedes the cell membrane damage as assessed by propidium iodide (PI) staining. Adapted from Kribben et al.[99]

normalized cytosolic calcium. The 10 minutes of cytosolic calcium rise correlated significantly with subsequent cell damage observed at the 20 minute mark. The pivotal role of the rise in cytosolic calcium during hypoxia was further demonstrated by using the intracellular Ca^{2+} chelator BAPTA to prevent the rise in cytosolic calcium; this approach resulted in marked cytoprotection against hypoxic tubular injury.

What are the mechanisms whereby increases in cytosolic free calcium could lead to cell membrane injury? Potential calcium-dependent mechanisms include changes in the actin cytoskeleton of proximal tubule microvilli,[119] activation of calcium-dependent PLA_2,[40] and activation of the calcium-dependent cysteine protease, calpain.[116,117,120,121]

Calcium-Dependent Changes in the Actin Cytoskeleton

The role of calcium in pathophysiologic alterations of the proximal tubule microvillus actin cytoskeleton was studied in freshly isolated tubules.[119] Precisely defined medium calcium levels were defined using a combination of the metabolic inhibitor, antimycin, and the ionophore, ionomycin, in the presence of glycine to prevent lethal membrane damage. Increases of intracellular calcium to 10 μM were sufficient to initiate concurrent actin depolymerization, fragmentation of F-actin into forms requiring high-speed centrifugation for recovery, redistribution of villin to sedimentable fractions, and structural microvillar damage consisting of severe swelling and fragmentation of actin cores. However, during ATP depletion induced by antimycin alone or hypoxia alone, initial microvillar damage was calcium independent. This study suggests that both ATP depletion-dependent but Ca^{2+}-independent, as well as Ca^{2+}-mediated processes, can disrupt the actin cytoskeleton during acute proximal tubule cell injury; that both types of change occur, despite protection afforded by glycine and reduced pH against lethal membrane damage; and that Ca^{2+}-independent processes primarily account for prelethal actin cytoskeletal alterations during simple ATP depletion of proximal tubule cells.

In normal proximal tubule cells, actin is concentrated in apical brush border microvilli, along with the actin-binding protein, villin. Villin plays an important role in actin bundling and in microvillar assembly but can also act as an actin-fragmenting protein at higher calcium concentrations. The effects of ischemic injury and reperfusion on the distribution of villin and actin in the proximal tubule cells of rat kidneys were examined.[122] This study demonstrated that villin may be involved in the initial disruption of the actin cytoskeleton during reperfusion injury and that its migration back to the apical domain of these cells accompanies the reestablishment of a normal actin distribution in the brush border.

ATP depletion results in the conversion of monomeric G-actin to polymeric F-actin during tissue ischemia.[123] This conversion results from altering the ratio of ATP-G actin and ADP-G actin, causing a net decrease in the concentration of thymosin actin complexes as a cosequence of the differential affinity of thymosin beta 4 for ATP and ADP-G actin.[124] Recent studies suggest that the actin-binding protein tropomyosin binds to and stabilizes the apical actin microvilli under physiologic conditions in proximal tubules.[125]

Activation of Phospholipase A_2

Phospholipase A_2 (PLA_2) enzymes are important regulators of prostaglandin and leukotriene synthesis and can directly modify the composition of cellular membranes.[126] PLA_2 enzymes are also potent regulators of inflammation. The cytosolic form, $cPLA_2$, preferentially releases arachidonic acid from phospholipids and is regulated by changes in intracellular calcium concentration.[127]

PLA_2 enzymatic activity was measured in cell-free extracts prepared from rat renal proximal tubules.[40] Both soluble and membrane-associated PLA_2 activity were detected. All PLA_2 activity detected during normoxia was calcium dependent. The fractionation of cytosolic extracts by gel filtration revealed three peaks of PLA_2 activity. Exposure of tubules to hypoxia resulted in stable activation of soluble PLA_2 activity, which correlated with the disappearance of the highest molecular mass form (>100 kDa) and the appearance of a low–molecular-mass form (approximately 15 kDa) of PLA_2. Hypoxia also resulted in the release of a low–molecular-mass form of PLA_2 into the extracellular medium. Pretreatment of tubules with glycine before hypoxia blocked this release of PLA_2 but not the activation of soluble PLA_2 activity. This study provides direct evidence for calcium-dependent PLA_2 activation during hypoxia. However, calcium-independent forms of PLA_2 have also been found to play a role in hypoxic proximal tubular injury.[128]

The mechanism of PLA_2-induced cell membrane damage is interesting. Membrane phospholipid breakdown has been observed to occur in a number of tissues during ischemia.[129] In proximal tubules, hypoxia has been shown to

cause an increase in free fatty acids, which was initially believed to contribute to cell injury.[130] However, a study from our laboratory has shown that unsaturated free fatty acids protect against hypoxic injury in proximal tubules and that this protection may be mediated by a negative feedback inhibition of PLA_2 activity.[41] This protective effect of unsaturated free fatty acids has been confirmed by Zager et al.[131] The injurious effect of PLA_2 could be related to a direct disruption of cell membrane integrity by attacking the phospholipid component of cell membranes or through the accumulation of lysophospholipids, which have been shown to disrupt cell membranes and cause cytotoxicity.[132]

Activation of Calpain

The cysteine proteases are a group of intracellular proteases that have a cysteine residue at their active site. The cysteine proteases consist of three major groups: cathepsins, calpains, and the newly discovered caspases. The major groups of cysteine proteases are shown in Table 29.4. The cathepsins are non–calcium-dependent lysosomal proteases that do not appear to play a role in lethal cell injury.[133–135] Calpain is a calcium-activated neutral protease (CANP).[136] It has absolute dependence on calcium. There are two major ubiquitous or conventional isoforms of calpain, the low calcium sensitive μ-calpain and the high calcium sensitive m-calpain.[137,138] The isoenzymes have the same substrate specificity but differ in affinity for Ca^{2+}. μ-Calpain is activated by micromolar concentrations of Ca^{2+}, and m-calpain is activated by millimolar concentrations of Ca^{2+}. The millimolar concentrations of intracellular calcium needed for the activation of m-calpain are not seen in normal cells, and phosphatidylinositol is thought to lower the calcium concentration required for half the maximal autolysis of m-calpain.[139] Procalpain exists in the cytoplasm as an inactive proenzyme and becomes active proteolytically only after it has become autolysed at the cell membrane. Activity of the autolysed calpain is subject to a final regulation by calpastatin.[140,141] Calpastatin is a specific endogenous inhibitor of calpain. It is as widely distributed in nature as the enzyme itself. Calcium is required for calpastatin to bind to calpain and thus for the inhibitory effect of calpastatin on calpain.

Postulated functions of calpain include platelet activation and aggregation, cytoskeleton and cell-membrane organization,[142] and the regulation of cell growth.[143–146]

The calcium-dependent calpains have been shown to be mediators of hypoxic/ischemic injury to the brain, liver, and the heart.[147–150] The role of the calcium-dependent cytosolic protease, calpain, in hypoxia-induced renal proximal tubular injury has also been demonstrated.[43] Tubular calpain activity increased significantly by 7.5 minutes of hypoxia, before there was significant LDH release, and further increased during 20 minutes of hypoxia. Chemically dissimilar cysteine protease inhibitors markedly decreased LDH release after 20 minutes of hypoxia and completely prevented the rise in calpain activity during hypoxia. This role of calpain in proximal tubule injury has subsequently been confirmed by other groups.[151,152] This increased calpain activity has subsequently been shown to be associated with a breakdown of the cytoskeletal protein, spectrin, both in vitro[45] and in vivo,[153] as well as increasing Na-K-ATPase into the cytoplasmic fraction of the cell.

Recent studies have demonstrated that calpain mediates progressive plasma membrane permeability and the proteolysis of cytoskeleton-associated paxillin, talin, and vinculin during antimycin A or hypoxia-induced proximal tubular cell death.[154] Novel nonpeptide calpain inhibitors are protective against antimycin A–induced calcium influx and hypoxia/reoxygenation-induced proximal tubular cell death.[155] In novel in vivo studies, calpastatin transgenic mice that had a decreased activation of calpain in the kidney

29.4 The Major Groups of Cysteine Proteases

	Cathepsins	Calpains	Caspases
Family	B,H,L,S (lysosomal)	μ and m Calpain Tissue specific isoforms	1–14
Location	Lysosome	Cytoplasm	Cytoplasm
Activation	Calcium-independent	Calcium dependent	Caspase activated
Optimal pH	5-6	7.4	7.4
Functions	Intracellular protein degradation	Intracellular signaling Cytoskeletal stability Necrosis and apoptosis	Apoptosis/necrosis Cytokine activation

Adapted from Edelstein[121] with permission.

were generated.[156] In an anti-glomerular basement membrane (GBM) model, calpastatin-transgenic mice had less severe glomerular injury and a reduction in nuclear factor kappa-B (NF-κB) activation, suggesting a role for calpain in inflammation.

Caspases

Caspases are another group of intracellular cysteine proteases. Caspases participate in two distinct signaling pathways: (1) the activation of proinflammatory cytokines and (2) the promotion of apoptotic cell death.[9,157–162] Caspases 3 and 7 are the major mediators of apoptosis. The term "caspase" embodies two properties of these cysteine proteases in which "c" refers to "cysteine" and "aspase" refers to their specific ability to cleave substrates after an aspartate residue. The members of the caspase family are divided into subfamilies based on substrate specificity and function.[163] Caspase-1 (previously known as IL-1–converting enzyme [ICE]) plays a major role in the activation of proinflammatory cytokines. For many years it was not known how caspase-1 was activated. It has recently been discovered that procaspase-1 is activated in a complex called the inflammasome.[164,165] The inflammasome is a protein scaffold that contains NALP (NACHT, LRR, and pyrin domain–containing) proteins, an adaptor protein called ASC (apoptosis-associated specklike protein containing a caspase-recruiting domain [CARD]), procaspase-1, and caspase-5. The interaction of the CARD of procaspase-1 is mediated by the CARD of ASC and the CARD present in the C-terminus of NALP-1. Active caspase-1 in the inflammasome is a regulator of the unconventional protein secretion of leaderless proteins like IL-1α and fibroblast growth factor (FGF)-2.[166] In a recent study, the inflammasome components NLRP3 and ASC were highly expressed in the renal tubular epithelium of humans and mice.[167] The absence of Nlrp3, but not ASC, protected against ischemic AKI.[167] Activation of caspases-1, 8, 9, and 3 have been described in hypoxic renal epithelial cells[168–170] and cerebral ischemia.[171] Although cells contain many caspases, the targeted disruption of specific caspase genes in mice has provided much insight into the functions of individual caspases during cell death.[172]

Although caspases play a crucial and extensively studied role in apoptosis, there is now considerable evidence that the caspase pathway may also be involved in necrotic cell death.[173] The inhibition of caspases protects against necrotic cell death induced by the mitochondrial inhibitor, antimycin A, in PC12 cells, Hep G2 cells, and renal tubules in a culture.[174,175] Caspases are also involved in hypoxic and reperfusion injury in cultured endothelial cells.[176] Rat kidneys subjected to ischemia demonstrate an increase in both caspase-1 and caspase-3 mRNA and protein expression.[177] Caspases play a role in hypoxia-induced necrotic injury of isolated rat renal proximal tubules.[48] In this study, caspase activity was increased in association with cell membrane damage as assessed by LDH release. A specific caspase inhibitor attenuated the increase in caspase activity and markedly protected against cell membrane damage. To specifically identify the caspase involved in proximal tubular injury, proximal tubules were isolated from capsase-1 knockout mice and exposed to hypoxia.[178] Proximal tubules from caspase-1 knockout mice were protected against hypoxic injury, demonstrating the role of caspase-1 in directly causing cell membrane damage in proximal tubules.[178]

A study[179] investigated the role of caspase inhibition and apoptosis in ischemic AKI in mice in vivo. A relationship between apoptosis and subsequent inflammation was found. At the time of reperfusion, administration of the antiapoptotic agents insulin-like growth factor 1 (IGF-1) and ZVAD-fmk (a caspase inactivator) prevented the early onset of not only renal apoptosis, but also inflammation and tissue injury. Conversely, when the antiapoptotic agents were administered after the onset of apoptosis, these protective effects were completely abrogated.

There appears to be an interaction between caspases and calpain during hypoxia-induced injury in the proximal tubule, because caspase inhibition was shown to decrease calpain activity during hypoxia.[48,121] Recent in vivo studies[153] suggest that caspase-mediated degradation of the endogenous inhibitor of calpain, calpastatin, is a mechanism whereby the calcium-mediated activity of calpain is increased.

Caspases in Cold Ischemia

Preservation injury, also known as cold ischemia, is an important clinical problem in kidney transplantation. Significant damage to the kidney may occur during harvest, cold storage, and transport. An ongoing area of interest is identifying methods to reduce organ injury during this process. The primary consequence of cold ischemic injury is delayed graft function (DGF) in kidney transplants.[180–182] These consequences have both short-term and long-term effects. In a kidney transplant, for example, DGF increases patient morbidity in the short term because the hospital stay is longer and dialysis may be required. In the long term, DGF independently predicts reduced 1- and 5-year graft survival.[183]

Both human and animal studies suggest that the adverse impact of cold ischemia may be associated with apoptosis. In human kidney transplant biopsies performed after 1 hour of reperfusion, apoptosis of tubular cells correlated significantly with cold ischemic time.[184] Biopsies of human donor kidneys, which subsequently developed postoperative AKI, demonstrated increased renal tubular epithelial cell apoptosis.[185] Prolonged cold ischemia has also been shown to increase apoptotic cell death in rat kidney allografts at 24 weeks posttransplant.[186] Mitochondria undergo significant changes during ischemia and may contribute to preservation injury.[187]

Caspases have been studied in cold ischemic kidneys.[188] Kidneys were stored for 48 hours at 4°C to produce cold ischemia. Caspase-3 activity was massively increased (100-fold) in cold ischemic kidneys compared to controls. On immunoblot analysis, the processed form of caspase-3

was increased in cold ischemic kidneys compared to controls. The increase in caspase-3 was associated with significantly more renal tubular apoptosis and brush border injury. The pan-caspase inhibitor prevented the formation of the processed form of caspase-3 and the increase in caspase activity, and reduced apoptosis and brush border injury. The results of this study suggest that caspase inhibition may prove useful in kidney preservation.

In a pig model of cold ischemia in which the heart was stopped to mimic donation after cardiac death (DCD), there was a massive increase in apoptosis, caspase-3/7 activity, and caspase-3 protein expression.[189] Apoptosis was compared in DCD kidneys subjected to static versus pulsatile perfusion for 24 hours. Pulsatile perfusion significantly reduced proximal tubular apoptosis and was associated with increased Bcl-2 and hypoxia-inducible transcription factor-1α in the kidney.

The relevance of these studies to organ preservation and the subsequent risk of graft dysfunction is substantial. Caspase inhibitors are a particularly attractive approach to reduce the incidence of DGF in kidney transplantation.

