SECTION 28

Critical Care Nephrology in Pediatrics

CHAPTER 198

Cellular and Molecular Mechanisms of Acute Kidney Injury

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OBJECTIVES

This chapter will:

1. Describe cellular and molecular mechanisms at the base of acute kidney injury pathophysiology.
2. Detail alterations occurring at genetic, histologic, tubular, glomerular, and vascular levels.
3. Describe the role of nephrotoxins, inflammation, and organ cross-talk during critical illness.

Acute kidney injury (AKI) is a common clinical problem with increasing incidence, serious consequences, and unsatisfactory therapeutic options in children and adults.[1,2] AKI may be classified as *prerenal* (the functional response of structurally normal kidneys to hypoperfusion), *intrinsic* or intrarenal (involving structural damage to the renal parenchyma), or *postrenal* (urinary tract obstruction). The focus of this chapter is on intrinsic structural AKI, which is the most common and clinically significant subtype in critically ill patients and can be associated with acute tubular necrosis. The prognosis for patients with intrinsic AKI remains poor, with a mortality rate of 40% to 80% in the intensive care unit (ICU) setting. Mortality rates increase to 60% to 80% when AKI is associated with distant organs, such as the heart or lungs, in a state of cross-talking dysfunction.[3] Two major problems have plagued the field. First, well over 20 definitions for AKI have been used in published studies, ranging from "dialysis requirement" to "subtle increases in serum creatinine." In an attempt to standardize the definition, the term *acute kidney injury* (AKI) has been proposed to replace the former definition, acute renal failure.[4]

The second problem is an incomplete understanding of the cellular and molecular mechanisms underlying AKI. Current advances in basic and translational research that hold promise for elucidation of the pathogenesis of human AKI are the primary focus of this chapter. Although the emphasis is on ischemic AKI, additional mechanisms pertinent to nephrotoxins and sepsis also are explored briefly. However, AKI in the ICU setting frequently is multifactorial, with concomitant ischemic, nephrotoxic, and septic components, and with overlapping pathophysiologic mechanisms.

Histologic Alterations

The term *acute tubular necrosis* is a misnomer, because frank tubule cell necrosis rarely is encountered in human AKI. Prominent morphologic features of AKI in humans include effacement and loss of proximal tubule brush border, patchy loss of tubule cells, focal areas of proximal tubular dilation, presence of distal tubular casts, and areas of cellular regeneration.[5] Necrosis is inconspicuous, very focal, and restricted to the highly susceptible outer medullar regions. Necrosis appears to preferentially affect the distal tubular segments in the outer medulla (thick ascending limbs and collecting ducts) and is seen less commonly in proximal tubule cells of the cortex. By contrast, apoptosis is a consistent finding in distal and proximal tubules in ischemic and nephrotoxic forms of human AKI.[6] In addition, peritubular capillaries display a striking vascular congestion, endothelial damage, and leukocyte accumulation.[7] The mechanisms underlying these morphologic findings, and their implications for the ensuing profound renal dysfunction, are detailed next.

Hemodynamic Alterations

An intense and persistent renal vasoconstriction that reduces overall renal blood flow to approximately 50% of the normal rate has long been considered a hallmark of intrinsic AKI.[5] In addition, the postischemic kidney displays regional alterations in blood flow patterns.[7] Marked congestion and hypoperfusion of the outer medulla persists, even though cortical blood flow improves during reperfusion after an ischemic insult, leading to prolonged cellular injury and cell

death in these predisposed tubule segments. Mechanisms underlying these hemodynamic alterations relate primarily to endothelial cell injury.[7] The result is a local imbalance of vasoactive substances, with enhanced release of vasoconstrictors such as endothelin and decreased abundance of vasodilators such as endothelium-derived nitric oxide. Endothelin receptor antagonists ameliorate ischemic AKI in animals, but human data are lacking. Similarly, carbon monoxide and carbon monoxide-releasing compounds are protective in animal models of ischemic AKI, probably through vasodilation and preservation of medullary blood flow. This approach is undergoing preliminary tests in humans with AKI.[8] These hemodynamic abnormalities, however, cannot fully account for the profound loss of renal function, and several human trials of vasodilators such as dopamine have failed to demonstrate improvement in glomerular filtration rate established during AKI, despite augmentation of overall renal blood flow.

Alterations in Tubule Dynamics

Known derangements in tubule dynamics include obstruction, backleak, and activation of tubuloglomerular feedback. The consistent histologic findings of proximal tubule dilation and distal tubular casts in human biopsy specimens indicate that obstruction to tubular fluid flow certainly occurs in ischemic AKI. The intraluminal casts contain Tamm-Horsfall protein (uromodulin), which normally is secreted by the thick ascending limb as a monomer. Conversion into a gel-like polymer is promoted by the increased luminal sodium concentration typically encountered within the distal tubule in AKI. This provides the ideal environment for cast formation, with desquamated tubule cells and brush border membranes contributing to obstruction. However, it is unlikely that obstruction alone can account for intense renal dysfunction, because human studies using forced diuresis have not demonstrated an impact on survival and renal recovery rates in patients with AKI.

The various cell types along the nephron display segment-specific susceptibilities to different types of injury. Proximal tubule cells need a steady oxygen supply to remain viable, whereas those in the thick ascending limb are relatively resistant to hypoxia. Epithelial cells in the proximal tubule are injured most commonly in septic, ischemic, or nephrotoxic AKI; the S3 segment is most vulnerable to ischemic injury, whereas the S1 and S2 segments are affected most commonly by toxic nephropathy because of their high rates of endocytosis. Tubular cells also differ in their ability to generate hypoxia inducible transcription factors (HIFs), which mediate cellular responses to hypoxia and are generally cell protective. The collecting duct is most effective at generating HIFs, whereas the proximal tubule has a moderate capacity and the thick ascending limb is least effective. Segments of the nephron also produce differing biomarkers in response to injury. For example, kidney injury molecule-1 (KIM-1) originates from the proximal tubule, whereas neutrophil gelatinase-associated lipocalin (NGAL) arises from the collecting duct. Detecting biomarker changes and localizing them to specific tubule segments may provide more information about the type of injury affecting the kidney and may aid in administering a precise therapeutic intervention.[9]

A role for activation of tubuloglomerular feedback has been proposed as a mechanism for the reduction in glomerular filtration rate in AKI. The increased delivery of sodium chloride to the macula densa as a result of cellular abnormalities in the proximal tubule would be expected to induce afferent arteriolar constriction by means of A_1 adenosine receptor (A1AR) activation, thereby decreasing glomerular filtration rate. However, a knockout of the A1AR resulted in a paradoxical worsening of ischemic AKI, and exogenous activation of A1AR was protective.[10] Thus tubuloglomerular feedback activation secondary to ischemic injury may represent a beneficial phenomenon that limits delivery of ions and solutes to the damaged proximal tubules, thereby reducing the demand for adenosine triphosphate (ATP)-dependent reabsorptive processes. Any salutary effect of exogenous A1AR activation in human AKI remains to be determined.

Alterations in Tubule Cell Metabolism

A profound reduction in intracellular ATP content invariably occurs early after ischemic renal injury, which sets in motion a number of critical metabolic consequences in tubule cells.[5,11] Oxygen deprivation leads to rapid degradation of ATP to adenosine diphosphate (ADP) and adenosine monophosphate (AMP). With prolonged ischemia, AMP is metabolized further to adenine nucleotides and to hypoxanthine. Adenine nucleotides freely diffuse out of cells and their depletion precludes resynthesis of intracellular ATP during reperfusion. Nevertheless, although provision of exogenous adenine nucleotides or thyroxine (which stimulates mitochondrial ATP regeneration) can mitigate AKI in animal models, this approach has yielded disappointing results in human AKI.

ATP depletion leads to impaired calcium sequestration within the endoplasmic reticulum, as well as diminished extrusion of cytosolic calcium into the extracellular space, resulting in increased free intracellular calcium after AKI. Potential downstream complications include activation of proteases and phospholipases and cytoskeletal degradation. Calcium channel blockers may provide some protection from renal injury in the transplantation setting, but evidence for their efficacy in other forms of human AKI is lacking.

