

Pathophysiology of ischemic acute kidney injury

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Abstract | Acute kidney injury (AKI) as a consequence of ischemia is a common clinical event leading to unacceptably high morbidity and mortality, development of chronic kidney disease (CKD), and transition from pre-existing CKD to end-stage renal disease. Data indicate a close interaction between the many cell types involved in the pathophysiology of ischemic AKI, which has critical implications for the treatment of this condition. Inflammation seems to be the common process that links the various cell types involved in this process. In this Review, we describe the interactions between these cells and their response to injury following ischemia. We relate these events to patients at high risk of AKI, and highlight the characteristics that might predispose these patients to injury. We also discuss how therapy targeting specific cell types can minimize the initial and subsequent injury following ischemia, thereby limiting the extent of acute changes and, hopefully, long-term structural and functional alterations to the kidney.

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Introduction

The kidney is comprised of heterogeneous cell populations that function together to perform a number of tightly controlled and complex processes. Acute kidney injury (AKI) is a common clinical event that disrupts this homeostasis, leading to unacceptably high morbidity and mortality. One cause of AKI is ischemia, which can occur for a number of reasons, for example, with the use of vasoconstrictive drugs or radiocontrast agents; hypotension linked to surgery/trauma blood loss and sepsis is also a known cause of ischemia. The body is able to adapt to a reduction in blood flow to a certain level, but when delivery of oxygen and metabolic substrates becomes inadequate, cellular injury leads to organ dysfunction. In this Review, we describe the interactions between the various cell types and processes involved in the pathophysiology of AKI. We also outline an approach that will facilitate patient care and development of therapies for ischemic AKI in the future.

Patients at high risk of ischemic AKI

A cardiovascular event can lead to ischemic AKI in any patient, but there are certain individuals who are at inherently high risk of developing AKI following mild to moderate reductions in kidney perfusion. Thakar *et al.*¹ were the first to emphasize the importance of understanding and quantifying this increased risk. Subsequent studies have validated clinical variables, such as age, existing chronic kidney disease (CKD), and proteinuria,

that contribute to this increased risk,^{2–5} and clinical staging systems, including the RIFLE system, have been developed to classify patients according to their risk or injury.^{6,7}

These parameters should be used together with traditional biomarkers when evaluating risk in a patient undergoing elective procedures or receiving therapies that potentially reduce renal blood flow.⁸ Rather than a single characteristic, patients often have multiple associated risk factors that result in a cumulative risk of developing ischemic AKI (Box 1).¹ Identifying patients at risk is an essential component of the medical workup, involving both history taking and a physical examination, as this will maximize care and reduce risk. For example, improving the hemodynamic status of a patient is important to minimize AKI from nephrotoxic drugs such as cisplatin and aminoglycosides, or use of radiocontrast agents, which reduce kidney perfusion.⁹ Additional factors, such as high levels of oxidative stress or inflammation, also increase a patient's risk of developing AKI.¹⁰

Prerenal azotemia is the most common cause of AKI and accounts for 40–55% of all cases.^{11–13} This condition results from kidney hypoperfusion owing to a reduced effective arterial volume, that is, the volume of blood effectively perfusing the organs. Prerenal azotemia is divided into volume responsive and volume non-responsive forms. In patients with the volume non-responsive form, additional intravascular volume does not restore kidney perfusion and function. For example, patients with conditions such as congestive heart failure and sepsis might not respond to intravenous fluid therapy, as decreased cardiac output or total vascular resistance prevent kidney perfusion.^{14–16} Compensation for prerenal azotemia includes activation of baroreceptors, which

Competing interests

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Key points

- During ischemic acute kidney injury (AKI), ATP depletion results in cytoskeletal changes in epithelial and endothelial cells, causing disruption of function, and a decrease in glomerular filtration rate
- Apoptosis and necrosis are major mechanisms of cell death that have important roles in ischemic injury, with the contribution of each pathway depending on the extent of the injury
- Under physiological conditions, endothelial cells regulate permeability, vascular tone, coagulation, and inflammation; endothelial cells that are dysfunctional substantially contribute to the extension phase of AKI
- Inflammation and its mediators orchestrate the extension phase of ischemic AKI, but can also limit injury to tubular epithelial cells and vascular endothelial cells, thereby hastening repair
- Complex interactions between epithelial cells, endothelial cells, inflammatory mediator cells, and cytokines can result in persistent injury during acute tubular necrosis in AKI
- Stem cells, mesenchymal cells, and endothelial progenitor cells contribute to the repair and regeneration of tubular and endothelial cells following injury, and could provide attractive targets for therapeutic intervention