Caspase-1 and IL-18

Caspase-1 is a proinflammatory caspase that cleaves precursor IL-1β and precursor IL-18. Caspase-1-/- mice developed less ischemic AKI as judged by renal function and renal histology (Fig. 29.6A).[190] IL-1β receptor knockout mice or mice treated with IL-1β receptor antagonist (IL-1Ra) are not protected against ischemic AKI.[30] Because caspase-1 also activates IL-18, lack of the mature form of IL-18 in these caspase-1-/- mice was investigated as a possible mechanism of this protection against AKI. Kidney IL-18 was more than 100% increased in wild-type AKI as compared to sham-operated controls. On an immunoblot analysis, there was

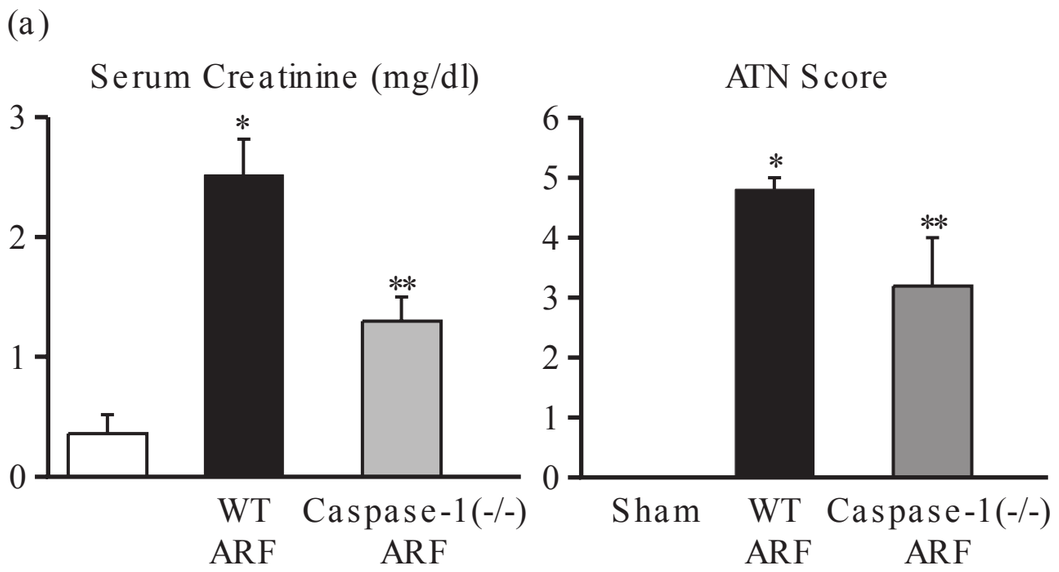


FIGURE 29.6 Caspases and interleukin (IL)-18 in ischemic acute kidney injury (AKI). **A:** Caspase-1-/- mice are protected against ischemic AKI. Caspase-1-/- mice developed less severe AKI, as determined by the serum creatinine and AKI scores compared with wild-type (WT) mice with AKI. * $P < .001$ versus sham; ** $P < .01$ versus WT AKI. **B:** Caspase-1 converts the pro to mature IL-18. Mice treated with IL-18 antiserum (AS) are functionally protected against ischemic AKI. In vehicle-treated mice with ischemic AKI (Veh AKI), serum creatinine and blood urea nitrogen (BUN) levels were significantly increased at 24 hours compared with sham-operated controls. In mice treated with neutralizing IL-18 AS, the serum creatinine and BUN levels were significantly reduced. * $P < .01$ versus sham, ** $P < .01$ versus vehicle-treated mice with Veh AKI. **C:** Mice treated with the pan-caspase inhibitor OPH-001 are protected against ischemic AKI. In vehicle-treated mice with ischemic AKI, the serum creatinine and AKI scores were significantly increased at 24 hours of postischemic reperfusion compared with sham-operated controls. In mice treated with OPH-001 (OPH) before the induction of ischemic AKI, the serum creatinine and AKI scores were significantly decreased compared with sham-operated controls. * $P < .001$ versus sham; ** $P < .01$ versus vehicle-treated mice with Veh AKI; not significant versus sham. ARF, acute renal failure; ATN, acute tubular necrosis. Reproduced from Melnikov et al.,[191] with permission.

a conversion of the precursor to the mature form of IL-18 in AKI wild-type mice, but not in the caspase-1-/- AKI mice and sham-operated controls. To further analyze the role of IL-18, wild-type mice were injected with rabbit antimurine IL-18 neutralizing antiserum prior to the ischemic insult. These mice were protected against AKI to a similar degree as caspase-1-/- mice (Fig. 29.6B).

Caspase-deficient mice have provided extensive information on the role of individual caspases in disease processes. The study of caspase inhibitors is an important step toward the possible therapeutic effect of caspase inhibition in ischemic AKI. Mice with ischemic AKI treated with newly developed caspase inhibitor, Q-VD-(Ome)-OPH (OPH-001) had a marked reduction (100%) in BUN and serum creatinine and a highly significant reduction in the morphologic AKI score compared with vehicle-treated mice (Fig. 29.6C).[191] OPH-001 significantly reduced the increase in caspase-1 activity and IL-18 and prevented neutrophil infiltration in the kidney during ischemic AKI. To further investigate whether this lack of neutrophil infiltration was contributing to the protection against ischemic AKI, a model of neutrophil depletion was developed. Neutrophil-depleted mice had a small (18%) reduction in serum creatinine during ischemic AKI but no reduction in the AKI score despite a lack of neutrophil infiltration in the kidney. Remarkably, caspase-1 activity and IL-18 were still significantly increased in the kidney in neutrophil-depleted mice with AKI. Thus, to investigate the role of IL-18 in ischemic AKI in the absence of neutrophils, neutrophil-depleted mice were treated with an IL-18–neutralizing antiserum. IL-18-antiserum–treated neutrophil-depleted mice with ischemic AKI had a significant reduction (75%) in serum creatinine and a significant reduction in the AKI score compared to vehicle-treated neutrophil-depleted mice. These results suggest a novel neutrophil-independent mechanism of IL-18–mediated ischemic AKI.

In other studies, it was determined whether IL-18–binding protein transgenic (IL-18BP Tg) mice are protected against ischemic AKI.[192] IL-18 function is neutralized in IL-18BP Tg mice. IL-18BP Tg mice with AKI had significantly lower BUN, serum creatinine, and ATN score than wild-type mice. The number of macrophages in the kidney was significantly reduced in IL-18BP Tg compared with wild-type mice. The proinflammatory chemokine, CXCL1 (also known as KC or IL-8), was significantly reduced in the kidneys of IL-18BP Tg mice compared to wild-type mice. This study demonstrates that protection against ischemic AKI in IL-18BP Tg mice is associated with less macrophage infiltration and less production of CXCL1 in the kidney.

The effects of different caspase inhibitors on ischemic AKI in the rat kidney have been studied.[193] A caspase-1 inhibitor significantly reduced functional and histologic evidence of ischemic AKI compared to a caspase-3 inhibitor. Another group of investigators found that caspase-1–deficient mice were not protected against renal ischemia.[194] In this study, the model of renal ischemia was 45 minutes of unilateral renal pedicle clamping with contralateral nephrectomy. This model produces a milder form of functional injury than bilateral clamping. At 24 hours, BUN and creatinine were lower in the caspase-1-/- mice than in the wild-type, but the decrease was not statistically significant.

Matrix Metalloproteinases

Matrix metalloproteinases are a large family of zinc-dependent matrix-degrading enzymes that include interstitial collagenases, stromelysins, gelatinases, elastases, as well as membrane-type matrix metalloproteinases. They play a crucial role in remodeling the extracellular matrix, which is an important physiologic feature of normal growth and development. In the kidney, interstitial sclerosis and glomerulosclerosis have been associated with an imbalance of extracellular matrix synthesis and degradation.[195] Alterations in renal tubular basement membrane matrix proteins, laminin and fibronectin, occur after renal ischemia-reperfusion injury.[196] The role of matrix metalloproteinases in this process has been studied.

In endothelial cells isolated from ischemic kidneys, the proteolytic activity of proMMP-2, proMMP-9, and MMP-9 was increased. Occludin, an in vivo MMP-9 substrate, was partly degraded in the endothelial fractions during ischemia, suggesting that the upregulation of MMP-9 had a functional effect to degrade occluding. These data suggest that AKI leads to the degradation of the vascular basement membrane and to increased permeability related to the increase of MMP-9.[197] In renal cells, in vitro cleavage of cadherins in normal rat kidney (NRK) cells requires active membrane-type (MT)1-MMP (MT1-MMP), also known as MMP-14.[198] The disruption of cadherin/catenin complexes in AKI may be associated with the transtubular back-leak of glomerular filtrate. In contrast to the potential injurious role of some MMPs, MMP9 protects the S3 segment of the proximal tubule and the intercalated cells of the collecting duct from apoptosis in AKI, most likely by releasing soluble stem cell factor (sSCF), an MMP9 substrate.[199]

Meprin A is a zinc-dependent metalloendopeptidase that is present in the brush border membrane of renal proximal tubular epithelial cells. The redistribution of this metalloendopeptidase to the basolateral membrane domain during AKI results in degradation of the extracellular matrix and damage to adjacent peritubular structures. The effect of meprin A, the major matrix degrading metalloproteinase in rat kidney, on the laminin–nidogen complex was examined. Nidogen-1 (entactin) acts as a bridge between the extracellular matrix molecules, laminin-1 and type IV collagen, and thus participates in the assembly of basement membranes. Following ischemic injury, meprin A undergoes redistribution and/or adherence to the tubular basement membrane. Nidogen breakdown products are produced as the result of partial degradation of tubular basement membrane by meprin A following renal tubular ischemia–reperfusion injury.[200]

The susceptibility of inbred strains of mice to ischemic and nephrotoxic acute renal failure was studied in mice with normal and low meprin A activity.[201] The strains of mice with normal meprin A developed more severe renal functional and structural injury following renal ischemia or the injection of hypertonic glycerol compared to the two low meprin A strains. These findings suggest that meprin A plays a role in the pathophysiology of AKI following ischemic and nephrotoxic acute renal failure insults to the kidney.[201] A recent study[202] demonstrated that meprin inhibition protects against ischemic AKI in vivo in rats.

Nitric Oxide

NO is a lipophilic, highly reactive free radical gas with diverse biomessenger functions.[203] NO mediates diverse functions including vasodilatation, platelet aggregation inhibition, neurotransmission, inflammation, antimicrobial and antitumor actions, and apoptosis.[203] Whether the net effects of NO are beneficial or deleterious is determined by the cell type, the concentration of NO, the duration of production, and the composition of the surrounding microenvironment.[203] There are three major nitric oxide synthase (NOS) isoforms in the kidney: neuronal NOS or nNOS (also known as NOS1), inducible NOS (iNOS, also known as NOS2), and endothelial NOS or eNOS (also known as NOS3) (Table 29.5).[204] The macula densa is the principal site of nNOS expression in the kidney.[205,206] In situ hybridization studies in the NRK demonstrate iNOS mRNA in the S3 segment of the proximal tubule, the cortical and medullary thick ascending limb, the distal convoluted tubule, and the cortical collecting duct and the inner medullary collecting duct.[207] eNOS mRNA has been detected in glomeruli, preglomerular vasculature, and proximal and distal tubules.[208] eNOS protein is mainly present in the endothelium of intrarenal, afferent, efferent, and glomerular arterioles and the medullary vas recta.[205] Expression of eNOS protein in tubules has not yet been reported.[204] nNOS and eNOS are continuously present, are activated by calcium, and are also termed constitutive NOS (cNOS).[209,210] In contrast, iNOS is induced when the cells have been stimulated by certain cytokines, microbes, and microbial products, and thus is called iNOS.[211,212] The time course of both calcium-dependent and -independent NOS activity in the rat renal cortex and medulla has been studied.[213] Calcium-dependent NOS activity in the cortex and the medulla decreased in the early phase of AKI and then increased in the recovery phase in the cortex. iNOS activity increased in the early phase of AKI in both the cortex and the medulla and was maintained at higher levels in the medulla. However, in another study, L-arginine improved the deficiency of constitutive NOS activity and improved the recovery phase of ischemic AKI in rats.[214]

Studies in freshly isolated proximal tubules from knockout mice have also revealed the role of NO in hypoxic/ischemic tubular injury. Hypoxia-induced proximal tubule damage, as assessed by LDH release, was no different between wild-type and mice in which eNOS and nNOS had been knocked out. However, proximal tubules from iNOS knockout mice demonstrated resistance to the same degree of hypoxia.[42] The iNOS knockout mice also had less renal failure and better survival than the wild-type mice after renal artery clamping.[215] An induction of heat shock protein was also observed in the iNOS knockout mice as a potential con-

29.5 Nitric Oxide Synthase (NOS) Isoforms

	Tissue Distribution		
Isoform	**Body**	**Renal**	**Phenotype of Knockout Mouse**
nNOS (Type 1)	Neurons, skeletal muscle, penis	Macula densa	Protection against cerebral ischemia[478,479]
iNOS (Type 2)	Constitutive: ileum, uterus, skeletal muscle. Induced: macrophage, VSMC	Constitutive: mTAL, proximal tubule	Less hypotensive response to LPS[480] Increased mortality in polymicrobial sepsis[481] No protection against LPS-induced AKI[375] Protection against ischemic AKI[215]
eNOS (Type 3)	Endothelium	Glomerular vessels, intrarenal arteries	Hypertension[482] Increased susceptibility to stroke[483] and myocardial ischemia[484]

nNOS, neuronal nitric oxide synthase; iNOS, inducible nitric oxide synthase; eNOS, endothelial nitric oxide synthase; VSMC, vascular smooth muscle cell; mTAL, medullary thick ascending limb; LPS, lipopolysaccharide; AKI, acute kidney injury.

tributor to the protection. Chiao et al.[216] produced further results in a renal artery clamp model in mice in which alpha melanocyte-stimulating hormone (αMSH) was shown to block the induction of iNOS, decrease neutrophil infiltration, and afford functional protection. A subsequent study examined the relative importance of αMSH on the neutrophil pathway by examining the effects of αMSH in ICAM-1 knockout mice and neutrophil-poor isolated perfused kidneys where neutrophil effects are minimal or absent.[217,218] In this study, it was found that αMSH decreases renal injury when neutrophil effects are minimal or absent, indicating that αMSH inhibits neutrophil-independent pathways of renal injury.

Hypoxia was found to increase NO release from freshly isolated proximal tubules and this effect was blocked by L-NAME, a nonspecific NOS inhibitor, but not by the inactive D-NAME compound.[39,219] The NO release during hypoxia was accompanied by LDH release and was reversed by L-NAME administration. Interestingly, however, the administration of L-NAME, the nonspecific NOS inhibitor, to the rat kidney clamp model actually worsened the renal failure.[220] This result was interpreted as an overriding blocking effect of eNOS activity with the nonspecific effects of L-NAME.[68] This would worsen the renal vasoconstriction and the resultant injury, thus obscuring any salutary effect at the level of the proximal tubule.[221] Thus, opposing abnormalities in NO production within the endothelial and tubular compartments of the kidney may contribute to renal injury.[68] Reduced eNOS-derived NO production causes vasoconstriction and worsens ischemia; increased iNOS-derived NO production by tubular cells adds to the injurious effects of ischemia on these cells. Therapeutic interventions to modulate NO production in ischemic AKI may require the selective modulation of different NOS isoforms in the tubular and vascular compartments of the kidney.[222] On this background, Noiri and colleagues[220] performed studies using a specific antisense oligonucleotide to iNOS. The ischemia-induced upregulation of iNOS and nitrite production were both blocked by the antisense oligonucleotide. Most importantly, the BUN and serum creatinine did not rise after the renal ischemic insult in the animals treated with the antisense oligonucleotide against iNOS.

Noiri and colleagues also studied the relationship between NO and osteopontin during ischemic AKI. Osteopontin is a negatively charged glycosylated phosphoprotein that is expressed in many tissues, including the renal epithelial cells. Osteopontin serves both a cell attachment function and a cell signaling function via the alpha-v beta-3 integrin. Effects on gene expression include suppression of the induction of NOS by inflammatory mediators. Osteopontin may play an important role in the pathophysiology of AKI. Osteopontin knockout mice subjected to renal ischemia developed worse renal failure and more structural damage than wild-type controls.[223] This was associated with the augmented expression of inducible NOS and the prevalence of nitrotyrosine residues in kidneys from osteopontin knockout mice versus wild-type counterparts. This study[220] provides strong evidence of the renoprotective action of osteopontin in acute renal ischemia.

The protective effect of 17 β-estradiol against ischemic AKI in rats is due to the activation of the PI3K/Akt pathway followed by increased eNOS phosphorylation.[224]

The microvillar actin and cellular integrins are potential substrates of NO action, which could contribute to the ischemia-mediated sloughing of the brush border membrane and the detachment of the proximal tubule epithelial cells from their extracellular matrix.[11,225–227] Such an effect would not only result in impaired tubular sodium reabsorption, but would also provide intraluminal cellular debris as a component of tubular cast formation.

Heat Shock Proteins

The stress response is a highly conserved homeostatic mechanism that allows cells to survive a variety of different stresses.[228] Stresses that trigger the heat shock response include hyperthermia, hypothermia, the generation of oxygen radicals, hypoxia/ischemia, and toxins.[229] On a molecular level, their function is to protect cells from environmental stress damage by binding to partially denatured proteins, dissociating protein aggregates, regulating the correct folding, and cooperating in transporting newly synthesized polypeptides to the target organelles.