The role of reactive oxygen species in the pathogenesis of AKI is supported by substantial evidence. During reperfusion, the conversion of accumulated hypoxanthine to xanthine generates hydrogen peroxide and superoxide. In the presence of iron, hydrogen peroxide forms the highly reactive hydroxyl radical. Concomitantly, ischemia induces nitric oxide synthase in tubule cells. The nitric oxide generated interacts with superoxide to form peroxynitrate, which results in cell damage via oxidant injury as well as protein nitrosylation.[5,11] Reactive oxygen species cause renal tubule cell injury by oxidation of proteins, peroxidation of lipids, damage to DNA, and induction of apoptosis and autophagy. Studies have documented a dramatic increase in oxidative stress and autophagy in experimental and human AKI.[12] Scavengers of reactive oxygen molecules (such as superoxide dismutase, catalase, and *N*-acetylcysteine) protect against ischemic AKI in animals, but human studies have been inconclusive. A promising advance in the field is the protective effect of Edaravone (a potent scavenger of free radicals and inhibitor of lipid peroxidation) observed with administration at the time of reperfusion in a rat model of ischemic AKI.[13] Edaravone was approved for human use in the treatment of cerebral ischemia. However, results with its use in human AKI are awaited.

Free iron derived from red cells or other injured cells is one of the most potent factors in the generation of reactive oxygen species, and the iron scavenger deferoxamine alleviates ischemia-reperfusion injury in animal models. However, the systemic toxicity (primarily hypotension) of

this agent precludes its routine clinical use in human AKI. Three major molecules are under study in the area of iron chelation. The first is human apotransferrin, an iron-binding protein, which protects against AKI in animals by abrogating renal superoxide formation.[14] Apotransferrin has been used successfully for the reduction of redox-active iron in patients undergoing hematologic stem cell transplantation without any adverse effects. The second is neutrophil gelatinase-associated lipocalin (NGAL), a major iron-transporting protein complementary to transferrin, and one of the most highly induced genes and proteins in the kidney after AKI.[15] Administration of NGAL provides remarkable structural and functional protection in animal models.[16] The potential use of these endogenous iron chelating agents (apotransferrin and NGAL) in human AKI is under investigation. Third, deferiprone, which also acts as an iron chelator, currently is being investigated as a potential therapeutic agent in human AKI. Oral deferiprone completed a phase 2 randomized clinical trial to test the efficacy and safety of the treatment, and a phase 3 trial with patients with AKI on preexisting chronic kidney disease is underway.

Alterations in Tubule, Endothelial, and Glomerular Cell Cytoskeleton

The structural response of the tubule cell to injury is multifaceted and includes loss of cell polarity and brush borders, cell death, dedifferentiation of viable cells, proliferation of tubule cells, and restitution of a normal epithelium. Cellular ATP depletion leads to a rapid disruption of the apical actin cytoskeleton and redistribution of actin from microvilli into the cytoplasm.[17] ADF (cofilin) is a cytosolic protein that is normally maintained in the inactive phosphorylated form by Rho GTPases. In cultured tubule cells, ATP depletion leads to Rho GTPase inactivation, with resultant activation and relocalization of ADF to the surface membrane and membrane-bound vesicles. Concomitantly, ATP depletion dissociates the actin-stabilizing proteins tropomyosin and ezrin, allowing the activated ADF to bind and consequently sever actin, which in turn leads to microvillar breakdown. Similarly, ATP depletion of cultured endothelial cells has been shown to activate ADF/cofilin in a direct and concentration-dependent fashion, which results in abnormal F-actin aggregation.[18] Thus inactivation of ADF may represent a promising but unexplored direction for AKI.

ATP depletion induces p38-MAPK-HSP27 (heat shock protein-27) signaling, which is implicated in actin rearrangement and reduced cell adhesion. p38-MAPK signaling is activated by stress stimuli, such as ischemic injury, and likely induces epithelial cell shedding. ATP depletion increases phosphorylation of p38-MAPK and HSP27, and translocation of HSP27 from the cytoskeleton to the cytosol. HSP27 normally acts as an F-actin cap-binding protein and inhibits actin polymerization. HSP27 translocation renders actin filaments more susceptible to rearrangement. Mice pretreated with a p38-MAPK inhibitor did not display increased HSP27 translocation, suggesting that the p38-MAPK pathway is involved with HSP27 translocation, cytoskeletal changes, and loss of cell adhesion. Further research is needed to determine whether these cytoskeletal changes are toxic or protective during AKI.[19]

Disruption of the apical cytoskeleton by ATP depletion also results in loss of tight (zonula occludens) junctions and zonula adherens junctions. Reduced expression, redistribution, and abnormal aggregation of a number of key proteins that constitute the tight and adherens junctions have been documented after ischemic injury in cell culture, animal models, and human studies.[5] Loss of cadherin staining in the vascular endothelium also suggests that cadherin junctions are altered during injury. The consequent loss of tight junction barrier function can potentially magnify the transtubular backleak of glomerular filtrate induced by obstruction.

Studies have shown that ischemic injury also can result in podocyte-specific molecular and cellular changes. In healthy podocytes, Neph1 (a component of the podocyte slit diaphragm) complexes with ZO-1 (an actin-related tight junction protein), to link tight junctions to the cortical actin skeleton, thereby providing a structural framework for the slit diaphragm. Slit diaphragms are an important component of the glomerular filtration barrier, and loss of the structure can lead to impaired filtration. Ischemia induces the dissociation of Neph1 from ZO-1, which results in podocyte effacement and loss of the Neph1-ZO-1 interaction. Although the interaction can be restored after reperfusion, the recovery of podocyte structure is often incomplete.[20]

Other changes occur in the glomerulus during AKI. Podocyte foot processes coarsen during injury, and there is a decrease in heparin sulfate proteoglycan and sialic acid on the endothelial surface layer, which can lead to albuminuria and decrease glomerular filtration rate (GFR). Tumor necrosis factor-alpha (TNF-α) recently was implicated as a mechanism mediating AKI via glomerular abnormalities; studies showed that TNF-α receptor (TNFR1) knockout mice exhibit fewer glomerular alterations and an ameliorated GFR reduction after ischemic injury. TNF-α is a key cytokine involved with AKI and is implicated especially in septic AKI. The glomerular endothelial barrier is often dysfunctional in septic AKI; in TNF-induced AKI, the glomerular fenestrae and endothelial surface layer detach from the basement membrane and swell. Mice deficient in TNFR1 have normal glomerular morphology and density with minimal cellular detachment, suggesting that TNF-α mediates damage to the glomerular endothelium and is a key effector in septic AKI.[21]

Recent studies also have noted increased microvascular permeability during AKI, with the observation of dextran leaking into the interstitial space, pointing to the loss of the capillary barrier. Matrix metalloproteinase (MMP) 2 and 9 may contribute to this breakdown, as deletion of MMP-9 stabilized microvasculature in mice. Peritubular capillary loss occurs in the weeks after kidney injury and causes persistent renal hypoxia, which can mediate progression to chronic kidney disease (CKD). MMP enzymes degrade and modify the extracellular matrix, inducing loss of microvascular density and preventing blood vessel stability. Deleting MMP-9 in mice preserved microvascular density, when measured 3 weeks after ischemic injury. The deletion also stabilized vascular endothelial growth factor (VEGF) levels, which decreased in mice that retained the MMP-9 gene. Deleting MMP-9 does not promote angiogenesis but does help stabilize existing vascular structures during ischemic injury, likely because of its association with VEGF.[22]

Ischemia results in the early disruption of at least two basolaterally polarized proteins: namely, Na^+, K^+ ATPase and integrins. The Na^+, K^+ ATPase normally is tethered to the spectrin-based cytoskeleton at the basolateral domain by the adaptor protein ankyrin. In cell culture, animal models, and human studies, ischemia leads to a reversible cytoplasmic accumulation of Na^+, K^+ ATPase, ankyrin, and spectrin in viable tubule cells.[23] The mislocated Na^+, K^+ ATPase remains bound to ankyrin but is devoid of spectrin. Postulated mechanisms for loss of Na^+, K^+ ATPase polarity include hyperphosphorylation of ankyrin, with consequent loss of spectrin binding, and cleavage of spectrin by ischemia-induced activation of proteases such as calpain.

A physiologic consequence of the loss of basolateral Na^+, K^+ ATPase is impaired proximal tubule sodium reabsorption and a consequent increase in fractional excretion of sodium, which are diagnostic signatures of intrinsic AKI.