The proteins induced by these stresses belong to a family of proteins called heat shock proteins (HSP). The proteins are identified by their molecular weight. The most important families include proteins of 90, 70, 60, and 27 kDa.[229] HSP 90 is essential for cell viability. It is associated with the steroid hormone receptor and is a general chaperone with ATPaselike activity. In stressed cells, it associates with the cytoskeletal protein, actin. The HSP 70 family includes proteins that are both constitutively expressed and induced by stress. They are the most highly induced proteins by stress and function as chaperones, binding to unfolded or misfolded proteins. The HSP family is restricted to the mitochondrial matrix where it functions as an unfoldase. The HSP 27 family has functions similar to HSP 70. Ubiquitin is a stress protein that binds denatured proteins and targets them for proteolysis by the proteasome.

Renal ischemia results in both a profound fall in cellular ATP and a rapid induction of the 70 kD heat shock protein family, HSP-70.[230,231] The relationship between cellular ATP and the induction of the stress response in the renal cortex during renal ischemia has been studied. Van Why et al.[232] demonstrated that a 50% reduction in cellular ATP in the renal cortex must occur before the stress response is detectable, that a reduction of ATP below 25% of control levels produces a more vigorous response, and that reperfusion is not required for the initiation of a heat shock response in the kidney.[232]

Ischemic AKI also induces differential expression of small HSPs. In sham-operated kidneys, HSP 25 localized to glomeruli, vessels, and collecting ducts, whereas another

stress protein, αB-crystallin, localized primarily in medullary thin limbs and collecting ducts. After ischemia, HSP 25 accumulated in proximal tubules in the cortex and the outer medulla, whereas αB-crystallin labeling became nonhomogeneous in the outer medulla, and increased in the Bowman capsule. This study demonstrates that there is striking differential expression of HSP 25 and αB-crystallin in various renal compartments.[233]

In vitro studies have demonstrated that HSP induction protects cultured renal epithelial cells from injury. It has been determined that prior heat stress protects opossum kidney (OK) cells, a cultured renal epithelial cell line, from injury mediated by ATP depletion.[234] Also, HSP 70 overexpression is sufficient to protect cultured proximal tubule (LLC-PK1) cells from hyperthermia but is not sufficient to protect against hypoxia.[235]

During ischemic AKI, the question of whether prior HSP induction by hyperthermia is protective is controversial. One study[236] found that prior heat shock protected kidneys against warm ischemia. Another study[237] investigated the protective effect of heat shock proteins on ischemic injury to renal cells in two different experimental models: ischemia-reflow in intact rats and medullary hypoxic injury as seen in the isolated perfused rat kidney. The prior induction of HSP by hyperthermia was not protective against the functional and morphologic parameters of ischemic AKI in either of these models.[237] These variable results may be explained by the complexity of the intact animal compared to cultured cells; the degree, duration, and timing of the hyperthermic stimulus; and the differential response of mature and immature kidneys.[24,238]

Pharmacologic agents have been used to increase stress protein expression. In a recent study, the induction of HSP 70, a potent antiapoptotic agent, inhibited ischemic renal injury in mice.[239] Recently, inhibitors of the proteasome have been identified that can block the rapid degradation of abnormal cytosolic and ER-associated proteins. The hypothesis that proteasome inhibitors, by causing the accumulation of abnormal proteins, might stimulate the expression of cytosolic heat shock proteins and/or ER molecular chaperones and thereby induce thermotolerance was tested in Madin-Darby canine kidney cell cultures.[240] The inhibition of proteasome function induced heat shock proteins and ER chaperones and conferred thermotolerance in these cells. Thus, these agents may have applications in protecting against cell injury.[240] Another study[241] determined that proteasome inhibition protects against the morphologic and functional abnormalities in ischemic AKI in rats.[241] However, the effect of proteasome inhibition on HSP induction during ischemic AKI was not determined in this study.

The mechanism of HSP protection against ischemic AKI is interesting. It has been suggested that HSPs participate in the postischemic restructuring of the cytoskeleton of proximal tubules.[242] It was found that HSP 72 complexes with aggregated cellular proteins in an ATP-dependent manner, suggesting that enhancing the HSP 72 function after an ischemic renal injury assists refolding and stabilization of Na(+)-K(+)-ATPase or aggregated elements of the cytoskeleton, allowing reassembly into a more organized state.[243] Another study examined the temporal and spatial patterns of HSP 25 induction in relation to the actin cytoskeleton.[244] This study suggested that there are specific interactions between HSP 25 and actin during the early postischemic reorganization of the cytoskeleton. In another study,[245] the Brown Norway rat was resistant to renal failure and AKI compared to the Sprague-Dawley rat. The Brown Norway rat had no distribution of Na-K-ATPase into detergent soluble cortical extracts, and the immunohistochemistry showed that baseline HSP 72 and 25 expression was increased in proximal tubules of the Brown Norway rats compared to the Sprague-Dawley rats.

Another potential mechanism of HSP protection against proximal tubular injury is the inhibition of apoptosis. OK proximal tubule cells exposed to ATP depletion develop apoptosis by morphologic and biochemical criteria. Prior heat stress reduced the number of apoptotic-appearing cells, significantly decreased DNA fragmentation, and improved cell survival compared with controls.[246] This study demonstrated that novel interactions between HSP 72 and the antiapoptotic protein Bcl2 may be responsible, at least in part, for the protection afforded by prior heat stress against ATP depletion injury.

Altered Gene Expression

During renal ischemia in vivo, the reaction of the renal epithelial cells is heterogeneous.[247] Some cells, especially those of the proximal tubule, undergo necrosis. Other cells undergo apoptosis, and still others survive the ischemic injury intact. In addition, injured tubules are relined with new cells actively engaged in DNA repair and synthesis. Thus, surviving tubular cells can reenter the cell cycle and replicate. These cells may undergo partial dedifferentiation that allows them to undergo mitosis.[248] The complex events that mediate this heterogeneous response of tubular cells are being studied. This response of tubular cells may involve the early immediate gene response.

Immediate early genes and proto-oncogenes are induced during the early reperfusion period after renal ischemia.[249] There is c-Fos and c-Jun activation as well as an increase in DNA synthesis.[250] There is an accumulation of early growth response factor 1 (Egr-1) and c-Fos mRNAs in the mouse kidney after occlusion of the renal artery and reperfusion.[251,252] Transient expression of the genes c-Fos and Egr-1 may code for DNA binding transcription factors and initiate the transcription of other genes necessary for cell division.[253] JE and KC, growth-factor–responsive genes with cytokinelike properties that play a role in inflammation, are also expressed during early renal ischemia.[254] These genes may code for proteins with chemotactic effects that can attract monocytes and neutrophils into areas of injury.[252] Studies demonstrate that c-Fos and c-Jun are expressed following renal ischemia as a typical immediate

early gene response, but they are expressed in cells that do not enter the cell cycle.[248,255] The failure of the cells to enter the cell cycle may depend on the coexpression of other genes.

DNA synthesis occurs in the proximal tubule, whereas the induction of the early gene response is restricted to cells of the thick ascending limb and collecting duct.[252] Thus, the immediate early gene response does not always occur in cells that undergo DNA synthesis, suggesting that the role of the early gene response is not necessarily proliferative in this setting. The role of the stress response during renal ischemia and the fate of the cells undergoing it are unknown. This immediate early gene response may play a role in the protection of tubular cells against injury. Alternatively, it may be important in mounting a response that will later help the regeneration of other tubular cells because the products of some of these genes are localized to cells that do not undergo cell death from apoptosis or necrosis.[256] The immediate early gene response may be the response to sublethal injury, allowing the cell to dedifferentiate.[253]

The pathways that lead to the early gene response are interesting. At least two quite different pathways lead to the activation of c-Jun.[257–259] Growth factors activate c-Jun via the mitogen-activated protein kinases (MAPKs), which include extracellular-regulated kinases (ERKs) 1 and 2. This pathway is proliferative in nature. In contrast, the stress-activated protein kinase (SAPK) pathway is separate from the MAPK pathway. These kinases include c-Jun N-terminal kinase (JNK) 1 and 2. Activation and the effect on cell fate of the SAPK pathway is very different from the MAPK pathway. The SAPK pathway is essentially antiproliferative and can lead to either cell survival or cell death. During renal ischemia, SAPKs are activated, and the inhibition of SAPK after ischemia protects against renal failure.[260,261] Thus, it is possible that manipulation of this pathway could lead to therapies that may ameliorate AKI.

Numerous recent studies have analyzed gene expression during ischemic AKI. Cell communication, apoptosis, and inflammation genes distinguish primary allograft function in human kidney transplantation.[262] In renal ischemia–reperfusion in mice, there was an increase in genes involved in cell structure, extracellular matrix, intracellular calcium binding, and cell division/differentiation.[263] In another study in mice, there were consistent patterns of altered gene expression in the first 24 hours of postischemic reperfusion.[264] These genes included transcription factors, growth factors, signal transduction molecules, and apoptotic factors. In ischemia–reperfusion in the rat, alterations in the expression of 18 genes were identified by microarray analysis.[265] Nine genes were upregulated (ADAM2, HO-1, UCP-2, and thymosin β4 in the early phase, and clusterin, vanin1, fibronectin, heat-responsive protein 12, and FK506-binding protein in the established phase). Nine genes were downregulated (glutamine synthetase, cytochrome p450 IId6, and cyp 2d9 in the early phase, and cyp 4a14, Xist gene, peroxisome proliferator-activated receptor gamma (PPARγ), α-albumin, uromodulin, and ADH B2 in the established phase). Changes in the gene expression of ADAM2, cyp2d6, fibronectin, HO-1, and PPARγ were confirmed by quantitative real-time polymerase chain reaction (PCR). One of the problems with microarray analysis in the whole kidney during ischemic AKI in vivo is identifying which of the numerous cell types in the kidney is the source of the gene alteration. Laser capture microdissection of immunofluorescently defined cells (IF-LCM) can isolate pure populations of targeted cells from a sea of surrounding cells with excellent preservation of mRNA.[266] This technique has been used to label and isolate thick ascending limb cells in the kidney for mRNA analysis.[266]

In ischemic AKI in mice, 24,600 genes were tested by transcriptional analysis.[267] At days 3, 10, and 28 after ischemic AKI, 242, 146, and 46 genes were upregulated, respectively, and 85, 35, and zero genes were downregulated, respectively. Gene expression changes were primarily related to immune and inflammatory pathways both early and late after AKI. The most highly upregulated genes late after AKI were hepatitis A virus cellular receptor 1 (Havcr1) and lipocalin 2 (Lcn2), which code for KIM-1 and NGAL, respectively.

The PPARs are a group of nuclear receptor proteins that function as transcription factors regulating the expression of genes. PPARs play crucial roles in the regulation of cellular differentiation, development, and metabolism and tumorigenesis. Increased expression of PPARα[268] or PPARβ/δ[269] protects aginst ischemic AKI. Maintenance of free fatty acid oxidation in the proximal tubule may be the mechanism of the protection against ischemic AKI.[270]

Apoptosis

Apoptosis was first described by Kerr et al.[271] The term comes from the ancient Greek word that means "the dropping off as of leaves from a tree." The term stresses the facts that apoptosis is a physiologic form of cell death, occurs in the individual cell (or leaf) in a programmed pattern, and can be triggered according to a program regulated by external stimuli (autumn).[7] Thus, apoptosis is the name given to the process of physiologic or programmed cell death. Apoptotic cells undergo a series of morphologically identifiable changes in their pathway to cell death.[272] The morphologic, biochemical, and molecular characteristics of apoptosis versus necrosis are very different (Table 29.6). Autophagy has been proposed as a third mode of cell death.[273] Autophagy is a process in which cells generate energy and metabolites by digesting their own organelles and macromolecules. Autophagy permits a starving cell, or a cell that is deprived of growth factors, to survive. There are differences in the mode of death and different morphologic, biochemical, and molecular attributes between apoptosis, necrosis, and autophagy.[273]

The triggers of apoptosis include (1) cell injury (e.g., ischemia, hypoxia, oxidant injury, nitric oxide, cisplatin); (2) loss of survival factors (e.g., deficiency of renal growth factors, impaired cell-to-cell or cell-to-matrix adhesion);

29.6 Morphologic, Biochemical, and Molecular Differences Between Apoptosis and Necrosis

Apoptosis	Necrosis
Individual cells shrink and detach from other cells	Multiple cells swell but remain attached
Plasma membranes remain intact	Plasma membrane disruption
Cell excludes DNA-specific dye, propidium iodide	Propidium iodide enters cell and stains nucleus
Nuclear condensation, fragmentation, and pyknosis	Nuclear swelling and autolysis
Apoptotic bodies	No apoptotic bodies
Nuclear DNA fragmentation	Nuclear DNA fragmentation
Programmed by gene activation	No gene activation
Phagocytosis of cellular fragments	Cellular lysis
No inflammation	Inflammation

Autophagy has been proposed as a third mode of cell death.[273] Autophagy is a process in which cells generate energy and metabolites by digesting their own organelles and macromolecules, permitting a starving cell or a cell that is deprived of growth factors to survive.

and (3) receptor-mediated apoptosis (e.g., Fas [CD 95] and transforming growth factor [TGF]β).[274]

The two major pathways of apoptosis involve Fas and p53.[9,10,275] In the tumor necrosis factor (TNF) receptor superfamily, Fas antigen (CD 295) is the most important factor. Engagement of Fas by its ligand (FasL) results in apoptosis. The tumor suppressor gene, p53, mediates apoptosis in cells in which the DNA has been damaged. The cascades involving Fas and p53, which are centrally important in cell death, are shown in Figure 29.7.

Caspases are the major mediators of the cell death in apoptosis and also play a role in necrotic cell death. The central role of caspases in cell death is supported by caspase-8, 9, and 3 knockout mice that have strong phenotypes based on apoptotic cell death defects, developmental defects, and usually fetal/perinatal mortality.[162] Caspase-7, like caspase-3, is an executioner caspase and is downstream of the initiators, caspase-8 and 9. Both the intrinsic and extrinsic pathways activate caspase-7. Caspase-3 and 7 exhibit very similar substrate specificities in peptide hydrolysis assays in vitro.[276] However, the role of caspase-7 during the execution phase of apoptosis is obscure.[277] Caspase-7 is unable to cleave the well-known caspase-3 substrates including fodrin, gelsolin, DNA fragmentation factor 45 (DFF45), inhibitor of apoptosis proteins (IAP), and signal transducer and activator of transcription 1 (STAT-1).[277] Also, caspase-7 is unable to activate caspases that would normally be activated by caspase-3.[278] It is known that caspase-3 and 7 can act independently as executioners of apoptosis. Both caspase-3 and caspase-7 deficient mice are perinatally lethal due to a lack of apoptosis.[279] Caspase-7 may play a more specialized role in apoptosis than caspase-3.[277] Both caspase-3 and 7 are critical mediators of mitochondrial events of apoptosis.[280] Caspases have been described in detail earlier in this chapter.

Caspase-dependent or independent endogenous endonuclease activation, resulting in DNA fragmentation, is considered a characteristic biochemical marker for apoptosis.[49] However, DNA fragmentation also occurs in cellular necrosis.[49,281,282] The differentiation of apoptosis from necrosis in tubular cells, therefore, is still difficult[283] and requires both the demonstration of DNA fragmentation, usually using a histochemical technique based on terminal deoxynucleotidyl transferase (TdT) reactivity with DNA breaks, as well as morphologic evidence of apoptosis by light and electron microscopy. Pathways usually associated with apoptosis (e.g., endonuclease activation, increased mitochondrial permeability) may also be associated with necrosis, suggesting that apoptotic and necrotic cell death may share the same pathways.[284] Both apoptosis and necrosis can occur in tissues exposed to ischemia–reperfusion or cultured cells exposed to hypoxia.[285]

The number of in vitro and in vivo studies where apoptosis is described in renal tubules is increasing. These studies are summarized in Tables 29.7 and 29.8. A feature of in vitro prolonged ATP depletion leads to necrosis, whereas milder and shorter ATP depletion leads to apoptotic cell death. A similar pattern has emerged from the in vivo studies (Table 29.8) (i.e., the same insult in a mild form can lead to apoptosis and, when severe, can lead to necrosis).