The β1 integrins normally are polarized to the basal domain, where they mediate cell-substratum adhesions. Ischemic injury leads to a redistribution of integrins to the apical membrane, with consequential detachment of viable cells from the basement membrane. The exfoliated cells display abnormal adhesion within the tubular lumen, mediated by an interaction between apical integrin and the Arg-Gly-Asp (i.e., RGD) motif of integrin receptors. Administration of synthetic RGD compounds attenuates tubular obstruction and renal impairment in animal models, and the recent development of orally active integrin antagonists holds promise for clinical application in human AKI.[24]

Heat shock protein (Hsp)-90 can protect against ischemic injury by regulating vascular tone and preventing cytoskeletal disruptions. Prior studies have shown a reduction in endothelial cell eNOS (endothelial nitric oxide synthase) after ischemia, which consequently decreases NO synthesis. Hsp-90 was found to couple with eNOS, promoting NO production; when the coupling is disrupted, eNOS produces superoxide anions, which have deleterious effects during AKI. Intrarenal transfection of Hsp90α or Hsp90β in mice before ischemia protected against injury and preserved tubular architecture. Administering Hsp-90 has downstream effects on eNOS and induces NO production, which improves renal blood flow. Developing a therapy that induces Hsp-90α/β expression could prevent ischemic injury in clinical settings for patients who are especially susceptible to AKI.[25]

Sphingosine-1 phosphate receptor 1 ($S1P_1R$) is another molecule that helps maintain proximal tubular cell structure during ischemia. Selective activation of $S1P_1R$ on proximal tubule cells in mice blocked apoptosis and induced Akt and MAPK survival pathways. When $S1P_1R$ is knocked down with siRNA or deleted from proximal tubule cells, there is an increase in apoptotic cell death. A selective $S1P_1R$ agonist may have clinical efficacy in treating patients with ischemic AKI.[26] Sphingosine-1 phosphate receptors 2 and 3 ($S1P_2R$/$S1P_3R$) exacerbate kidney injury, because they induce inflammatory cytokines and mediate neutrophil infiltration. Dendritic cells (DCs) with $S1P_3R$ are required to activate natural killer cells and initiate neutrophil infiltration. Mice with $S1P_3R$-deficient DCs produce more antiinflammatory cytokines and have reduced tubular injury and neutrophil invasion. The absence of $S1P_3R$ makes DCs immature and tolerized, which promotes renal repair.[27] Blocking $S1P_2R$ before and after ischemic injury reduces tubular damage, expression of inflammatory genes, and necrosis.[28]

Alterations in Cell Viability

Injured tubular epithelial cells suffer one of three distinct fates after AKI. A majority of cells remain viable, suggesting that they either escape injury or are only sublethally injured and undergo recovery. A subset of tubule cells display patchy cell death resulting from at least two pathophysiologic mechanisms: Necrosis is an explosive, chaotic process characterized by loss of membrane integrity, cytoplasmic swelling, and cellular fragmentation. Apoptosis is a quiet, orderly demise typified by cytoplasmic and nuclear shrinkage, DNA fragmentation, and breakdown of the cell into membrane-bound apoptotic bodies that are rapidly cleared by phagocytosis. These two forms of cell death can coexist and are considered to present two ends of a spectrum. In AKI, the mode of cell death depends primarily on the severity of the insult and the resistance of the cell type. Necrosis occurs after more severe injury and in the more susceptible nephron segments and often is characterized by the activation of phospholipase A2, calpain, and eicosanoids. In contrast, apoptosis predominates after less severe injury, especially in the ischemia-resistant distal nephron segments. Apoptosis can be followed by "secondary necrosis," especially if the apoptotic cells are not removed rapidly.

Apoptosis is a major mechanism of early tubule cell death in contemporary clinical AKI, and considerable attention has been directed toward dissecting the molecular mechanisms involved.[6,29] Several pathways, including the intrinsic (Bcl-2 family, cytochrome *c*, caspase 9) extrinsic (Fas, FADD, caspase 8), and regulatory (p53, NF-κB) factors, appear to be activated by ischemic AKI. The extrinsic pathway functions by binding death ligands to cell-surface receptor, resulting in procaspase-8 activation, often through mediation with adaptor proteins such as Fas-associated protein with death domain (FADD) or TNF receptor 1-associated death domain. The role of the Fas-FADD pathway in animal models was suggested by demonstration of upregulation of these proteins in apoptotic tubule cells after ischemia,[30] and the functional protection afforded by small interfering RNA duplexes targeting the Fas gene. However, convincing human data are lacking, because the induction of the Fas gene shown in one study of human cadaveric kidney transplants was not reproduced in two subsequent publications.[6,29] On the other hand, growing evidence implicates an imbalance between the pro-apoptotic (Bax, Bid) and anti-apoptotic (Bcl-2, Bcl-xL) members of the Bcl-2 family in animals and humans so affected.[6,29] Disequilibrium between Bac/Bcl-2 and Bad/Bid can lead to the formation of mitochondrial pores, which alters cell viability. The proapoptotic transcription factor p53 is activated by HIF-1α and induced at the mRNA and protein levels, and inhibition of p53 by pifithrin-α suppresses ischemia-induced apoptosis by inhibiting transcriptional activation of Bax and mitochondrial translocation of p53.[31] However, pifithrin-α is an unlikely candidate for therapeutic consideration in humans, because generalized inhibition of p53-dependent apoptosis is likely to promote survival of damaged or mutation-bearing cells in other organ systems. Recent studies have examined the efficacy of p53 siRNA as a potential therapeutic agent, as administration of the treatment decreased serum creatinine and tubular necrosis in animal models. siRNA administered via IV rapidly accumulates in the kidney, after being endocytosed by proximal tubule cells. A dose of anti-p53 siRNA given 2 to 4 hours after ischemic injury in mice was effective in reducing tubular damage and apoptosis.[32] A clinical trial examining the effects of p53 siRNA in human AKI is underway.

Inhibition of other apoptotic pathways holds promise for clinical application in human AKI. Caspase activation is by and large the final common "execution" step in apoptosis, and cell-permeant caspase inhibitors have provided particularly attractive targets for study. Currently available inhibitors largely have been investigated only in animals, provide only partial protection, and are most effective when administered before the insult. Erythropoietin, α_1-acid glycoprotein, minocycline, TNF-α antagonists, A_1 adenosine receptor agonists, peroxisome proliferator-activated receptor-β ligands, geranylgeranylacetone, and poly(ADP-ribose) polymerase inhibitors have provided encouraging functional protection from AKI, with inhibition of apoptosis and inflammation.[5] Some of these agents are already widely available and have been used safely in other human conditions, and results with their use in AKI should be forthcoming. Challenges for the future clinical use of

apoptosis inhibition in AKI include determining the best timing of therapy, optimizing the specificity of inhibitor, minimizing the extrarenal side effects, and tubule-specific targeting of the apoptosis-modulatory maneuvers.

The study of autophagy as another mechanism of altered cell viability AKI has been a topic of considerable recent attention. Pathways leading to autophagy are upregulated in experimental AKI. In models of AKI, inhibition of autophagy in proximal tubules worsened tubular injury and renal function, suggesting a protective role for autophagy. Recent progress in identifying the interplay of autophagy, apoptosis, and regulated necrosis has revealed common pathways and molecules in this cross-talk during the pathogenesis of AKI. Autophagy and its associated pathways pose potentially unique targets for therapeutic interventions in AKI.

The role of micro-RNAs (miR) in AKI recently has been elucidated. Micro-RNAs regulate heme oxygenase-1 (HO-1) and H2A histones. HO-1 is protective, whereas H2A histones induce apoptosis in endothelial and tubular epithelial apoptotic pathways. In animal models, miR-24 was upregulated in the kidney 24 and 168 hours after IRI. miR-24 enrichment caused increased apoptosis, reactive oxygen species production, and defects in tubular migratory capacity. miR-24 targets Sphingosine 1-Phosphate Receptor 1 and H2A histone family mRNAs, and inhibits their translation. Silencing miR-24 with LNA-24 treatment improved cell migratory ability, endothelial tubule formation, and protected against endothelial and epithelial apoptosis. Heme oxygenase-1 (HO-1) was upregulated significantly after miR-24 silencing, suggesting that HO-1 inhibition is part of the miR-24 mediated-apoptotic pathway. Silencing miR-24 is a promising therapeutic treatment that lessens apoptosis and can reduce fibrosis, suggesting that it may be effective in inhibiting the development of CKD.[33] HO-1 activity also can be increased by hemin-pretreatment, which increases HO-1 in the glomeruli, and upregulates expression of thrombomodulin and activated protein C.[34]

The mechanisms whereby a majority of tubule cells escape cell death and either emerge unscathed or recover completely after AKI remain under active investigation. Heat shock proteins (HSPs) have surfaced as prime mediators of this cytoprotection. Induction of HSPs is part of a highly conserved innate cellular response that is activated swiftly and robustly subsequent to ischemic AKI. The heat shock response is particularly robust in immature kidneys and may form the basis for the common observation that subsequent AKI is less likely to develop in premature infants than adults. HSPs promote cell survival by inhibiting apoptosis, and liposomal delivery of HSP72 into cultured renal tubule cells blocks ischemia-induced apoptosis by increasing the Bcl-2 to Bax ratio. HSPs also facilitate the restoration of normal cellular function by acting as molecular chaperones that assist in the refolding of denatured proteins, as well as proper folding of nascent polypeptides. In cultured tubule cells, inhibition of the heat shock response by gene silencing techniques has been shown to produce profound impairment of cellular integrity and Na^+, K^+ ATPase polarity, and overexpression of HSP70 was noted to mitigate the loss of Na^+, K^+ ATPase polarity after ATP depletion.[35] Inducing HSP70 in dendritic cells and macrophages can expand the population of antiinflammatory $T_{regulatory}$ cells, which helps protect renal tissue.[36] Collectively, these findings suggest that maneuvers that enhance the innate HSP response have potential benefit in human AKI.

Surviving renal tubule cells possess a remarkable ability to regenerate and proliferate after AKI. Morphologically, repair is heralded by the appearance of dedifferentiated epithelial cells that express vimentin, a marker for multipotent mesenchymal cells. These cells most likely represent surviving tubule cells that have dedifferentiated. In the next phase, the cells upregulate genes encoding a variety of growth factors, such as insulin-like growth factor-1 (IGF-1), hepatocyte growth factor (HGF), and fibroblast growth factor (FGF), and undergo marked proliferation. In the final phase, cells express differentiation factors, such as NCAM and osteopontin, and undergo redifferentiation until the normal fully polarized epithelium is restored. Thus, during recovery, renal tubule cells recapitulate phases and processes very similar to those during normal kidney development.[37] Understanding the molecular mechanisms of repair may provide clues toward accelerating recovery from AKI. For example, HGF is renoprotective and renotrophic in animal models of AKI, as a consequence of its proliferative, antiapoptotic, and antiinflammatory actions. The use of HGF in humans, however, has been hampered at least in part by the widespread expression of its receptor, raising the possibility of serious extrarenal side effects.[38] Recent studies have explored the effects of BB3, a small molecule with strong HGF-like activity, which, when first administered at 24 hours after renal ischemia in rats, improved survival, augmented urine output, and improved kidney function in an animal model. BB3 is being tested in clinical settings with renal transplant patients.[39]

In the case of IGF-1, enthusiasm for its renoprotective effects has been dampened by its exacerbation of inflammation and neutrophil infiltration in the post-ischemic kidney in animals. Human trials with recombinant IGF-1 have not demonstrated a beneficial effect. New growth factors have emerged as potential therapeutic targets that may accelerate renal recovery, including epidermal growth factor and α-melanocyte stimulating hormone (α-MSH), which likely acts via direct hemodynamic effects. α-MSH is an efficacious antiinflammatory and antiapoptotic cytokine and protects from ischemic, nephrotoxic, and septic AKI.[40] However, although α-MSH therapy seemed effective in animal models, it was not effective in human AKI trials.

Identification of the source of multipotent mesenchymal cells involved in the regeneration and repair process has been a matter of intense contemporary research. It is now apparent that renal tubule cells are capable of regeneration, instead of regeneration being driven by an extrarenal progenitor population. Current literature suggests that proximal tubule cells undergo transient de-differentiation after injury and then proliferate to repopulate the tubule.[41] However, mesenchymal stem cells (MSCs) may have important autocrine, paracrine, and growth factor-like effects on kidney regeneration. MSCs are drawn to renal tubules that produce stromal cell-derived factor 1 (SDF-1), and remain in the injured kidney for a short period of time. They secrete various growth factors that mediate kidney repair.[42] Administered MSCs clearly enhance recovery from ischemic AKI in animals and recently were tested in clinical settings. Modified MSCs, which were modified to be immune privileged and genetically stable, were tested as a therapy for human AKI, but the treatment was deemed ineffective.[43]

Endothelial progenitor cells (EPCs) are bone marrow–derived precursors that promote tubular regeneration in the kidney after ischemic injury. Microvesicles from EPCs can protect the kidney from ischemic injury, likely by delivering microRNA that reprograms resident renal cells to a regenerative program. Rats treated with EPC microvesicles had a reduced number of tubular lesions, decreased creatinine and blood urea nitrogen (BUN) levels, reduced apoptosis, and increased tubular proliferation. Six months after ischemic injury, rats showed less fibrosis and glomerulosclerosis, suggesting that

EPC vesicles have long-term beneficial renoprotective effects.[44]

Alterations in the Microvasculature

The role of endothelial alterations in the initiation and extension of AKI has received increased attention in recent years. Morphologically, disruption of the actin cytoskeleton and junctional complexes, similar to those previously described in tubule epithelial cells, have now been documented in endothelial cells in experimental AKI. Consequent endothelial cell swelling, blebbing, and death, with detachment of viable cells, have been observed, and circulating endothelial cells have been demonstrated in humans with septic shock. Sites of endothelial denudation are prone to prolonged vasoconstriction; in one study, systemic or intrarenal administration of fully differentiated endothelial cells into postischemic rat kidneys results in functional protection.[5] Furthermore, ischemic injury leads to a marked upregulation of angiostatin, a well-known antiangiogenic factor that induces apoptosis of endothelial cells. Collectively, these findings provide a rationale for the use of proangiogenic agents that can increase the pool of or mobilize endothelial progenitor cells. These agents include erythropoietin, bone morphogenic protein (BMP), vascular endothelial growth factor (VEGF), and statins. A clinical trial is testing the efficacy of THR-184, a specific agonist of Alk3 (a major BMP receptor expressed in the proximal tubule). THR-184 was protective against ischemic and nephrotoxic AKI in animal models and is being investigated as a therapy for patients at high risk of cardiac-surgery AKI.

AKI also leads to increased endothelial expression of a variety of adhesion molecules that promote endothelium-leukocyte interactions. These include intracellular adhesion molecule-1 (ICAM-1), P-selectin, E-selectin, B7-1, vascular adhesion molecule-1 (VCAM-1), and thrombomodulin (TM). Although ablation of ICAM-1 gene and pretreatment with ICAM-1 antibody was shown to render mice resistant to ischemic AKI, human trials with anti-ICAM-1 monoclonal antibody administered after ischemic insult did not prevent AKI in cadaveric transplant recipients. Similarly, gene knockouts, monoclonal antibodies, and pharmacologic inhibitor studies have suggested a role for E-, L- and P-selectin.[5] Selectin inhibitors mimic a fucosyl sugar that is present on a shared P, E, and L-selectin ligand. However, subsequent studies have shown that it is platelet P-selectin, not endothelial P-selectin, that is the key component leading to AKI. Possible mechanisms include (1) adhesion of platelets to the endothelium, with subsequent leukocyte adhesion, and (2) adhesion of platelets to neutrophils, with consequent rouleaux formation and trapping in narrow peritubular capillaries.[5] Studies have elucidated mechanisms involved in coagulation and neutrophil-mediated ischemic injury. B_2 integrin (CD18) is thought to be involved in leukocyte-endothelial adhesion, as is B7-1, a leukocyte adhesion molecule whose expression is increased after injury. Endothelial cells are involved with coagulation processes via interactions with protein C and thrombomodulin (TM). TM is a glycoprotein present on the membrane surface of endothelial cells and serves as a cofactor to thrombin, which cleaves protein C to produce activated protein C (APC). Ischemic injury downregulates TM expression, which leaves the kidney microvasculature in a procoagulant state. Recombinant TM administered to rats before ischemia significantly reduced injury and dysfunction and improved renal histology. Rats treated with TM had faster renal blood flow rates, more free-flowing leukocytes, and a smaller percentage of rolling and static blood cells. TM administered after ischemic injury also was observed to decrease renal dysfunction. TM treatment increased APC activity, and induced EPCR/PAR-1 antiinflammatory signaling, while having its own renoprotective effects that are separate from the molecule's antithrombic properties.[45] APC likely suppresses induction of ACE-1 during renal injury by inhibiting thrombin generation, which promotes antiinflammatory signaling.[46] Administering APC to mice increases renal blood flow and ameliorates septic AKI.