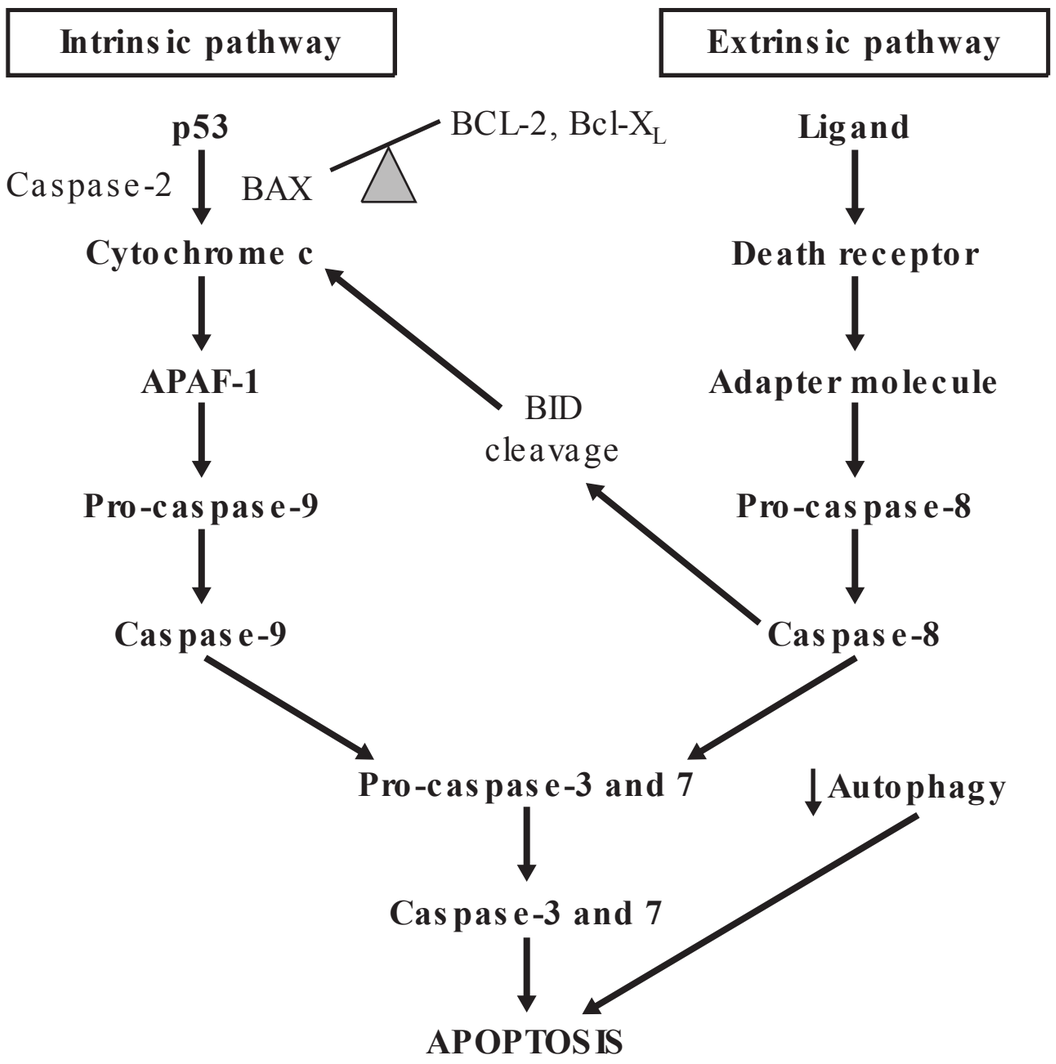


FIGURE 29.7 The major pathways of apoptosis. There are two major pathways of caspase-mediated apoptosis.[461] In the mitochondrial or intrinsic pathway, stress-induced signals (e.g., p53) act via Bcl-2 proteins to cause cytochrome c release from mitochondria. In the intrinsic pathway, there is the binding of a ligand (e.g., Fas ligand) to its death receptor (e.g., Fas) that recruits an adaptor protein. In the intrinsic pathway, the death receptors are a subset of the tumor necrosis factor (TNF) receptor family of cell surface molecules. Cytochrome c binds to a protein called apoptotic protease activating factor-1 (APAF-1). This binding allows APAF-1 to activate caspase-9, an initiator caspase, which then activates caspase-3 and -7. The presence of an excess of the antiapoptotic protein Bcl-2 on mitochondria inhibits cytochrome c release. Caspase-2 is a recently discovered caspase that is a critical initiator of the mitochondrial apoptosis pathway.[463] The activation and increased activity of caspase-2 is required for the permeabilization of mitochondria and the release of cytochrome c.[463] In the extrinsic pathway, the death receptors (CD95/Fas/APO-1, TNFR1, DR3/WSL-1/TRAMP, DR4/TRAIL-R1, DR5/TRAIL-R2, and DR6) are a subset of the TNF/NGF receptor family of cell surface molecules that possess a common motif within their cytoplasmic tails, called the death domain. The death domains of these receptors recruit adapter molecules that, in turn, recruit caspases to the receptor complex. For example, Fas antigen (CD 95) is engaged by its ligand (FasL) resulting in apoptosis. The activation of procaspase-8 requires association with its cofactor Fas-associated death domain (FADD). The pathways may be linked because caspase-8 may cleave a member of the Bcl-2 family, BH3-interacting domain death agonist (BID), which can release cytochrome c. Apoptosis mediated by both pathways has been described during renal ischemia–reperfusion in rats.[464,465] Both caspase-3 and -7 play a crucial and extensively studied role in the promotion of all forms of apoptotic cell death.[160] Caspase-7, like caspase-3, is an executioner caspase and is downstream of the initiators caspase-8 and -9. In general, cells may be more sensitive to apoptosis if autophagy is inhibited.[273] Bax, Bcl-2-associated X protein.

Numerous recent studies have demonstrated that erythropoietin (EPO) protects against ischemic AKI by affecting apoptotic cell death.[286–288] A single dose of EPO either pre-ischemia or just before reperfusion improves renal function and tubular injury; prevents the activation of caspase-3, 8, and 9; and reduces apoptotic tubular cell death.[286] EPO also protects against hypoxia-induced apoptosis in human proximal tubule cells.[287] In the same study, EPO functionally protected against ischemic AKI in rats in vivo and reduced outer medullary thick ascending limb apoptosis while potentiating tubular mitosis and proliferation.[287] In another study in rats with ischemic AKI, EPO decreased serum creatinine, decreased tubular apoptosis and necrosis, decreased tubular cell proliferation, increased antiapoptotic Bcl-2 protein expression, decreased caspase-3 activity, and increased heat shock protein 70 (HSP70) expression.[288] In a model of endotoxemia-induced AKI in mice, EPO significantly decreased renal superoxide dismutase and attenuated the renal dysfunction as assessed by inulin-GFR.[289] EPO receptors are expressed on mesenchymal stem cells (MSCs).[290] EPO results in an expansion of MSCs in bone marrow and the spleen that may mediate protection against ischemic AKI.[290] Thus, EPO may be a potential new therapy for AKI in humans.

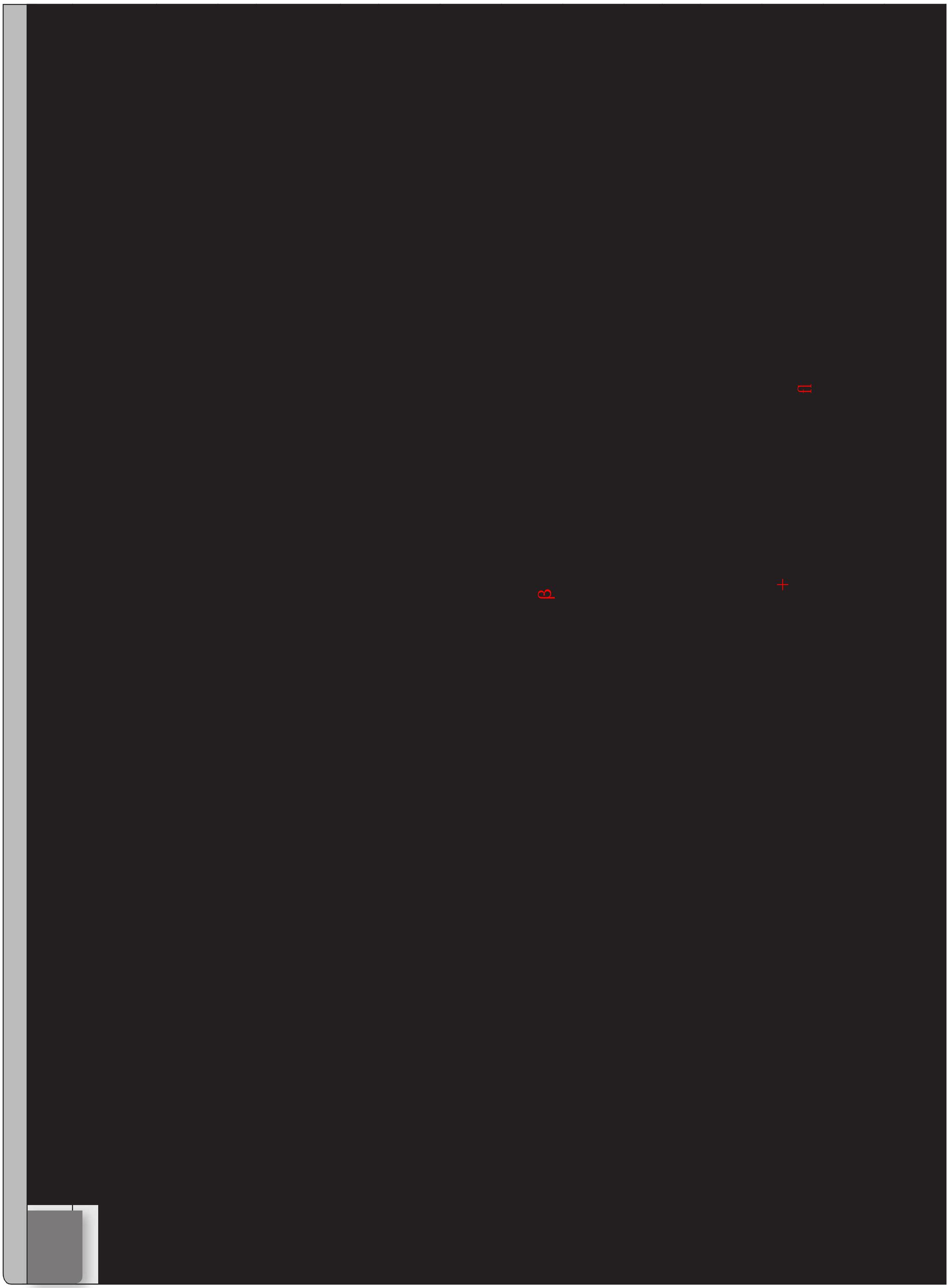
Complement

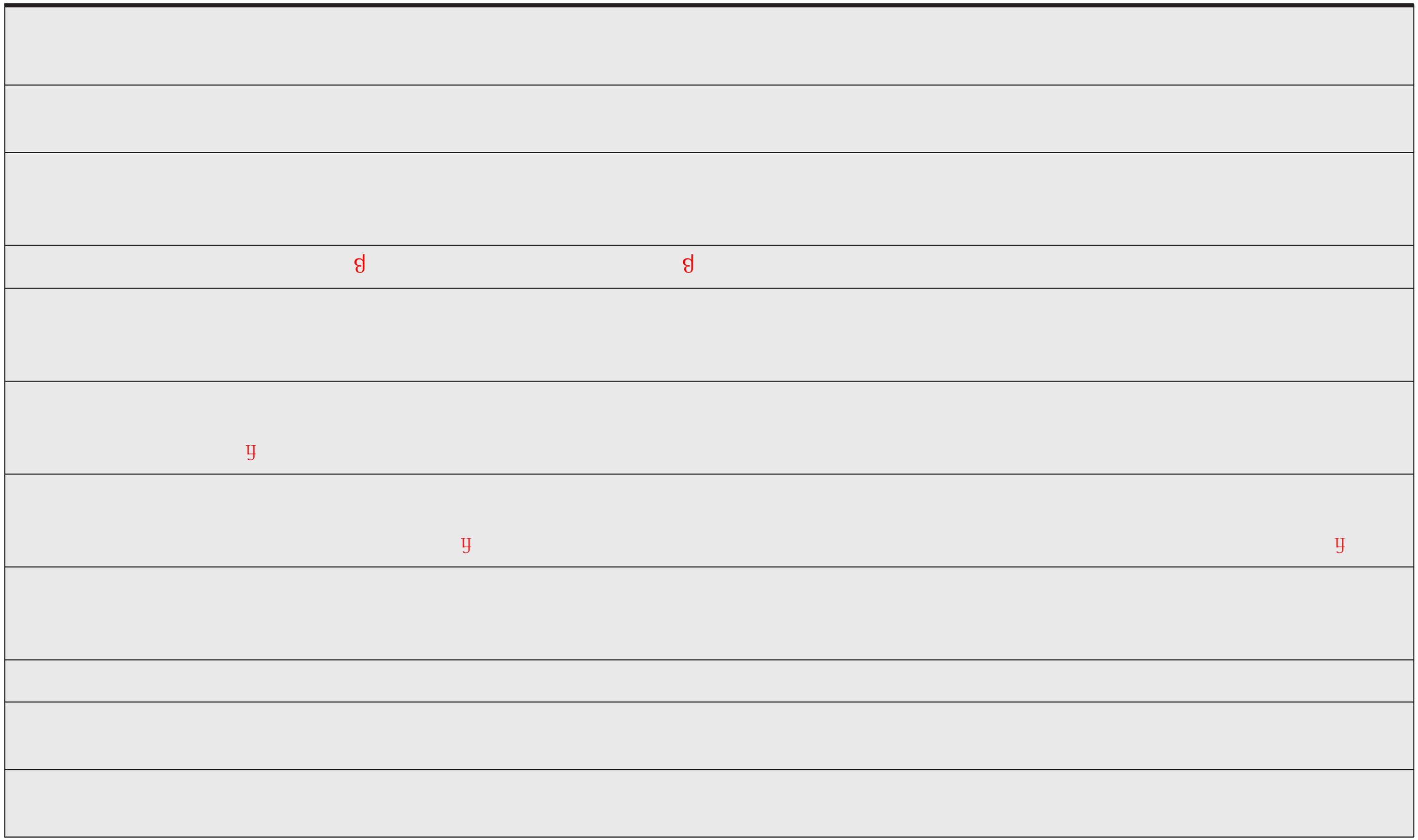
The complement system is a mediator of ischemia–reperfusion injury in the heart, lung, brain, intestines, and muscle.[291] A predominant role for C5b-9 in renal ischemia–reperfusion injury has been demonstrated.[292] In this study, the primary damaging effect of complement was on parenchymal cells rather than vascular endothelial cells. In another study, lack of a functional alternative complement pathway ameliorated ischemic AKI in mice.[293] In this study, mice deficient in factor B, an essential protein in the alternative complement pathway, were functionally and histologically protected against ischemic AKI. Treatment of mice with an inhibitory antibody to mouse factor B prevented the deposition of C3b on the tubular epithelium and systemic generation of C3b and protects against apoptosis and AKI in mice.[294] Loss of polarity of complement receptor 1–related protein y (Crry) in the tubular epithelium precedes activation of the alternative pathway along the basolateral aspect of the tubular cells, and heterozygous gene-targeted mice that expressed lower amounts of Crry are more sensitive to ischemic injury.[295] Also, the inhibition of Crry expression in proximal tubular epithelial cells in vitro resulted in alternative pathway–mediated injury to the cells.[295] Alternative complement pathway activation after renal ischemia reperfusion induces the production of macrophage inflammatory protein-2 (MIP-2) and mouse homolog of IL-8 (KC) by proximal tubule epithelial cells.[296] In addition, the alternative complement pathway, an innate immune system, recognizes hypoxic injury and triggers a systemic inflammatory response through the generation of C3a and a subsequent activation of the NF-κB system.[296]

29.7 Apoptosis in Hypoxic/Anoxic Tubular Injury In Vitro (Cultured Cells)

Cell Type	Type of Injury	Signals	Comment	Reference
MDCK and primary culture rat proximal tubules	Hypoxia		Necrosis also observed	485
Primary culture mouse proximal tubules	Partial ATP depletion (Antimycin A)	Renal growth factors did not ameliorate apoptosis	Severe ATP depletion caused necrosis	36
LLC-PK1 (proximal) and MDCK cells	ATP depletion		Same pattern in LLC-PK1 and MDCK	486
MDCK (distal)	Partial ATP depletion (Antimycin A)	Fas, FADD, caspases, PARP	Severe ATP depletion caused necrosis	487
Opossum kidney (proximal)	ATP depletion (cyanide, 2-deoxy-D-glucose)	Bcl-2/Bax	Prior heat shock attenuated apoptosis	246
Rat proximal tubules	Hypoxia	Caspases Bcl-2	Serine protease inhibitors suppressed caspase-9 activation and apoptosis	488
Rat proximal tubules	Antimycin A	Mitochondrial Ca^{2+} and permeability	L-type calcium channel blocker decreased apoptosis	489
Human tubular cells	Fas transfection	Fas	High but not basal Fas expression caused apoptosis	490
MDCK	Overexpression of ankyrin death domain	Fas	Inhibition of ankyrin Fas interaction decreased apoptosis	491
Rat proximal tubules	Hypoxia Cisplatin Staurosporine	Mitochondrial cytochrome c release	Minocycline upregulates Bcl-2 and decreases apoptosis	492
Rat proximal tubules	ATP depletion	Bid cleavage	Bid cleavage and apoptosis blocked by antiapoptotic Bcl-2 overexpression and caspase-9 inhibition	493
HK-2 cells	Mitochondrial dysfunction	Apoptosis antagonizing transcription factor (AATF)	Silencing of AATF worsened mitochondrial dysfunction	494

MDCK, Madin Darby canine kidney; LLC-PK1, cultured proximal tubule; FADD, fas-associated death domain; PARP, poly ADP ribose polymerase domain; Bax, Bcl-2-associated X protein; HK-2, human kidney-2.





Klotho

Klotho is a transmembrane protein that provides some control over insulin sensitivity and aging. It is increased in the kidney, blood, and urine.[297] Ischemic AKI reduced Klotho in the kidney, blood, and urine. Klotho deficiency resulted in worse AKI, and Klotho overexpression resulted in less AKI. This study suggests that AKI is a state of reversible Klotho deficiency.[297]

The Relative Importance of Proximal Versus Distal Tubular Injury

There is an ongoing debate regarding which nephron segments are most severely injured in ischemic AKI.[84] The target zone for hypoxic injury has also been extensively studied in the isolated perfused rat kidney (IPRK). In this model the target zone predominantly involves the S3 segments of the proximal tubule and also the distal tubules located within the outer stripe of the outer medulla and their cortical equivalent, the medullary rays, those straight sections of the proximal and distal tubules draining the superficial cortical glomeruli. Although the sensitivity of the proximal tubules to injury is well recognized in all models of AKI, the debate over whether the proximal or distal nephron segments are the primary target for hypoxic/ischemic injury has been well reviewed.[84] Of interest in the IPRK model of injury is the presence of a consistent artifact as a result of the absence of an oxygen carrier during erythrocyte-free perfusion. This artifact is the necrosis of mTAL cells first described by Alcorn et al.[298] It had been observed by Leichtweiss et al.[299] that tissue oxygen tension fell sharply in the region of the corticomedullary junction. Studies by Brezis et al.[300] demonstrated that the mTAL lesion resulted from hypoxia and provided support for the hypothesis that countercurrent diffusion of oxygen from descending to ascending limbs of the vasa recta is responsible for the prevailing low oxygen tension of the renal medulla. Subsequently, this group was able to induce a similar lesion in a number of models of renal injury. They have championed the notion that the mTAL segment lies on the brink of hypoxia as a result of the unique architecture of the kidney, which facilitates the countercurrent multiplier required for the formation of concentrated urine.[301] Most recently, it has been demonstrated that the induction of apoptosis targeted only in TAL cells in the absence of neutrophil inflammation in the mouse kidney results in renal failure, oliguria, and an impaired urine concentrating ability.[302]

Other groups have also suggested that the arteriovenous diffusion of oxygen between adjacent parallel arteries and veins is responsible for lowering tissue PO_2 in the corticomedullary region and for maintaining the very low medullary PO_2.[303,304] However, Endre et al.[305] demonstrated in the IPRK in the presence of low concentrations of erythrocytes that mTAL injury was prevented both under control conditions with high perfusate oxygen tension and in the presence of hypoxia.[305] The proximal tubule continued to be injured by hypoxia in the presence of erythrocytes, confirming that mTAL necrosis is an artifact of cell-free perfusion in this model. Nevertheless, coupled with the evidence for preglomerular arteriovenous diffusion of oxygen, which reduces average cortical PO_2 to subvenous levels,[306] the IPRK studies suggest that an even greater amount of the kidney is under threat from hypoxia when renal perfusion is reduced. Such widespread borderline hypoxia may be adaptively useful in priming the oxygen sensor in renal erythropoietin producing cells. However, when there is reduced renal perfusion, critically low levels of oxygen can be reached in tubular regions, particularly where there is high energy demand from transepithelial transport. Clearly, both proximal straight tubules (S3) and mTAL exist in such a region under constant threat of hypoxia. Magnetic resonance (MR) microscopy studies of the IPRK have demonstrated swelling of the cells in these interbundle regions in the outer medulla and their cortical equivalent, the medullary rays, restricting flow through the vascular bundles.[307] These MR observations complement the earlier observations by Thiel et al.,[308] Mason et al.,[309,310] and others that there is erythrocyte aggregation and stasis in the outer stripe of the model after reperfusion following ischemia. This reduction in perfusion may in part mediate the continued injury in this area.

Of greater interest is the observation that frank necrosis of mTAL cells is rarely seen in vivo. However, several studies in the IPRK have observed DNA fragmentation in mTAL cells after brief hypoxia[54] and after 15 or 60 minutes of reperfusion after ischemia.[311] DNA fragmentation has been observed after 24 hours reperfusion following ischemia in vivo in rats,[312,313] although little or no morphologic evidence of apoptosis has been observed in any of these studies. Similar DNA fragmentation was observed in human autopsy specimens after renal hypoperfusion.[314] Recent studies of the Bcl-2 multigene family and growth factors by Gobé et al.[312,314] in a 30 minute bilateral arterial clamp model of ischemia–reperfusion have suggested a way of reconciling the observations of proximal cell necrosis and DNA fragmentation without apoptosis in nearby mTAL. After 24 hours of reperfusion, distal tubules showed a marked increase in the expression of antiapoptotic Bcl-2 and a moderate increase in antiapoptotic Bcl-X_L and proapoptotic Bax. Proximal tubules showed a marked increase in Bax expression and a moderate increase in Bcl-X_L. Twenty-four hours after the expression of the Bcl-2 proteins was increased, IGF-1 and epidermal growth factor (EGF) protein levels were increased in the distal tubule, similar to the Bcl-2 antiapoptotic proteins, and were also detected in the adjacent proximal tubules, suggestive of paracrine action in these tubules. TGF-β expression was moderately increased in regenerating proximal tubules, but no relationship was seen with the pattern of expression of the Bcl-2 genes. An explanation of these results is that the distal tubule is adaptively resistant to ischemic injury via the promotion of survival by antiapoptotic Bcl-2 genes, and its survival allows for the expression of growth factors critical not only to the maintenance and regeneration of its own cell population (autocrine action), but also to the adjacent ischemia-sensitive proximal tubular cells (paracrine action).

Therefore, the hypothesis has been proposed that both the S3 proximal tubule and mTAL cells reside in regions where oxygen availability is borderline. Hypoxia induces both necrosis and apoptosis in proximal tubular cells. Hypoxia triggers apoptosis in mTAL cells but the presence of antiapoptotic Bcl-2 genes prevents the completion of programmed cell death and the DNA fragmentation is repaired. The induction of the growth factors EGF and IGF in these mTAL and distal tubule (DT) cells then provides both autocrine and paracrine mechanisms, respectively, for the recovery of the mTAL and proximal tubules. Because proximal cells are necrotic or have sloughed due to a loss of cell adhesion, proximal tubule recovery is delayed compared to the mTAL. This hypothesis also provides a mechanism for tubular obstruction by casts because viable mTAL cells are the source of Tamm-Horsfall protein.

Tubuloglomerular Feedback

Tubuloglomerular feedback ("tubular communication with the glomerulus") operates within the juxtaglomerular apparatus (JGA) of each nephron where changes are sensed in the salt content of fluid at the luminal macula densa and that information is transmitted to the afferent arteriole to cause compensatory changes in single nephron GFR.[315] nNOS (NOS 1) is expressed in the macula densa and may influence tubuloglomerular feedback. However, micropuncture experiments using NOS antagonists have shown that NO may modulate tubuloglomerular feedback.[315] Local NOS blockade causes the curve that represents tubuloglomerular feedback to shift leftward and become more steep. Changes in macula densa NO production may underlie the resetting of tubuloglomerular feedback, which is required in order to keep the tubuloglomerular feedback curve aligned with ambient tubular flow as tubular flow changes to accommodate physiologic circumstances. Also, macula densa NO production may be substrate limited and dissociated from NOS protein content. The importance of NO to tubuloglomerular feedback resetting and the substrate dependence of NO production have both been found during changes in dietary salt consumption.[316,317] In addition, nNOS inhibition sensitizes the tubuloglomerular feedback mechanism after volume expansion.[318] Macula densa cells detect changes in distal sodium chloride concentration, at least in part, through an apical Na:2Cl:K cotransporter.[319] Macula densa NO directly inhibits Na:2Cl:K cotransport, and NO and angiotensin (AT)II independently alter cotransporter activity.[319] To determine the role of the local renin–angiotensin system on tubuloglomerular feedback, mice with absent renal tissue expression of angiotensin converting enzyme (ACE) were studied.[320] Tubuloglomerular feedback was absent in mice without ACE in the kidney, suggesting that renal tissue ACE is an important contributor to tubuloglomerular feedback.[320] Mice deficient in adenosine A_1 receptors lack tubuloglomerular feedback.[321] Mice deficient in ecto-5′nucleotidase/CD73, the enzyme responsible for adenosine formation from AMP, have an impairment of the tubuloglomerular feedback regulation of GFR.[322]

Taken together, the proximal tubular injury and resultant dysfunction could contribute to the drastic fall in GFR, the hallmark of ischemic AKI. One potential mechanism is increased tubuloglomerular feedback. Specifically, in AKI, decreased proximal tubule reabsorption would increase solute delivery to the macular densa with the resultant constriction of the afferent arteriole and a fall in GFR.[323] In normal nephrons, the maximal fall in GFR with increased solute delivery to the macular densa is approximately 50%. Thus, increased tubuloglomerular feedback could be a major factor in mediating the pathway whereby proximal tubule damage could lower GFR. However, because clinical AKI or ischemic AKI is associated with a 90% fall in GFR, either additional factors or increased sensitivity of tubuloglomerular feedback postischemic injury to the kidney must occur. In that regard, dissected afferent arterioles from ischemic kidneys have been shown to have increased cytosolic calcium concentrations and enhanced vasoconstriction responses to AT-II and endothelin.[324,325] It is thus theoretically tenable that the sensitivity of the tubuloglomerular feedback is indeed enhanced postischemia. However, the role of tubuloglomerular feedback in ischemic AKI remains controversial.[326]

In support of a pathogenic role of tubuloglomerular feedback in ischemic AKI are studies by Brian Meyer and colleagues[55,56] that demonstrated the following: (1) the translocation of NaK-ATPase to the cytoplasm results in depolarization confined to the proximal tubule; (2) the fractional excretion of lithium, a surrogate measure for the fraction of filtered sodium that is delivered to the macula densa, the site of tubuloglomerular feedback, is massively increased; and (3) that these abnormalities persist for the duration of the maintenance phase of postischemic AKI. This study provides evidence for decreased proximal reabsorption of sodium, resultant increased sodium delivery to macula densa, tubuloglomerular feedback, and a resultant filtration failure that accompanies ischemic AKI.

Another pathway whereby tubular injury can contribute to a fall in GFR is by causing intraluminal cast formation and tubule obstruction. This will be the next topic discussed.

TUBULAR CAST FORMATION AND OBSTRUCTION

The classic radiologic findings in early AKI, prior to the realization that contrast is nephrotoxic, was an early dense nephrogram not followed by a pyelogram. Because the nephrogram phase represents contrast entering the tubules by filtration, a persistent nephrogram suggests a tubular obstruction with ongoing glomerular filtration.

Kidneys with ischemic AKI are swollen and, therefore, it was suggested that interstitial edema may lead to tubular collapse secondary to extraluminal-mediated compression.

However, it is clear that recovery from AKI can occur when the kidneys are still enlarged and swollen. An increased excretion of tubular epithelial casts are, however, a hallmark of recovery from AKI.[24] The presence of tubular casts on a renal biopsy, as well as urinary casts, has provided morphologic support for a role in tubular obstruction due to intraluminal cast formation in the pathogenesis of ischemic AKI.[327] As noted previously, although earlier micropuncture studies failed to consistently demonstrate increased tubular pressures postischemia, several subsequent studies provided convincing evidence for the presence of tubular obstruction in experimental ischemic AKI. Arendhorst et al.,[328] using micropuncture techniques during saline loading, demonstrated clear evidence of increased tubular pressures in postischemic, as compared to normal, kidneys. Renal vasodilation to restore renal blood flow also demonstrated increased tubular pressures in ischemic AKI in the rat. Perhaps the most compelling studies, however, were those micropuncture experiments performed by Tanner and Steinhausen.[329] They found that perfusing the proximal tubule with artificial tubular fluid at a rate that did not increase tubule pressure in normal animals increased tubule pressures in animals after a renal ischemic insult. Moreover, venting those obstructed tubules led to improved nephron filtration rates. Burke et al.[330] also demonstrated that the prevention of ischemic AKI in dogs with mannitol led to a decrease in intratubular pressures, suggesting that the induced-solute diuresis led to the relief of the cast-mediated tubular obstruction.

Although it is clear that brush border membranes, necrotic cells, viable cells, and perhaps apoptotic tubular epithelial cells enter tubular fluid after an acute renal ischemic insult, the actual process and predominant location of the cast formation is less clear. It is known that the casts uniformly stain for Tamm-Horsfall protein.[327]

Integrins

Integrins are heterodimeric glycoproteins consisting of different combinations of alpha and beta subunits; they recognize the most common universal tripeptide sequence, arginine-glycine-aspartic acid (RGD), which is present in a variety of matrix proteins.[12] These integrins can mediate cell–cell adhesion via an RGD inhibitable mechanism.[11]

In normal kidneys, proximal tubular cells are stained by the RGD peptide, RhoG-RGD, basolaterally in a punctate pattern and with Bt-RGD only minimally. On the other hand, ischemic kidneys labeling with RhoG-RGD and Bt-RGD occurred at the basolateral and apical aspect of tubular cells as well as on desquamating or desquamated cells within the tubular lumen and also on the vasa rectae.[331] In ischemic kidneys, antibodies to β1 and αV subunits of integrins stained glomeruli and the apical aspect of the proximal and distal tubules. Desquamated cells and cellular conglomerates obstructing the tubular lumina were intensely stained with RGD peptides.[332] Dual labeling experiments with Bt-RGD and antibodies against integrin receptors demonstrated αVβ3 binding sites for RGD peptides in the vasculature and some desquamated cells, whereas the majority of the desquamated cells bind Bt-RGD via β1 integrins.[331]

Experimental results support a role for adhesion molecules in the formation of casts. It has been shown that a translocation of integrins to the apical membrane of tubular epithelial cells may occur with ischemia.[11,226,227] Possible mechanisms for the loss of the polarized distribution of integrins include cytoskeletal disruption, state of phosphorylation, activation of proteases, and the production of NO.[333,334] These integrins are known to recognize RGD tripeptide sequences.[13,332] Thus, viable intraluminal cells could adhere to other luminal or paraluminal cells. The Goligorsky group provided experimental evidence for this cell–cell adhesion process as a contributor to tubule obstruction in ischemic AKI.[335–337] Synthetic cyclical RGD peptides were infused prior to the renal ischemic insult in order to block cell-to-cell adhesion as a component of tubule obstruction.[14,335–338] Using micropuncture techniques, the cyclic RGD tripeptides blocked the rise in tubular pressure postischemic insult.[13] An in vivo study of RGD peptides (cyclic RGDDFLG and RGDDFV) in ischemic AKI in rats demonstrated the attenuation of renal injury and an accelerated recovery of renal function.[14] The systemic administration of fluorescent derivatives of two different cyclic RGD peptides, a cyclic Bt-RGD peptide and a linear RhoG-RGD peptide, infused after the release of a renal artery clamp ameliorated ischemic AKI in rats.[14,337] The staining of these peptides suggests that cyclic RGD peptides inhibited tubular obstruction by predominantly preventing cell-to-cell adhesion, rather than cell-to-matrix adhesion.[332]

In addition to cell–cell adhesion, it is worthy to note that Zuk et al.[339] have demonstrated increased fibronectin in the tubular lumen after an ischemic insult, and fibronectin is known to possess arginine-glycine-aspartic acid (RGD) sequences that are recognized by cellular integrins. Moreover, Tamm-Horsfall protein (THP) is known to possess an RGD sequence, which may or may not be in a position to be recognized by integrins. This possibility, however, led to in vitro cellular adhesion studies in which $LLCPK_1$ cell adhesion to several different matrices (i.e., collagen I and collagen IV) was examined.[333] Interestingly, THP diminished cell adhesion in artificial fluid, mimicking distal tubular fluid but not tubular fluid similar to AKI or collecting duct fluid, which have significantly higher ionic concentrations.[340] In this regard, it has been suggested that THP becomes a polymeric gel in the presence of high ionic strength fluid, but is a non-gel monomeric substance in low ionic strength fluid. Studies have documented that the gel formation by THP is an active process that can be abolished by boiling. A role of the oligosaccharide component of THP in the gel formation was demonstrated because N-glycanase treatment to remove the oligosaccharide abolished the gel formation.[340]

Thus, the intraluminal presence of brush border membranes and viable and nonviable cells in association with the extracellular matrix (eg, fibronectin, THP, adhesion molecules) support their involvement in cast formation in ischemic AKI. The actual tubular obstruction by the casts, however, may only occur in the presence of the impaired vascular responses to renal ischemia. More specifically, if net glomerular filtration pressure was normal, the majority of the tubular casts may be excreted in the urine rather than lodging in the collecting duct and other nephron sites. Tubular factors in the pathogenesis of ischemic AKI are shown in Figure 29.7. The various perturbations in the renal vasculature that occur in association with a renal ischemic insult will now be discussed.