Derangements in the coagulation cascade, such as alterations in tissue-type plasminogen activator and plasminogen activator inhibitor-1 in the kidney, may account for the fibrin deposits characteristically found in the renal microvasculature after ischemic injury. The pathways and mechanisms that are involved in formation of interstitial fibrosis after AKI have been examined. Impaired endothelial proliferation and mesenchymal transition processes contribute to vascular dysfunction after AKI. In contrast with tubule epithelial cells, cells of the renal vasculature lack an efficient regenerative capacity, which results in a persistent 30% to 50% reduction in vascular density after ischemic injury.[47] Vascular dropout likely promotes hypoxia and impairs hemodynamic and sodium regulatory responses in the kidney after AKI and may augment the progression of CKD. Endothelial-to-mesenchymal transition may explain the loss of renal microvessels and the deposition of interstitial fibroblasts, which are seen during ischemic AKI recovery. Numerous genes and molecules have been identified as contributing to kidney fibrosis, including platelet-derived growth factor receptor-β (PDGFR-β), metalloproteinase inhibitor 3 (encoded by the *TIMP-3* gene), a disintegrin and metalloproteinase with thrombospondin motifs 1 (encoded by *ADAMTS1* gene), transforming growth factor β (TGF- β), and angiotensin II (Ang II).

Reduced endothelial proliferation is considered a factor that contributes to vascular loss after AKI. Expression of VEGF (which stimulates endothelial cell proliferation in vitro) decreases after AKI. Immunoneutralization of TGF-β was observed to preserve vasculature, whereas administering VEGF up to 3 weeks postischemic injury also protects from vascular dropout. Administration of VEGF to rats with ischemic injury decreased vessel loss, but did not change endothelial cell proliferative capacity, suggesting that renal vascular loss likely is due to a lack of vascular trophic support after injury.[48]

Pericyte and vascular stability after kidney injury also is compromised, because pericytes differentiate into scar-forming myofibroblasts and lose their stabilizing functions. A recent study observed upregulated expression of ADAMTS-1, a metalloprotease reported to cleave capillary basement membrane proteins and inhibit angiogenesis, and downregulated expression of TIMP-3, which stabilizes capillary tube networks. Kidney pericytes are unable to stabilize capillary tubes in the presence of ADAMTS-1, which may mediate pericyte detachment. TIMP-3 deficiency increases myofibroblast expansion and microvascular permeability. ADAMTS-1 blockade may have promising therapeutic uses in limiting interstitial fibrosis, although more research is necessary to explore ADAMTS-1 blockade in a clinical setting.[48]

TGF- β, a cytokine that drives organ fibrosis, stimulates profibrotic signaling, and helps activate pericyte-to-myofibroblast transition, which drives interstitial kidney fibrosis. Activation of VEGF receptor 2 and PDGFR-β are key steps in activating pericyte-to-myofibroblast transition and can drive microvascular rarefaction, inflammation, and fibrosis. Fibrosis was induced in mice with a unilateral

ureteral obstruction (UUO) model; after injury, pericytes lost their connection with endothelial cells and transitioned to myofibroblasts after injury. Recent studies have examined the role of epithelial-to-pericyte crosstalk in stimulating myofibroblast development, and have identified TGF- β as a key mediator in this process. Canonical TGF-β signaling was seen in postunilateral ureteric obstruction mice, and inhibition of TGF-β attenuated the extent of interstitial fibrosis. TGF-β promotes cell cycle arrest and upregulates the p21/JNK pathway to stimulate profibrotic cytokine production. Blocking TGF-β receptors would promote normal cell cycle progression and may have relevance in clinical settings with human AKI.[49]

Vascular endothelial growth factor (VEGF) also is involved in endothelial-pericyte crosstalk that can lead to microvascular rarefaction and fibrosis. Administering VEGF during early AKI preserves microvascular density, although overexpression of VEGF later in the recovery process can lead to vascular dropout. Loss of VEGF expression is observed during ischemic injury. Rats treated with VEGF up to 3 days after ischemic injury did not alter the course of renal injury or tubular repair but prevented renal hypertrophy when rats were given a high-sodium diet. VEGF treatment can attenuate the loss of peritubular capillary density and improves long-term renal function, if administered during early injury or recovery.[50]

VEGF signaling can have a deleterious effect if overexpressed and can promote the progression of interstitial fibrosis. Overexpressed PDGF signaling from endothelial cells to pericytes promotes vascular rarefaction, while VEGF signaling from pericytes to endothelial cells is involved in developmental angiogenesis. Blocking VEGFR2 or PDGFRβ signaling promotes downregulation of pro-fibrotic transcripts and can limit pericyte proliferation, differentiation, and migration and downregulate recruitment of inflammatory macrophages. VEGFR2 blockade was observed to attenuate the early angiogenic response to injury, and prevented capillary rarefaction following AKI. Administering VEGFR2 blockade before or active injury was effective in preventing microvascular rarefaction in mouse models. VEGFR2 is expressed almost exclusively in the kidney endothelium, suggesting that a blockade may hold promise as a therapy in human AKI.[51]

Oxidative stress in the postischemic kidney increases angiotensin II (Ang II) activity, which acts as a vasoconstrictor and promotes renal fibrosis. The persisting oxidative stress may mediate CKD progression and increase interstitial fibrosis. Ischemic injury induces generation of superoxide and reactive oxidant species, which increases oxidant stress, resulting in hyperactive Ang II activity for at least 5 weeks after AKI. Ang II decreased renal blood flow and increased renal vascular resistant in rats, and increased tubulointerstitial space in post-AKI kidneys. Apocynin, an NADPH oxidase inhibitor, can reduce the enhanced vasoconstrictor response to Ang II and may protect against fibrosis in post-AKI kidneys.[52]

Alterations in the Inflammatory Response

A growing body of evidence indicates that the inflammatory response plays a major role in ischemic AKI. Inflammatory cascades initiated by endothelial dysfunction can be augmented by the generation of a number of potent mediators by the ischemic proximal tubule, which is thought to represent a "maladaptive response."[53] These include proinflammatory cytokines (such as TNF-α, interleukin [IL]-6, IL-1β, and transforming growth factor-β [TGF-β]), and chemotactic cytokines (such as monocyte chemoattractant protein-1 [MCP-1], IL-8, and RANTES). Elegant human studies have recently demonstrated that the levels of the pro-inflammatory cytokines IL-6 and IL-8 in the plasma predict mortality in patients with AKI, and the levels of CXCR3-binding chemokines in the urine predict AKI after kidney transplantation, attesting to the clinical significance of these mechanisms.[5] Toll-like receptor 2 (TLR2) may represent a major component of this proinflammatory response,[54] and Toll-like receptor 4 (TLR4) recently was suggested as another mediator of endothelial ischemic injury. Mice lacking TLR4 were observed to have reduced tubular damage and fewer proinflammatory cytokines after renal injury.[55]

Renal tubular expression of TLR2 and TLR4 is enhanced after ischemic AKI, and *TLR2* gene silencing by knockout and antisense treatment prevents ischemia-induced renal dysfunction, neutrophil influx, tubule apoptosis, and induction of MCP-1, TNF-α, IL-6, and IL-1β. Morphologically, several leukocyte subtypes have been shown to aggregate in peritubular capillaries and interstitial space even within the tubules after ischemic AKI, and their relative roles remain under investigation. Neutrophils are the earliest to accumulate in the postischemic kidney and often are found in the peritubular capillary network of the outer medulla, where they adhere to endothelial cells and can cause capillary plugging and congestion. Neutrophils also migrate into the interstitium, which increases vascular permeability and aggravates tubular injury. IL-17, which mediates neutrophil recruitment and migration, worsens ischemic injury. IL-17 secretion also induces IFN-γ production, which augments the inflammatory response. Blocking IL-17 or IFN-γ was observed to decrease renal injury in animal models.[56] A recent study found that using A2A receptor analogs, which decrease the number of endothelial adhesion molecules, inhibits neutrophil transmigration and assists in preserving renal function.[57] Neutrophil depletion or blockade of neutrophil function provides partial functional protection in some but not all animal models. Furthermore, neutrophils are not a prominent feature of ischemic AKI in humans, casting doubt on the clinical significance of neutrophil infiltration.