VASCULAR PERTURBATIONS

Ischemic AKI is associated with renal vasoconstriction with a resultant decrease in glomerular hydrostatic pressure and renal plasma flow.[341–343] Not only are circulatory vasoconstrictors, such as catecholamines, AT-II and endothelin, as well as renal sympathetic tone frequently increased in the setting of ischemic AKI,[68] but the renal vascular response to vasoconstrictors has been shown to be enhanced. This increased response to vasoconstrictors is due in part to the earlier mentioned increase in cytosolic calcium concentration in the afferent arterioles of the glomerulus. Endothelial damage is also associated with a diminution of the renal vasodilators, which oppose the action of vasoconstrictors. In experimental sepsis, the NO secondary to iNOS has been suggested to downregulate renal eNOS.[344] Moreover, the renal clamp model of AKI in the rat has been shown to be associated with downregulation of endothelial-derived nitric oxide (eDNO).[324,345] Recent studies have also shown that endothelin receptor antagonists ameliorate the diminution in renal hemodynamics associated with renal ischemia in the isolated perfused rat kidney[346,347] Further support for endothelin as an important mediator of ischemia–reperfusion-induced renal injury is the protective effect of an endothelin-A receptor antagonist in rats after clamping of the renal arteries.[348] Impairment of prostaglandin synthesis by the damaged endothelium can also profoundly enhance renal vascular resistance associated with renal ischemia. In this regard, infusion of prostaglandin E1 may protect against ischemic AKI.[349] Also, inhibition of thromboxane A2 improves renal function in rats exposed to warm ischemia–reperfusion.[350]

Intravital two-photon microscopy is an ideal method to study the microvascular events within the functioning kidney in vivo.[351–354] Intravital two-photon microscopy enables investigators to follow functional and structural alterations with subcellular resolution within the same field of view over a short period of time. Endothelial cell dysfunction within the microvasculature was observed and quantified using the infusion of variously sized, differently colored dextrans or proteins. Movement of these molecules out of the microvasculature and accumulation within the interstitial compartment are readily observed during AKI. The FVB-TIE2/GFP mouse, in which the endothelium is fluorescent, has been used to study morphologic changes in the renal microvascular endothelium during ischemia–reperfusion injury in the kidney.[355] Alterations in the cytoskeleton of renal microvascular endothelial cells correlated with a permeability defect in the renal microvasculature as identified using fluorescent dextrans and two-photon intravital imaging. Also, proximal tubule cell injury was increased in areas adjacent to areas of reduced endothelial injury and dysfunction.

Acute and chronic microvascular alterations are seen in a mouse model of ischemic AKI.[358] Three-dimensional reconstructions of microvascular networks obtained 24 hours following an acute ischemic injury demonstrate an intact endothelial monolayer in areas of increased microvascular permeability. There was no terminal deoxynucleotidyl transferase (TdT) mediated nick-end labeling (TUNEL) staining in microvascular endothelial cells despite the activation of caspase-3. This study demonstrates that detachment and a subsequent loss of endothelial cells following ischemic injury is not a major contributor to altered microvascular permeability.

In a more recent study, it was demonstrated that impaired endothelial proliferation and mesenchymal transition contribute to vascular rarefaction following AKI.[356] In the kidney of Sprague-Dawley rats after ischemic AKI, proliferating endothelial cells were undetectable for up to 2 days following ischemia/reperfusion (I/R). Endothelial mesenchymal transition states were identified.

Toll-like receptor 4 (TLR4) regulates early endothelial activation during ischemic acute kidney injury.[357] Increased TLR4 expression was seen on endothelial cells of the vasa recta of the inner stripe of the outer medulla of the kidney 4 hours after reperfusion.[357] The addition of hydrogen peroxide increased TLR4 expression in MS1 microvascular endothelial cells in vitro. TLR4 was localized to proximal tubules in the cortex and the outer medulla after 24 hours of reperfusion.

Acute alterations of the renal microvasculature, including altered microvascular permeability, are important contributors to the overall pathophysiology of AKI.[358,359] These acute microvascular alterations may have chronic consequences that result in the progression of AKI to chronic kidney disease (CKD).

Oxygen Free Radicals

Studies supported indirect evidence for injury induced by oxygen free radicals (OFRs) during reperfusion.[360,361] Although some studies have demonstrated that activated neutrophils produce OFR injury after ischemia,[362,363] other studies in isolated proximal tubules[364] and the studies in the cell-free isolated perfused rat kidney[365] indicated that OFRs were generated and contributed to the injury process even in the absence of neutrophils. The identification

of the actual species of OFRs involved in reperfusion injury required direct methods of detection rather than a reliance on scavengers.

The direct detection of hydroxyl radicals was initially achieved in isolated proximal tubules by using biochemical traps.[364] Similar studies were subsequently performed in the intact kidney using 0.5 mM salicylate to react with hydroxyl radicals during reperfusion for 15 minutes after an ischemia of 15 minutes.[366] An increase in 2,5 dihydroxybenzoic acid was observed using high performance liquid chromatography (HPLC) with electrochemical detection. Subsequent studies by Kadkhodaee et al.[367] used electron paramagnetic resonance (EPR) and 5,5-dimethyl-l-pyrroline N-oxide (DMPO) as a spin trap and confirmed that hydroxyl radicals were generated during a brief 3 minute reperfusion period following 20 minutes of ischemia. Interestingly, both studies demonstrated a significant generation of hydroxyl radicals in control kidneys, which was abolished by the addition of the scavenger, dimethyl-2-thiourea (DMTU). An increase in an unidentified carbon-centered radical was also identified during reperfusion in the EPR study and could represent an early lipid peroxidation product.[367]

With 60 minutes of reperfusion after 20 minutes of ischemia in the IPRK, tubular damage was prominent in both the cortical and medullary proximal tubule and in the mTAL.[311] Pretreatment with either allopurinol, which acts both to inhibit xanthine oxidase and acts as an OFR scavenger, or DMTU, reduced both the morphologic features of injury and the extent of DNA fragmentation in the mTAL. Taken together, these results suggest that hydroxyl radicals formed during reperfusion after ischemia play a significant role in both necrotic and apoptotic cell injury.

On the background of these postischemic vascular perturbations is the observation that a decrease in renal perfusion pressure is not associated with autoregulation of either GFR or renal blood flow.[324,341,342,368–370] In fact, rather than renal vasodilation, renal vasoconstriction occurs with a fall in renal perfusion pressure in the postischemic kidney. Thus, a degree of hypotension, which is of no clinical significance in the normal kidney, may cause renal damage in the kidney during the recovery phase of AKI. The same increased sensitivity in the postischemic kidney has also been shown to occur with nephrotoxic agents such as aminoglycosides.

Studies to examine the role of Ca^{2+} and calcium channel blockers (CCBs) in the vascular perturbations in experimental ischemic AKI have been performed. These studies demonstrate that intrarenal CCBs can reverse the increased sensitivity to renal nerve stimulation as well as the loss of renal autoregulation, both of which characterize experimental AKI.[371] In addition, other studies in the rat showed that atrial natriuretic peptide (ANP), which attenuates vasoconstrictor-induced increases in $[Ca^{2+}]_i$ in cultured vascular smooth muscle cells,[372] is also protective against ischemic AKI[373] despite the fact that its systemic administration causes a fall in arterial pressure.

Cisplatin-induced or ischemic AKI in vivo and hypoxia/reoxygenation of tubular epithelial cells in vitro induces the production of reactive oxygen metabolites (ROM).[374] The generation of ROM results in tubular epithelial cell death, which is mediated by caspases and/or endonucleases. The inhibition of ROM protects the tubular epithelium from caspase and endonuclease activation and from cell death.

Endotoxemia-Induced Acute Kidney Injury

Recent experimental studies in septic mice have incriminated still another mediator of renal vasoconstriction. The intraperitoneal administration of lipopolysaccharide (LPS) as an endotoxin was associated with a profound decrease in GFR and renal blood flow both in wild-type and iNOS knockout mice. A soluble receptor of TNF, however, was associated with profound improvement in renal hemodynamics in both wild-type and iNOS knockout mice.[375] Because septic patients with renal failure have a high mortality, this observation has potential therapeutic importance.

Sepsis in mice has also been shown to be associated with an impaired response of NO-mediated cyclic GMP in the renal cortex, the agent's secondary messenger for vasodilation. The renal nerves and the activation of the renin–angiotensin system contribute to renal vasoconstriction during sepsis. In this study, renal denervation decreased the high plasma renin levels during endotoxemia and was protective against the decreased GFR and renal blood flow in a normotensive model of endotoxemia-induced sepsis.[376]

Oxygen radicals may contribute to vasoconstriction in endotoxemia-induced AKI.[377] AKI during sepsis is associated with increased NO and oxygen radicals, including superoxide. Renal extracellular superoxide dismutase (EC-SOD) is decreased in endoteoxemia.[378] Antioxidant therapy with chemically dissimilar antioxidants, metalloporphyrin and tempol, preserved GFR and renal blood flow during endotoxemia.[378] This protective effect was reversed by the inhibition of iNOS, suggesting the importance of the bioavailability of NO for the preservation of renal function during endotoxemia.[378]

The demonstration of global renal vasoconstriction in sepsis may depend on the model used. In a nonlethal hyperdynamic model of sepsis in sheep injected with Escherichia coli, renal failure developed despite markedly increased renal blood flow.[379]

In another study in endotoxemia-induced AKI, the role of renal inflammation and apoptosis was determined.[380] In this study, LPS acted on extrarenal TLR4, leading to systemic TNF release and subsequent AKI. Mice with a mutation in TLR4 were resistant to LPS-induced AKI and had less neutrophil infiltration and renal cell apoptosis.[380]

The role of caspase-1 and its associated cytokines was investigated in a nonhypotensive model of endotoxemic AKI.[381] In mice with endotoxemic AKI, the GFR measured by fluorescein isothiocyanate (FITC)-labeled inulin was significantly higher in caspase-1-/- versus wild-type mice at 16 and 36 hours. IL-1 β and IL-18 protein were significantly increased in the kidneys of mice with endotoxemic AKI versus vehicle-treated mice. However, the inhibition of IL-1β with L-1Ra, or the inhibition of IL-18 with IL-18–neutralizing antiserum-treated or combination therapy with IL-1Ra plus IL-18–neutralizing antiserum did not improve the GFR in mice with endotoxemic AKI. These results suggest that neither IL-1β nor IL-18 are the mediators on endotoxemic AKI.[381]

Ghrelin is a stomach-derived growth hormone secretagogue. Ghrelin has been shown to have anti-inflammatory properties. Serum ghrelin levels were increased in endotoxemia AKI accompanied by increased ghrelin receptor (GHSR-1a) protein expression in the kidney.[382] Ghrelin administration significantly decreased serum cytokine levels (TNF-α, IL-1β, and IL-6), serum endothelin-1 levels, serum NO levels, and renal iNOS expression in endotoxemic AKI. When administrated before LPS, ghrelin protected against the fall in glomerular filtration rate. In another study, ghrelin was shown to improve renal function in mice with ischemic AKI.[383]

INFLAMMATION IN ISCHEMIC ACUTE KIDNEY INJURY

Ischemic AKI has been described as an inflammatory disease.[20] This is evidenced by numerous studies demonstrating endothelial injury, leukocyte infiltration in the kidney, and the generation of inflammatory mediators by tubular cells.[20]

Endothelial Cell Injury

Silver nitrate staining of blood vessels was studied in rats at 4 hours after the release of a renal artery clamp.[384] Ischemic AKI resulted in disorganization of endothelial integrity with areas of denudation, partial disappearance of cell–cell borders, and distortion of cell–cell contacts most prominent in the renal microvasculature. Intravital microscopy of blood flow in peritubular capillaries provided direct evidence for the existence of a no-flow phenomenon caused by endothelial injury.[384,385] The administration of endothelial cells or surrogate cells expressing endothelial NOS, either intravenously or intra-arterially, resulted in functional protection against ischemic AKI.[384] These studies suggested that endothelial cell injury is the primary cause of the no-flow phenomenon and that, when ameliorated, there is attenuation of renal function.

It has been demonstrated in mice that injury of renal microvascular endothelium alters the barrier function after ischemia.[355] In this study, circulating von Willebrand factor (vWF), a marker of endothelial injury, was increased in the circulation 24 hours after ischemia. In FVB-TIE2/GFP mice, in which the microvasculature can be visualized, there were alterations in the cytoskeleton and in the integrity of adherens junctions that correlated with a permeability defect identified using fluorescent dextrans and two-photon intravital imaging.[355]

The extension phase of AKI is marked by continued hypoxia and an inflammatory response, which are more marked in the corticomedullary junction.[19] Severely reduced blood flow, stasis, and the accumulation of red blood cells has been documented in the corticomedullary region.[19] Endothelial cell injury is thought to play an important role in the initiation and extension phase of ischemic AKI.[19,386]

Neutrophil Activation

Renal ischemia–reperfusion injury is associated with an increase in infiltrating neutrophils.[387] The adherence of neutrophils to the vascular endothelium is an essential step in the extravasation of these cells into ischemic tissue.[29] Therefore, leukocyte adhesion molecules have been studied in renal injury.[388] After adherence and chemotaxis, infiltrating leukocytes release reactive oxygen species and enzymes that damage the cells.[29] The infusion of normal neutrophils accentuates severe ischemia–reperfusion injury and decreases GFR during ischemia. Activated neutrophils have been shown to further decrease GFR in response to renal ischemia at least in part due to the release of oxygen radicals.[363,389–391] In contrast, the infusion of oxygen radical–deficient neutrophils from patients with chronic granulomatous disease did not worsen the course of ischemic injury.[390] The mechanism by which adherent leukocytes cause ischemic injury is unclear, but likely involves both the release of potent vasoconstrictors including the prostaglandins, leukotrienes, and thromboxanes,[392] as well as direct endothelial injury via the release of endothelin and a decrease in NO.[68,393]

Intracellular adhesion molecule 1 (ICAM-1) has been suggested to play an important role in the pathophysiology of ischemic AKI.[387,394] Increased systemic levels of the cytokines, TNF-α, and IL-1 may upregulate ICAM-1 after ischemia and reperfusion in the kidney.[394] ICAM-1 on endothelial cells promotes the adhesion of neutrophils to these cells and causes tissue damage. The administration of a monoclonal antibody against ICAM-1 protected against ischemic AKI in rats.[387,390] Pretreatment with an ICAM-1 antisense oligodeoxyribonucleotide ameliorated the ischemia-induced infiltration of granulocytes and macrophages and resulted in less cortical renal damage as assessed by a quantitative pathologic grading scale.[395] In parallel, ICAM-1–deficient mice are protected against renal ischemia.[394] Thus, ICAM-1 is a mediator of ischemic AKI, probably by potentiating neutrophil–endothelial interactions.