Macrophages are the next to accumulate in animal models, in response to upregulation of MCP-1 in tubule cells and induction of its cognate receptor CCR2 on macrophages. Selective macrophage depletion ameliorates ischemic AKI, but the induction of tissue injury by macrophages appears to additionally require the coordinated action of T cells and neutrophils. Recent studies have revealed that macrophages have two distinct phenotypes during AKI; the first phenotype contributes to injury, whereas the second promotes kidney repair. Classically activated macrophages, which predominate in early ischemic injury, produce proinflammatory cytokines (such as IL-12, IL-23, and LY-6C). Alternatively activated macrophages, also known as M2 macrophages, are believed to modulate the inflammatory response and promote tissue repair and are prevalent during the recovery and repair phase of AKI. Macrophages are thought to alter their expression profile in response to signals received in the local microenvironment, suggesting that their role in AKI is complex and nuanced.[58]

Wnt7b recently was suggested as a pathway involved in macrophage-mediated tubule repair. The Wnt pathway is known to regulate cell proliferation, and injury-induced enhancement of the Wnt pathway response was noted in epithelial and interstitial cells after kidney injury. The *Fzd4/Lrp6* pathway was upregulated in macrophages, suggesting that macrophages were a source of Wnt ligands. Macrophage ablation in mice resulted in failed regeneration of kidney tubule epithelium, a phenotype that was also observed

when expression of Wnt reporter was reduced. Dickkopf proteins, which are Wnt modulators, helped repair kidney function when administered to mice, suggesting that they hold promise as a potential therapy to assist tubule repair in human AKI.[59]

Dendritic cells (DCs), like macrophages, have pro- and antiinflammatory functions and work closely with other components of the immune system to respond to kidney injury. Dendritic cells help regulate immune effector cells, and present antigenic material to T cells. Macrophages and DCs share similar functions and have functional plasticity depending on the cues they receive from the microenvironment. There is a contiguous network of DCs, which are identified by presence of the chemokine receptor CX_3CR_1, in the kidney interstitium and mesangium. DCs are key initiators, potentiators, and effectors in the innate renal immune system. The role of DCs in renal injury is not resolved fully in current literature, because some studies suggest that depleting DCs has protective effects while other findings suggest that deleting dendritic cells in mice worsens injury. Dendritic cell A_{2A}Rs may dampen the immune response, as agonists have reduced ischemic injury in rat models. There is general consensus that the renal microenvironment determines the final DC phenotype.[60] DCs are the earliest producer of TNF-α and other proinflammatory cytokines but also can increase expression of antiinflammatory molecules such as IL-10. Proteinuria, which often occurs with kidney disease, allows DCs to capture and present antigens to T cells, which can cause kidney disease to persist. Cross-talk between renal DCs and mesenchymal stem cells is suggested to have antiinflammatory consequences. More research is needed to fully elucidate the role of DCs in a human AKI setting.[61]

T cells have been identified in animal as well as human models of ischemic AKI and often are observed to increase the production of proinflammatory molecules, such as TNF-α and IFN-γ. T cell depletion is protective in experimental AKI.[62] Double-$CD4^+/CD8^+$ knockout mice are protected from ischemic AKI, and adoptive transfer of wild-type T cells into the null mice abrogates this protective effect. Inconsistencies exist, however, and recent data suggest that the role of T cells in ischemic AKI may be complex, with the identification of both protective (T_H2 phenotype) and deleterious (T_H1 phenotype) subtypes of T cells. Moreover, animals deficient in both T and B cells are not protected from ischemic AKI, and depletion of peripheral $CD4^+$ T cells fails to bestow protection from ischemic AKI.

$T_{regulatory}$ cells recently were identified as a beneficial and reparative portion of the immune response and were suggested to suppress renal inflammation and assist in preserving renal function. $CD4^+CD25^{Hi}$ cells that express Foxp3 work via contact-dependent and soluble mediators and inhibit dendritic cell maturation and downstream immune responses. The receptor TIGIT, which is highly expressed on T_{reg} cells, induces dendritic cells to produce IL-10 and transforming growth factor-β (TBG-β), which dampens the inflammatory immune response. T_{reg} cells also can kill activated immune cells with Fas ligand and granzyme B-mediated mechanisms, or induce apoptosis of activated T cells. T_{reg} cell deficiency in mice resulted in increased renal inflammation and reduced renal function and tubular proliferation; these effects were reversed by adding wild type T_{reg} cells to lymph nodes. However, depleting T_{reg} cells in a sepsis model of AKI improved mice survival, suggesting that T_{reg} cells may contribute to septic AKI. T_{regs} administered to mice 24 hours after reperfusion injury reduced T cell production of proinflammatory molecules and accelerated recovery of renal function, suggesting that a drug that enhances T_{reg} cell count may have therapeutic significance. Preliminary experiments in which T_{reg} cells were infused into a mouse before ischemic injury helped protect against renal injury and dysfunction. Pharmacologic treatments that target intrinsic T_{reg} cells remain a promising therapeutic option for human AKI.[63]

Recent literature has focused on the proximal tubule's role in mediating the innate immune response. Toll-like receptors are a key portion of the innate immune system and function as pattern recognition receptors. Epithelial cells exhibit similar behavior as traditional innate immunity cells and assist in maintaining the integrity of the tissue microenvironment in normal conditions. The proximal tubule filters and takes up endotoxins, a process likely mediated by TLR4, but does not result in any apparent injury to the segment, in part because of increased production of cytoprotective molecules such as heme oxygenase-1 and sirtuin-1. The proximal tubule also secretes cytokines and chemokines, which contribute to vascular isolation of injured tissue. Cross-talk between the proximal tubule and the thick ascending limb (TAL) of the loop of Henle recently has been revealed, with TLR4 and Tamm-Horsfall Protein (THP) suggested as likely mediators of the process. THP, also known as uromodulin, is considered a protective molecule, and inhibits proximal tubule production of proinflammatory cytokines and chemokines. Cross-talk between the TAL and proximal tubule suppresses tubular activation of innate immunity and reduces inflammatory injury. The absence of THP in mice resulted in more severe inflammation, increased cast formation, reduced renal function, and diffuse tubular necrosis in the outer medulla. Neutrophil infiltration also was increased in THP-knockout mice, corroborating suggestions that THP functions as an antiinflammatory and protective molecule during ischemic injury.[64]

The potential role of B cells in ischemic AKI is intriguing. Compared with wild-type animals, B cell–deficient mice are protected partially from structural and functional ischemic renal injury, despite comparable neutrophil and T cell infiltrations. Wild type serum transfer, but not B cell transfer, into B cell–deficient mice was shown to restore susceptibility to ischemic AKI, implicating a soluble serum factor as a mechanism by which B cell deficiency confers renal protection.[5]

Activation of the complement system in AKI, with resultant amplification of the inflammatory response in the kidney, has received widespread attention in recent years. Whereas ischemia-reperfusion injury in most organs activates the complement cascade along classic pathways, studies in animals and humans have implicated the alternative pathway in AKI.[5] This evidence remains debatable, however, because other reports have identified a role for the mannose-binding lectin pathway after animal and human ischemic AKI. Also controversial is the identification of the final active complement component. Although earlier studies pointed to the C6b-directed formation of a membrane attack complex, recent observations have identified a predominant role for C5a in ischemic AKI.[65] C5a is a powerful chemoattractant that recruits inflammatory cells such as neutrophils, monocytes, and T cells. The kidney is one of the few organs in which the C5a receptor is normally expressed, in proximal tubule epithelial cells as well as in interstitial macrophages. C5a receptor expression in tubule epithelial cells is upregulated markedly after ischemia-reperfusion injury and sepsis. Inhibition of C5a generation using monoclonal antibodies was found to protect against renal dysfunction induced by ischemia, and in turn to inhibit neutrophil and macrophage influx in experimental models. Of importance, pretreatment with orally active small

molecule C5a receptor antagonists substantially reduced the histologic and functional impairment induced by ischemic AKI in animal models.[66] Small molecule antagonists for C5a receptors currently are undergoing a phase II clinical trial in rheumatoid arthritis and represent promising agents for the treatment or prevention of ischemic AKI.