Red blood cell swelling has been suggested to cause the medullary blood flow congestion, which occurs after renal ischemia and worsens the relative hypoxia in that region of the kidney. The restoration of renal blood flow in experimental renal ischemia in dogs, however, occurs with either an isotonic or hypertonic mannitol induced–diuresis. There is now evidence that upregulation of adhesion molecules may contribute to this impaired medullary blood flow postischemic injury.[388,396,397]

P-selectin, an important molecule involved in the adherence of circulating leukocytes to tissue in inflammatory states, also seems to be involved in the infiltration of the leukocytes during ischemic injury. In fact, renal ischemia has also been shown to be associated with upregulation of endothelial P-selectin with enhanced adhesion of neutrophils.[398] A soluble P-selectin glycoprotein ligand prevented the infiltration of leukocytes and ameliorated ischemia-induced renal dysfunction.[334] In contrast to P-selectin, L-selectin does not appear to mediate tubular damage in the postischemic kidney.[388] After adherence and chemotaxis, neutrophils release reactive oxygen species or oxygen free radicals. IL-17 produced by neutrophils regulates interferon (IFN)-γ–mediated neutrophil migration in the ischemic AKI in mice.[399]

There is evidence that neutrophils mediate tubular injury in AKI.[400] This evidence is derived from studies that show an accumulation of neutrophils in ischemic AKI and studies demonstrating a beneficial role of anti–ICAM-1 therapy in AKI.[394] Also, mice depleted of peripheral neutrophils by antineutrophil serum were protected against ischemic AKI.[394] However, in another study, rats depleted of peripheral neutrophils by antineutrophil serum were not protected against ischemic AKI.[401] In another study, mice were injected with 0.1 mg of the rat IgG2b monoclonal antibody RB6-8C5 (BD Pharmingen Inc., San Diego, CA) intraperitoneally 24 hours before a renal pedicle clamp.[191] This results in the depletion of neutrophils in the peripheral blood and in the kidney during ischemic AKI. In this study, there was slight functional protection and no histologic protection against ischemic AKI in neutrophil-depleted mice.[191]

The kinetics of margination and transmigration of neutrophils in vivo in the kidney and lungs following renal ischemia–reperfusion has been studied.[402] At 24 hours after an ischemic AKI, kidney neutrophil content increased threefold. The neutrophils were found primarily in the interstitium and, to a lesser degree, marginated to the vascular endothelium. Interstitial neutrophils had significantly lower levels of intracellular IFN-γ, IL-4, IL-6, and IL-10, and thus, a tendency for decreased amounts of IL-4 and TNF-α compared to the marginated neutrophils. Transmigration sites of neutrophils were directly associated with areas of increased vascular permeability. The activation of the adenosine 2A receptor significantly decreased both kidney neutrophil transmigration by about half and vascular permeability by about a third. This study suggests that there is a sequential recruitment and transmigration of neutrophils from the vasculature into the interstitium of the kidney at the site of tissue injury in ischemic AKI.

Lymphocytes

The role of lymphocytes in ischemic AKI is an ongoing area of study.[403,404] Lymphocytes have been examined in genetically altered immune-deficient mice. In one study, mice with a combined deficiency of both CD4 and CD8 cells were protected against ischemic AKI at 48, but not at 24 hours postischemic reperfusion.[405] In a follow-up report by the same investigators, nu/nu mice that are athymic and deficient in both CD4 and CD8 T cells were protected against ischemic AKI 24- and 48-hours postischemic reperfusion.[406] To determine the pathogenic T-cell type, mice with targeted genetic deficiencies of either CD4 or CD8 T cells were also studied. CD4-deficient mice, but not CD8-deficient, are protected against ischemic AKI.[406] Therefore, it appears that the pathogenic T-cell subtype in ischemic AKI is the CD4 T cell. However, RAG-1-/- mice, which lack mature T and B lymphocytes, are not protected against ischemic AKI despite lacking both CD4 and CD8 T cells.[407] In a recent study,[408] mice deficient in B lymphocytes alone were protected against ischemic AKI. In summary, CD4 T-cell deficient, nu/nu (lacking mature T cells), and B-cell deficient mice are protected against ischemic AKI, whereas CD8 T-cell and RAG-1-/- (lacking mature B and T cells) mice are not protected.

The effect of a complete depletion of CD4 T cells with a monoclonal antibody in ischemic AKI is not known. In one report,[409] the use of GK1.5 antibody alone to deplete CD4 T cells did not protect against ischemic AKI; however, the complete depletion of CD4 T cells as judged by fluorescence activated cell sorting (FACS) analysis did not occur. Protection did occur when GK1.5 antibody was used with two other antibodies, which resulted in the depletion of both CD4 and CD8 T cells.[409] In another study,[410] the complete depletion of CD4 T cells using the GK1.5 antibody was not protective against ischemic AKI in mice. CXCR3 plays an important role in the recruitment of Th1 cells into the kidney in ischemic AKI.[411] In summary, it is believed that CD4 T cells are important in the pathogenesis of ischemic AKI and that very few T cells in the kidney are enough to contribute to injury.[404,406,409]

Regulatory T cells (Tregs) are known to blunt the immune response. Foxp3+ regulatory T cells play a role in kidney repair after an ischemic AKI.[412] Tregs also contribute to the protective effect of ischemic preconditioning in the kidney.[413] Treatment of mice with a Treg cell–depleting antibody reversed the protective effect of preconditioning on kidney neutrophil infiltration, function, and histology. Sphingosine-1-phosphate receptor (S1PR) agonists reduce ischemic AKI in mice that lack T and B lymphocytes (Rag-1 knockout mice).[414] S1PR agonists also reduce hypoxia-induced

apoptosis in cultured mouse proximal tubule cells. This study shows that the protective effect of S1PR agonists is independent of T cells.

Natural killer (NK) cells are lymphocytes that mediate innate immunity against pathogens and tumors via their ability to secrete cytokines.[415] NK cells are unique in their constitutive expression of receptors for cytokines (eg, IL-18), which are produced by activated macrophages.[416] NK cells are activated by IL-18 independently of IL-12.[417] A model of NK cell activation in injured tissues has been proposed.[418] In this model, it is hypothesized that NK cells are recruited to sites of injury from the bloodstream. Once in the tissue, NK cells become activated and release cytokines like IL-18.[418] In support of this hypothesis, it is known that NK cells play a role in numerous disease processes.[419] NK cell depletion in wild-type C57BL/6 mice is protective against ischemic AKI.[420] An adoptive transfer of NK cells worsened injury in NK-, T-, and B-cell–null Rag2(-/-)γ(c)(-/-) mice with ischemic AKI. NK cell–mediated kidney injury was perforin (PFN) dependent because PFN(-/-) NK cells had a minimal capacity to kill tubular epithelial cells in vitro compared with NK cells from wild-type mice. Alternatively, B cells limit repair after ischemic AKI.[421]

Monocyte/Macrophages

Another inflammatory cell that is a potential mediator of injury in ischemic AKI is the monocyte/macrophage.[404] Macrophages infiltrate the postischemic rat kidney.[422] Macrophage chemoattractants (e.g., monocyte chemoattractant protein 1 [MCP-1]) are increased in the postischemic rat kidney.[404] In a model of macrophage depletion using liposomal clodronate, it was demonstrated that macrophages contribute to tissue damage during acute rejection.[423] It was determined that macrophages are a source of injurious IL-18 in ischemic AKI in mice.[424] Macrophage depletion in the kidney was achieved by using a tail vein injection of liposomal-encapsulated clodronate (LEC). The adoptive transfer of RAW 264.7 cells, a mouse macrophage line that constitutively expresses IL-18 mRNA, reversed the functional protection against AKI in LEC-treated mice. In addition, the adoptive transfer of peritoneal macrophages in which IL-18 function was inhibited also reversed the functional protection in macrophage-depleted mice, demonstrating that IL-18 from the adoptive transfer of macrophages is not sufficient to cause ischemic AKI. Possible sources of injurious IL-18 in AKI include the proximal tubule and lymphocytes. In this regard, freshly isolated proximal tubules from mice release IL-18 into the medium when exposed to hypoxia, and proximal tubules from caspase-1–deficient mice are protected against hypoxic injury.[178] An anti-B7-1 antibody blocks mononuclear cell adherence in the vasa recta of rats and attenuates ischemic AKI both functionally and histologically.[425] Gene therapy in rats expressing an amino-terminal truncated MCP-1 reduced macrophage infiltration and AKI.[426] Two recent studies have demonstrated that macrophage depletion using liposomal clodronate is protective against ischemic AKI in mice.[427,428]

Dendritic cells act as antigen-presenting cells and as messengers between the innate and adaptive immunity. The kidney has a rich network of resident dendritic cells.[429] Dong and colleagues[430] have identified the surveying renal dendritic cell network as the predominant source of TNF-α during the early stages of ischemic AKI.[430]

Proinflammatory cytokines increase the expression of the CX_3C chemokine, fractalkine, on injured endothelial cells. The fractalkine receptor (CX_3CR1) is expressed on NK cells, monocytes, and some CD8+ T cells.[431] Fractalkine has a mucinlike stalk that extends the chemokine domain away from the endothelial cell surface, enabling the presentation of the CX_3C-chemokine domain to leukocytes. Fractalkine serves the dual function of an adhesion molecule and a chemoattractant.[431] Fractalkine is a major chemoattractant for NK cells and monocytes, but not for neutrophils.[432] Fractalkine expression is increased in patients with renal tubulointerstitial inflammation, with the strongest expression localized to vascular sites near to macrophage inflammation.[433] Fractalkine is a strong candidate for directing mononuclear cell infiltration induced by vascular injury.[433] Fractalkine expression is increased in the endothelium of large blood vessels, capillaries, and glomeruli in ischemic AKI.[427] Fractalkine receptor inhibition is protective against ischemic AKI.[427]

Inflammatory Mediators

In renal ischemia–reperfusion, tubular epithelial cells produce TNF-α, IL-1, IL-6, IL-8, IL-18, TGF-β, MCP-1, RANTES, and fractalkines.[20,190,191] Leukocytes produce TNF-α, IL-1, IL-8, MCP-1, reactive oxygen species, and eicosanoids.[20] The anti-inflammatory cytokine, IL-10, inhibits TNF-α, ICAM-1, and iNOS and protects against ischemic and cisplatin-induced renal failure and AKI.[434]

Statins are potent anti-inflammatory drugs.[435] Both in vitro and in vivo studies suggest lipid lowering–independent anti-inflammatory functions of statins.[435] After adhesion to the vascular endothelium, inflammatory cells migrate to the site of inflammation.[435] Statins act independently of lipid lowering to selectively inhibit leukocyte adhesion by direct interactions with the leukocyte-function antigen 1 (LFA-1).[435] Statins reduce macrophage influx and chemokine expression in rat kidneys.[436] Statins are known to decrease the expression of proinflammatory cytokines by inflammatory cells.[435] Simvastatin reduces the expression of IL-6 and MCP-1 in monocytes from hypercholestrolemic patients and in cultured endothelial cells.[435] Pravastatin downregulates TNF-α and MCP-1[437] in human monocytes in vitro. Some of the anti-inflammatory effects of statins are mediated by NO.

Rats were treated with cerivastatin or a vehicle for 3 days before the induction of ischemic AKI.[438] Statin

treatment reduced the increase in serum creatinine by 40% and protected against tubular necrosis. In addition, monocyte and macrophage infiltration was almost completely prevented, ICAM-1 upregulation was decreased, and iNOS expression was reduced.[438] In another study, atorvastatin improved the course of ischemic AKI in aging rats by enhancing NO availability and improving renal hemodynamics.[439]

Proinflammatory cytokines are increased in the serum of animal models of AKI.[66] Data in animal models of AKI suggest that the inflammatory response in AKI is dysregulated. The effect of AKI on the production and elimination of proinflammatory cytokines may be a key mechanism by which patients with AKI have increased distant organ dysfunction and increased mortality. In animals with AKI, TNF-α,[440] IL-1β,[440] IL-6, KC, and granulocyte colony stimulating factor (GCSF) increase in the serum after AKI.[66] Cytokine production also increases in the kidney.[66] Renal cytokine production may contribute to renal injury and cause the increase in serum cytokines. Circulating cytokines may contribute to extrarenal organ injury.

Besides IL-18, there are other cytokines that play a role in ischemic AKI. The TNF-like weak inducer of apoptosis (TWEAK, TNFSF12) is a member of the TNF superfamily. TWEAK activates the Fn14 receptor and regulates apoptosis, proliferation, and inflammation. TWEAK and Fn14 expression was increased in experimental AKI induced by folic acid.[441] The IL-6/IL-6R axis plays a critical role in AKI.[442] High-mobility group box 1 (HMGB1), a nuclear factor released extracellularly as an inflammatory cytokine, is an endogenous ligand for TLR4. A neutralizing anti-HMGB1 antibody protected against ischemic AKI and reduced levels of IL-6, TNF-α, and MCP1.[443] Alternatively, the administration of recombinant HMGB1 after reperfusion exacerbated ischemic AKI. TLR4-deficient mice were protected against AKI and the administration of an anti-HMGB1 antibody or a rHMGB1 did not affect this renoprotection. This study concluded that endogenous HMGB1 promotes kidney damage after IRI, possibly through the TLR4 pathway.

RECOVERY FROM ISCHEMIC ACUTE KIDNEY INJURY

It has been demonstrated in rats that severe ischemic AKI results in a permanent alteration in renal capillary density that contributes to a urinary concentrating defect and renal fibrosis.[444,445] However, in contrast to other organs, the kidney can recover from tubular necrosis, at least in the short term. There are two potential mechanisms of recovery other than cell repair: (1) the dedifferentiation and proliferation of the surviving nonnecrotic tubular cells,[446] and (2) the mobilization and delivery of bone marrow stem cells to the injured kidney.[447]

After an ischemic AKI, there is a proliferation of tubular cells that mimic events in the developing kidney.[448] Epithelial cells are also dedifferentiated during the recovery period.[446] In the postischemic kidney, there is also an expression of genes that encode growth factors.[263,265,449] The dedifferentiation and the proliferation of tubular epithelial cells may result in the spreading of cells over the damaged basement membrane. Cell adhesion molecules like neural cell adhesion molecule (NCAM), cytokines, and KIM-1[59,450] may play a role in these processes. The targeted delivery of hepatocyte growth factor (HGF) to the proximal tubule in transgenic mice resulted in a marked protection against ischemic AKI.[451] Understanding the physiology of repair and the recovery of surviving tubular cells may lead to therapies to hasten the recovery process in humans.

Bone marrow stem cells have the capacity to migrate to other organs.[447] Bone marrow–derived cells can populate and contribute to the turnover of both the normal and injured renal tubular epithelium.[452] Cells from the bone marrow of adult mice are mobilized into the circulation by transient renal ischemia and home specifically to the injured kidney where they differentiate into tubular epithelial cells.[447] It was investigated whether an increase in circulating stem cells, using pharmacologic mobilization from the bone marrow, would improve renal function in mice with ischemic AKI.[453] The pharmacologic increase in stem cells was associated with marked granulocytosis and worsening of renal failure. In the future, therapies aimed at stimulating the proliferation, mobilization, and targeting of stem cells may enhance recovery from ischemic AKI.[447] However, in another study, it was shown that intrarenal cells, not bone marrow–derived cells, are the major sources for regeneration in postischemic AKI. Furthermore, a single injection of bone marrow cells did not make a significant contribution to renal functional or structural recovery.[454]

Mesenchymal stem cells (MSCs) play a role in regeneration and immunomodulation. The administration of MSCs protects against ischemic AKI in rats.[455] In this study, the expression of IL-1β, TNF-α, IFN-γ, and iNOS was significantly reduced by the intravenous administration of MSCs. In addition, the beneficial effects of MSCs were found to be mediated by paracrine actions and not by their differentiation into target cells. Human MSCs improve renal function and survival in mice with cisplatin-induced AKI.[456] The treatment of mice with autologous and allogeneic MSCs after AKI was safe and reduced renal fibrosis in mice that survived an AKI.[457] CD44 and hyaluronic acid interactions recruit exogenous MSCs to injured tissues to improve renal regeneration.[458] Also, MSCs exert beneficial effects on tubular cell repair in an ischemic AKI by the production of the prosurvival and mitogenic growth factor IGF-1.[459] A phase 1 study of MSCs in patients at risk for AKI after cardiac surgery is under way.

Colony stimulating factor (CSF-1) signals directly to renal tubular epithelial cells to mediate repair in mice.[460] Macrophages have been implicated in tissue repair, and CSF-1,

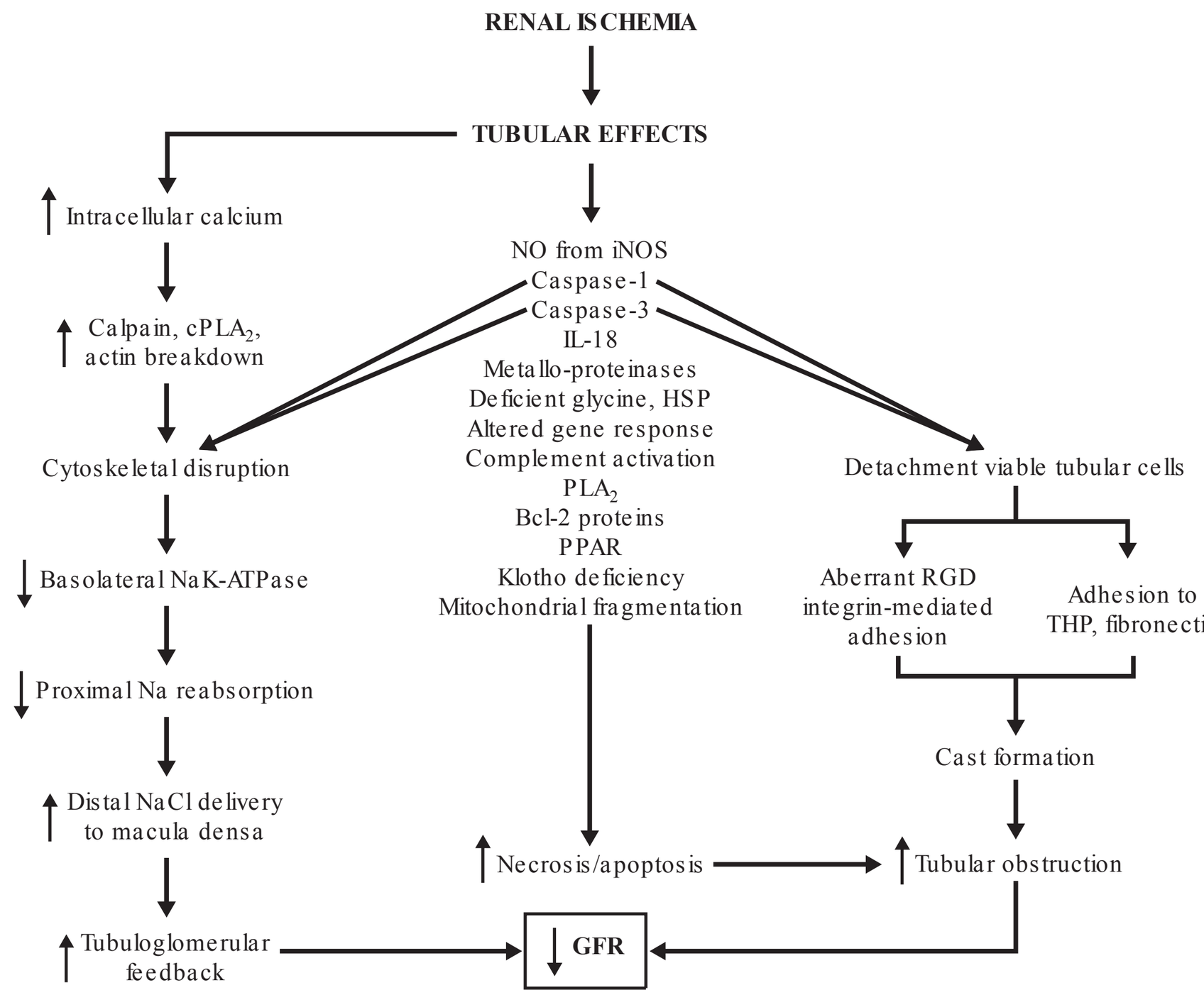


FIGURE 29.8 The tubular factors in the pathogenesis of ischemic acute kidney injury. NO, nitric oxide; iNOS, inducible nitric oxide; IL-18, interleukin 18; HSP, heat shock protein; PLA_2, phospholipase A_2; RGD, arginine-glycine-aspartic acid; THP, Tamm-Horsfall protein; PPAR, peroxisome proliferator–activated receptors; GFR, glomerular filtration rate. Reproduced from Kribben et al.[466] with permission.

the principal macrophage growth factor, is expressed by tubular epithelial cells. Mice injected with CSF-1 had decreased tubular pathology and improved renal function in an ischemic AKI. The study further demonstrated that CSF-1 mediates renal repair by both a macrophage-dependent mechanism and a direct autocrine/paracrine action on tubular epithelial cells (TECs).

SUMMARY

Tubular and vascular perturbations and inflammation combine to cause ischemic AKI. The tubular and vascular events in ischemic AKI are summarized in Figures 29.8 and 29.9. The inflammatory events are summarized in Figure 29.10. Recent laboratory studies using in vivo, cellular, and molecular approaches have provided substantial insight into the pathogenesis of the syndrome. These studies have identified several potential therapeutic interventions, which need to be tested with prospective clinical trials. Interventions that have attenuated experimental ischemic/hypoxic proximal tubule damage include cysteine protease inhibitors, melanocyte stimulating hormone (MSH), specific iNOS inhibition,

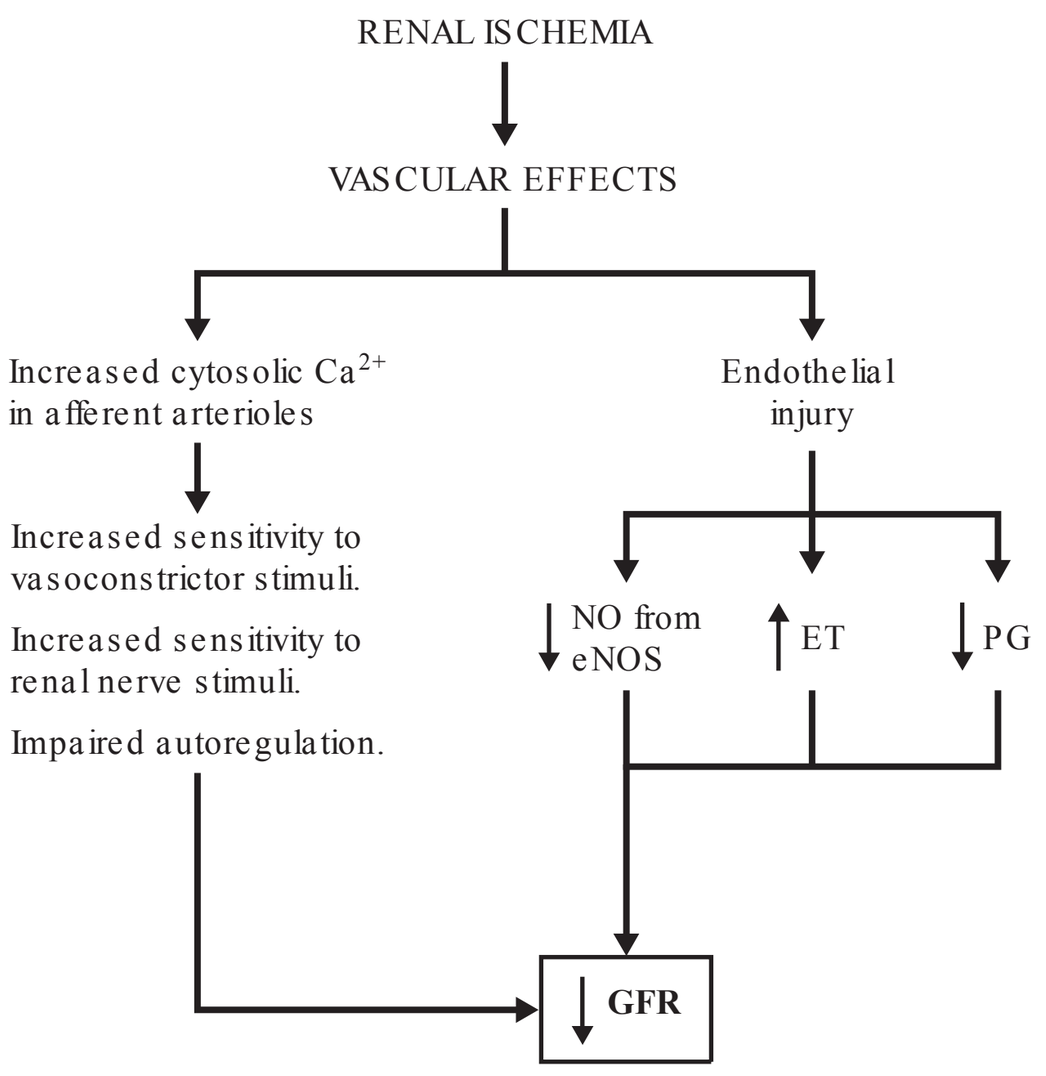


FIGURE 29.9 Vascular factors in the pathogenesis of ischemic acute kidney injury. NO, nitric oxide; eNOS, endothelial nitric oxide; ET, endothelin; PG, prostaglandins; GFR, glomerular filtration rate. Reproduced from Kribben et al.[466] with permission.

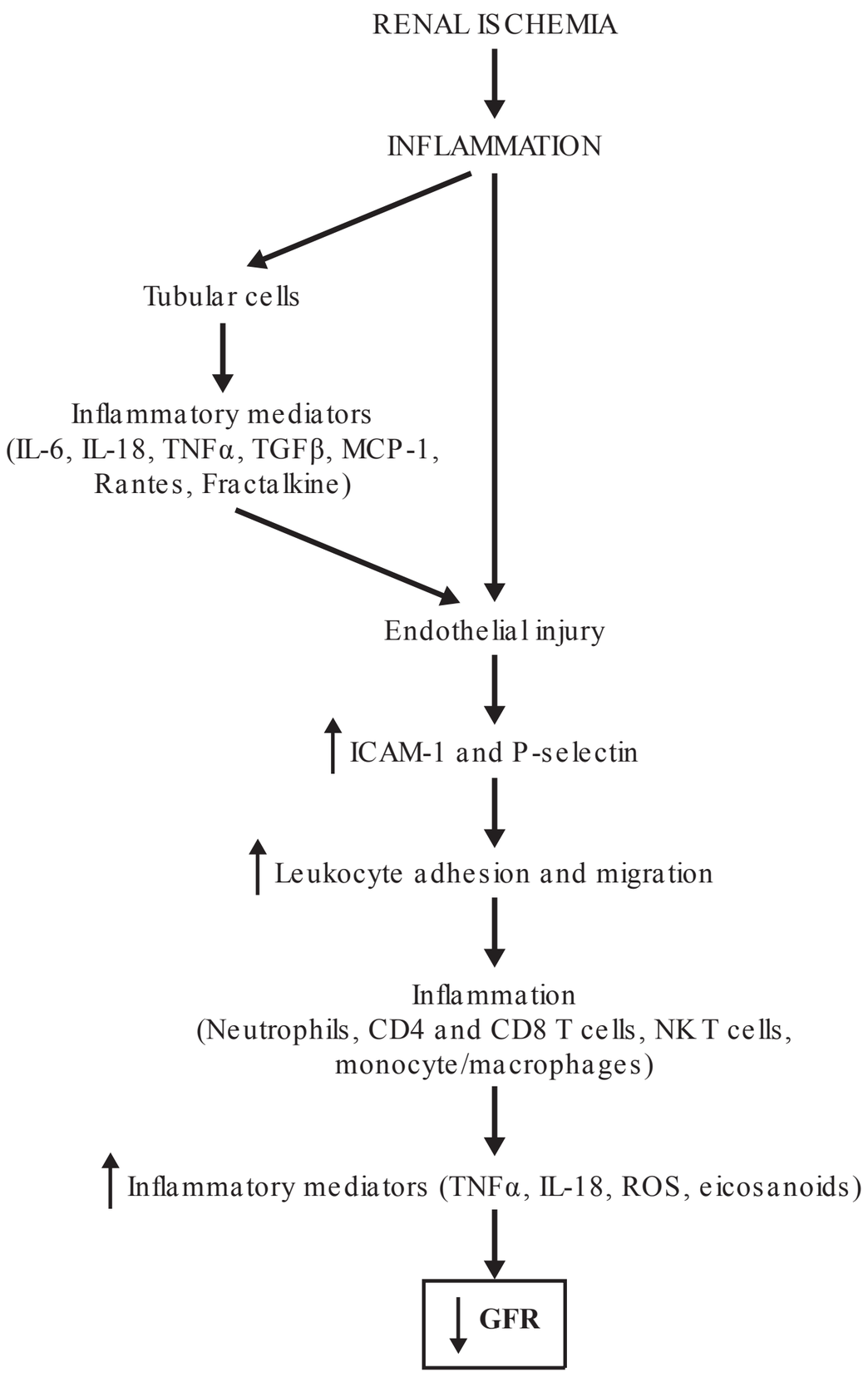


FIGURE 29.10 The inflammatory response in ischemic acute kidney injury. IL, interleukin; TNF-α, tumor necrosis factor alpha; TGF-β, transforming growth factor beta; MCP-1, monocyte chemoattractant protein 1; RANTES, regulated upon activation, normal T cell expressed and secreted; ICAM-1, intracellular adhesion molecule 1; NK, natural killer; ROS, reactive oxygen species; GFR, glomerular filtration rate .

synthetic cyclical arginine-glycine-aspartic acid (RGD) sequences, mannitol, oxygen radical scavengers, TNF soluble receptors, inducers of HSP-70 and anti-ICAM antibodies, endothelin antagonists, IL-18 antiserum, IL-6 inhibition erythropoietin, NGAL, CTLA4 immunoglobulin, fractalkine receptor inhibition, CSF-1, MSCs, IGF-1, macrophage stimulating protein, macrophage inhibition, hemeoxygenase-1, PPAR-α, β/δ activators, CXCR3 inhibition, sphingosine-1-phosphate receptor agonists, siRNA to p53, soluble thrombomodulin, IFN regulatory factor (IRF-1), fibrates, HMGB1 inhibition, ghrelin, Klotho, HIF-1 activation, complement factor B inhibition, and adenosine A1 receptor activation, to mention a few (Table 29.9).

29.9 Some Emerging Therapies for Ischemic Acute Renal Failure

Cysteine protease inhibitors
Caspase inhibitors
IL-18 inhibition
IL-6 inhibition
áMSH
Specific iNOS inhibition
Synthetic cyclical RGD sequences
Oxygen radical scavengers
TNF soluble receptors
Inducers of HSP
Anti-ICAM antibodies
Endothelin antagonists
Endothelial cell infusion
Mannitol with natriuretic peptides or calcium channel blockers
Erythropoeitin
NGAL
CTLA4 immunoglobulin
Fractalkine receptor inhibition
CSF-1
MSCs
IGF-1
Macrophage stimulating protein
Macrophage inhibition
Hemeoxygenase-1
Peroxisome proliferator–activated receptor (PPAR) α, β/δ activators
CXCR3 inhibition
Sphingosine-1-phosphate receptor agonsists
IFN regulatory factor (IRF-1)
Fibrates
High mobility group box (HMGB1) inhibition
Ghrelin
Klotho
HIF-1 activation
Complement factor B inhibiton
Adenosine A1 receptor agonist
Adenosine A2B receptor agonist
siRNA to p53
Soluble thrombomodulin

IL, interleukin; MSH, melanocyte stimulating hormone; iNOS, inducible nitric oxide; RGD, arginine-glycine-aspartic acid; TNF, tumor necrosis factor; HSP, heat shock protein; anti-ICAM, intercellular adhesion molecule-1; NGAL, neutrophil-gelatinase-associated lipocalin; CTLA4, cytotoxic T-lymphocyte antigen 4; CSF-1, colony stimulating factor; MSC, mesenchymal stem cell; IGF-1, insulin-like growth factor 1; CXCR3, chemokine CX-C-motif receptor 3; HIF-1, hypoxia-inducible factor 1; siRNA, small interfering RNA.

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