Other strategies that modulate the inflammatory response may also provide significant beneficial effects in human AKI, and several have already been tried in experimental situations. For example, IL-10 is a potent antiinflammatory cytokine that has been shown to provide functional protection against ischemic AKI by inhibiting maladaptive cytokine production by T_H1 cells. Injecting IL-10-overexpressing macrophages into mice reduced glomerular inflammation, although high doses likely are needed to induce normal creatinine and BUN levels. IL-10 likely functions by increasing intracellular iron concentrations and inducing heme-oxygenase expression. IL-10 treatment upregulated levels of antiinflammatory mediators, elevated intracellular iron concentrations, and increased cell proliferation.[67] Administration of a monoclonal antibody against the proinflammatory cytokine IL-6 ameliorated structural and functional consequences of ischemic AKI, decreased neutrophil infiltration, and reduced proinflammatory cytokine production. Bimosiamose, a pan-selectin inhibitor, was shown to provide protection from ischemic AKI in a kidney transplant model by reducing infiltration of macrophages and T cells and inhibiting intragraft expression of chemokines and cytokines. In addition to lowering cholesterol in humans, widely used statins possess several properties that may be beneficial in ischemic AKI, including profound antiinflammatory effects, inhibition of reactive oxygen species, and stimulation of endothelial nitric oxide production. In fact, several investigators have reported impressive structural and functional protection from ischemic AKI by short-term pretreatment with statins.[68] Finally, α-melanocyte-stimulating hormone (α-MSH), an antiinflammatory cytokine, protects against experimental ischemic AKI by inhibiting the maladaptive activation of genes that cause inflammatory and cytotoxic renal injury. Of interest, α-MSH potentiates the beneficial effect of erythropoietin, remains effective even when administered after renal ischemia, and also protects against the distal lung injury that occurs after ischemic AKI.[69] The overall safety records with some of these interventions render them promising candidates for the prevention and treatment of ischemic AKI.

Alterations in Gene Expression

Attempts at unraveling the molecular basis of the myriad pathways activated by AKI have been facilitated by advances in functional genomics and transcriptome profiling technologies. Several investigators have used these techniques in human and animal models of AKI to obtain expression profiles of thousands of genes. When combined with bioinformatics tools, these studies have identified novel genes with altered expression, new signal transduction pathways that are activated, and even new drug targets and biomarkers in AKI. One of the first induced molecules to be identified in the postischemic kidney using genomic approaches was kidney injury molecule 1 (KIM-1). KIM-1 protein subsequently was demonstrated to be upregulated in the postischemic animal and human kidney tubules, predominantly on the apical membranes of proximal tubule epithelial cells, where it may play a role in renal regeneration. An ectodomain is shed into the urine, making KIM-1 a promising noninvasive urinary biomarker of ischemic human AKI.[70]

Another example is NGAL, one of the most highly induced genes in the early postischemic kidney.[71] NGAL protein is markedly upregulated in kidney tubules very early after ischemic AKI in animals and humans and is excreted rapidly in the urine, where it represents a sensitive novel biomarker of early ischemic injury.[72] In the postischemic kidney tubule, NGAL protein is highly expressed in tubule cells that are undergoing proliferation, suggesting its protective or regenerative role subsequent to AKI. Exogenous administration of NGAL in experimental models before, during, or even shortly after ischemic or nephrotoxic injury provides remarkable protection at the functional and structural levels, with induction of proliferation and striking inhibition of apoptosis in tubule epithelial cells.[16] In this context, NGAL mitigates iron-mediated toxicity by providing a reservoir for excess iron and may provide a regulated source of intracellular iron to promote regeneration and repair. Exogenously administered NGAL also markedly upregulates heme oxygenase-1 (HO-1), a proven multifunctional protective agent in experimental AKI that works by limiting iron uptake, promoting intracellular iron release, enhancing production of antioxidants such as biliverdin and carbon monoxide, and inducing the cell cycle regulatory protein p21. Because of its multifaceted protective action, NGAL has emerged as a potential therapeutic target in AKI.

Tamm-Horsfall protein (THP), also known as uromodulin, is another kidney-specific glycoprotein whose expression is downregulated at the peak of kidney injury, and is upregulated significantly 48 hours after AKI. There is a major redistribution of THP from the apical membrane of the TAL towards the basolateral domain and renal interstitium during renal recovery. THP likely reduces inflammatory signaling in the proximal tubule, and THP-knockout mice had significantly different TNF-α levels and slower recovery after ischemic injury when compared with wild type mice. An increase in serum THP has been suggested as a biomarker for AKI recovery and holds promise as a clinically relevant biomarker.[73] Two SNPs within the promoter of the THP gene are associated with a decreased risk of chronic kidney disease and a lower level of THP excretion.[74]

Another maximally induced gene identified very early after ischemic injury is Zf9, a Kruppel-like transcription factor involved in the regulation of a number of downstream targets. Zf9 protein is markedly upregulated in the postischemic tubule cells, along with its major trans-activating factor, TGF-β1. Gene silencing of Zf9 was shown to abrogate TGF-β1 overexpression and mitigate the apoptotic response to ATP depletion in vitro.[75] Relevant studies thus have identified a hitherto unrecognized pathway that may play a critical role in the early tubule cell death that accompanies ischemic renal injury.

Extracellular signal-regulated kinases (ERK1 and ERK2) are activated 24 hours after injury and likely alter cytoskeletal organization and focal complex assembly. ERK1 and 2 are localized in damaged proximal tubule cells and are activated during renal reperfusion in response to reactive oxygen species and Ras signaling. ERK1 and 2 have negative downstream effects, and have been observed to phosphorylate proteins that induce the dissolution and restructuring of focal adhesions.[76] ERK inhibitors, such as U0126, may be relevant in clinical settings as a method that preserves cytoskeletal structure during AKI.

Another gene induced by AKI is pentraxin 3 (PTX3), which is released by many cells in response to inflammatory signals. PTX3 expression increases after ischemic injury and also may function as a biomarker for the early detection of

AKI. It is found on the glomerular endothelia and peritubular structures in the outer medulla. PTX3 also is linked to TLR4, because knocking out TLR4 prevents increase in PTX3 expression in mice. Knocking out PTX3 in mice reduces the expression of endothelial adhesion molecules and chemokines and reduces inflammatory responses.[77]

NEPHROTOXIC ACUTE KIDNEY INJURY

Drugs contribute to approximately 15% of all AKI cases in the adult critical care setting, but children are generally less prone to experience nephrotoxicity.[78] The nephrotoxic potential of pharmacologic agents is increased significantly in patients in the ICU setting, in whom renal blood flow may already be compromised by sepsis, cardiac dysfunction, AKI, or dehydration. Thus nonsteroidal antiinflammatory drugs commonly induce a hemodynamically mediated AKI by inhibiting cyclooxygenase (COX), the enzyme required for the synthesis of intrarenal vasodilatory prostaglandins. On the other hand, antibiotics such as aminoglycosides and antineoplastic agents such as cisplatin are primarily proximal tubular toxins. Radiocontrast agent-induced nephrotoxicity is thought to result from at least four pathophysiologic mechanisms: (1) direct toxic effects on tubule epithelial cells, (2) intrarenal vasoconstriction, (3) increased viscosity of the intrarenal blood flow, and (4) microshowers of atheroemboli.

ACUTE KIDNEY INJURY IN SEPSIS

Sepsis is one of the most common causes of AKI in the critical care setting. AKI occurs in more than 50% of patients with septic shock, in whom the mortality rate exceeds 70%. Sepsis-related AKI is primarily hemodynamically mediated, resulting from a potent combination of systemic vasodilation and renal vasoconstriction.[79] The generalized arterial vasodilation characteristic of sepsis is mediated at least in part by cytokines that enhance the expression of inducible nitric oxide synthase (iNOS) in the vasculature. Arterial underfilling leads to activation of the renin-angiotensin-aldosterone axis and to the nonosmotic release of vasopressin, all of which result in compromised renal perfusion. In addition, direct renal vasoconstriction results from cytokines, such as TNF-α, which induces endothelin release. However, a trial of a monoclonal antibody against TNF-α in sepsis did not improve patient survival, suggesting that other pathways contribute to sepsis-induced AKI. These include glomerular and vascular microthrombosis resulting from disseminated intravascular coagulation, generation of reactive oxygen species, activation of complement pathways, and hyperglycemia-induced alterations in the inflammatory response. Recent clinical trials of activated protein C to combat the procoagulant state, insulin for improved glycemic control, and antagonists of the C5a receptor have provided encouraging results in terms of improved survival in sepsis.

CLINICOPATHOLOGIC CORRELATIONS

The clinical course of AKI has been divided classically into three phases: initiation, maintenance, and recovery. To this paradigm, the addition of an "extension" phase after the initiation phase has been proposed, primarily to reflect previously underestimated amplification processes.[5,7] Recent advances in the pathogenesis of AKI allow postulation of temporal relationships between the clinical phases and the cellular alterations detailed in this chapter. The *initiation* phase is the period during which initial exposure to the ischemic insult occurs, kidney function begins to fall, and parenchymal injury is evolving but not fully entrenched. Intracellular ATP depletion is profound, sublethal injury to the tubule epithelial and endothelial cells predominates, generation of reactive oxygen molecules is initiated, and activation of inflammatory mechanisms commences. Intrarenal protective mechanisms such as induction of heat shock proteins in tubule cells also are brought to play during the initiation phase. If the injury is alleviated at this stage, complete restitution and recovery are the rule.

Prolongation of ischemia followed by reperfusion ushers in the *extension* phase. Blood flow returns to the cortex, and tubules undergo reperfusion-dependent cell death but also commence the regeneration process. By contrast, medullary blood flow remains severely reduced, resulting in more widespread tubule cell death, desquamation, and luminal obstruction. Injured endothelial and epithelial cells amplify the raging inflammatory cascades, and the endothelial denudation potentiates the intense vasoconstriction. The glomerular filtration rate continues to decline. This phase probably represents the optimal window of opportunity for early diagnosis and active therapeutic intervention.

During the *maintenance* phase, parenchymal injury is established, and the glomerular filtration rate is maintained at its nadir even though renal blood flow begins to normalize. Cell injury and regeneration occur simultaneously, and the duration and severity of this phase may be determined by the balance between cell survival and death. Repair of epithelial and endothelial cells appears to be critical to recovery. Measures to accelerate the endogenous regeneration processes may be effective during this phase.

The *recovery* phase is characterized functionally by an improvement in glomerular filtration rate and structurally by reestablishment of tubule integrity, with fully differentiated and polarized epithelial cells. The repair process may be incomplete, however, and microvascular and tubular dropout have been demonstrated in animal studies.

SYSTEMIC INJURY AND ORGAN CROSS-TALK

Systemic dysfunction and distant organ comorbidities—including the heart, lung, spleen, brain, liver, and gut—associated with AKI have been the subject of recent study. The prognosis for patients who have AKI and another organ in a state of dysfunction is especially poor, with a morality rate of 60% to 80%.[3] AKI in a setting of pulmonary dysfunction is best studied, as is cardiorenal syndrome.

AKI occurs in acute respiratory distress syndrome (ARDS) in about one fourth of patients, as the volume overload caused by AKI exacerbates lung injury. Renal injury results in increased pulmonary vascular permeability, aggravating the symptoms of respiratory distress. Impaired salt and water clearance, from dysregulated and downregulated epithelial sodium channel, Na^+/K^+ ATPase, and aquaporin 5,[80] further compromises gas exchange in ARDS. IL-6 is the major mediator of lung injury and inflammation after AKI, with increased neutrophil infiltration, vascular permeability, dysfunctional water and salt transporters, and inflammatory cytokine expression. Pulmonary endothelial cells are the main targets of AKI-induced lung injury, and studies have revealed major changes in TNFR1-dependent caspase activation and apoptosis in pulmonary cells. Toll-like receptor 4

(TLR4) also has been identified as a mediator for pulmonary neutrophil infiltration.

The cardiac and renal systems are in a complex, bidirectional relationship in which failure or injury in one organ can induce or exacerbate injury in the other. A diagnosis of cardiorenal syndrome, which is used in clinical settings to describe co-existing heart and renal failure, predicts higher mortality in patients. AKI can lead to cardiac hypertrophy and increased macrophage infiltration in cardiac tissue, as well as increased cardiac apoptosis.[3] Renal ischemia induces expression of proinflammatory molecules in cardiac tissue, including IL-1, TNF-α, and ICAM-1.

Acute uremic conditions can result in neurologic abnormalities, included increased microglial and neuronal pyknosis, elevated proinflammatory cytokine expression, and reduced locomotor function. Preliminary studies have suggested that the blood-brain barrier is disrupted in mice with AKI, which leads to inflammation, edema, and functional changes in the brain. AKI patients have more pronounced symptoms of acute uremic encephalopathy than with CKD, whereas patients with more severe AKI have more pronounced behavioral and motor changes. A study showed that there were increased pyknotic cells and activated microglial cells in the hippocampus. Inflammatory mediators that are increased in the kidney (including IL-1β, IL-6, G-SCF, MIP-1α, and MIP-1β) also were seen in the cerebral cortex and hippocampus.[81]

Brain injury or a brain-dead state leads to increased systemic cytokine production and inflammation. Patients who receive kidneys from brain-dead donors have more infiltrating T-lymphocytes and macrophages in the graft, whereas living and cardiac donors show reduced cytokine release after reperfusion. Reperfusion of kidneys from brain-dead donors was associated with the release of cytokines such as G-CSF, IL-6, IL-9, IL-16, and MCP-1, whereas kidneys from living and cardiac death donors showed only the release of IL-6 and MCP-1.[82] Brain death predisposes kidney grafts to be proinflammatory after reperfusion; developing a pretreatment that prevents the postreperfusion inflammatory response could increase graft survival and function in transplant patients.

Recent studies have focused on elucidating the relationship between AKI and dysfunction in other organ systems. Hepatorenal syndrome is a severe complication in AKI, with unacceptably low survival estimates of 90 days to 6 months. Hepatorenal syndrome can induce functional renal failure without structural damage to the kidney, compromised hemodynamic function, and activation of the renin-angiotensin-aldosterone axis, sympathetic system neurotransmitters, and vasopressin release. There is likely a reciprocal relationship between the liver and kidney—liver dysfunction upregulates proinflammatory cytokine expression in the kidney, and kidney injury leads to an increased neutrophil presence in the liver.[3] Patients with septic AKI have a high mortality rate, and studies have suggested that splenic apoptosis may increase blood HMGB1 (high mobility group box 1 protein), a potential mediator of sepsis. Splenocytes are reservoirs of transient endothelial progenitor cells and can conger protection from AKI when transferred into mice.[3] There is also emerging evidence of gut-kidney cross-talk, because germ-free mice have more severe renal injury after ischemia, and more T cell infiltration compared with mice with a robust microbiome. Acetate-producing bacteria have been suggested to protect against ischemic AKI, whereas Paneth cells in the small intestine may be potential mediators of intestinal, hepatic, and renal injury.[3]

Key Points

1. Acute kidney injury is a potentially lethal condition in the intensive care unit setting with multiple pathophysiologic mechanisms that interplay with and amplify one another.
2. Acute kidney injury in the intensive care unit setting is frequently multifactorial, with concomitant septic, ischemic, and nephrotoxic components, and with overlapping mechanisms.
3. Recent advances have brought new insight into the roles of apoptosis, autophagy, oxidant- and iron-mediated injury, endothelial changes, and the inflammatory response in the pathogenesis of acute kidney injury.
4. Conquering acute kidney injury will require a comprehensive approach, including making an early diagnosis and executing a multifaceted therapeutic approach based on a better understanding of the pathophysiology.
5. Novel strategies that have emerged from recent findings hold promise for the proactive treatment of human acute kidney injury.

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