Box 1 | Causes of reduced effective arterial volume and kidney hypoperfusion

Intravascular volume depletion

- Hemorrhage (e.g. following trauma, surgery, postpartum)
- Gastrointestinal losses (e.g. from diarrhea, vomiting, nasogastric loss)
- Renal losses (e.g. diuretics, osmotic diuresis, diabetes insipidus)
- Skin and mucous membrane losses (e.g. burns, hyperthermia)
- Nephrotic syndrome
- Cirrhosis
- Capillary leak

Reduced cardiac output

- Cardiogenic shock
- Pericardial disease (e.g. restrictive, constrictive, tamponade)
- Congestive heart failure
- Valvular heart disease
- Pulmonary disease (e.g. pulmonary hypertension, pulmonary embolism)
- Sepsis

Systemic vasodilation

- Cirrhosis
- Anaphylaxis
- Sepsis

Renal vasoconstriction

- Early sepsis
- Hepatorenal syndrome
- Acute hypercalcemia
- Drugs (e.g. norepinephrine, vasopressin, nonsteroidal anti-inflammatory drugs, angiotensin-converting enzyme, calcineurin inhibitors)
- Radiocontrast agents

initiates a cascade of neural and humoral responses, activation of the sympathetic nervous system, and an increase in the production of catecholamines, especially norepinephrine.¹⁷ Increased release of vasopressin is mediated by hypovolemia and a rise in extracellular osmolality, resulting in vasoconstriction, water retention, and back-diffusion of urea into the papillary interstitium.

Activation of the renin–angiotensin–aldosterone system leads to increased production of the potent vasoconstrictor angiotensin II, which preferentially increases efferent arteriolar resistance.¹⁸ Glomerular filtration rate (GFR) is preserved by the resultant increase in glomerular hydrostatic pressure. Angiotensin II activity is also increased during severe volume depletion, which leads to afferent arteriolar constriction, reducing both renal plasma flow, GFR, and the filtration fraction.¹⁹

Concomitantly, compensatory mechanisms of kidney autoregulation preserve glomerular perfusion.²⁰ Under physiological conditions, autoregulation of renal blood flow works above a mean systemic arterial blood pressure of 75–80 mmHg. Below this pressure, the glomerular ultrafiltration pressure and GFR decline abruptly.²¹ Renal production of prostaglandins, kallikrein, and kinins, as well as nitric oxide (NO) are increased and contribute to vasodilation.^{22,23} Nonsteroidal anti-inflammatory drugs inhibit the production of prostaglandins and worsen kidney perfusion in patients with hypoperfusion. Angiotensin-converting-enzyme inhibitors block the synthesis of angiotensin II and disturb the delicate balance between afferent and efferent arteriolar tone in patients who have severe reductions in effective arterial volume, such as severe congestive heart failure or bilateral renal artery stenosis, thus worsening prerenal azotemia. Conversely, very high levels of angiotensin II, as seen in circulatory shock, cause constriction of both afferent and efferent arterioles, which negates its protective effect.²⁴

Taken together, these data provide strong evidence that certain identifiable patients are at high risk of developing AKI. Increased attention to maximizing conditions, such as hemodynamic status, drug dosing based on GFR, and volume status is essential to prevent or ameliorate AKI in these patients.

Cellular changes during ischemic AKI
Acute epithelial cell injury

Following a reduction in effective kidney perfusion, epithelial cells are unable to maintain adequate intracellular ATP for essential processes. This depletion of ATP leads to cell injury and, if severe enough, cell death by necrosis or apoptosis. All segments of the nephron can be affected during an ischemic insult, but the most commonly injured epithelial cell is the proximal tubular cell. These cells are particularly susceptible for a number of reasons. First, this cell type has a high metabolic rate required for mediating ion transport and a limited capacity to undergo anaerobic glycolysis. Second, owing to the unique blood flow in the outer stripe of the S3 segment of the nephron, there is marked microvascular hypoperfusion and congestion in this region after injury that persists and mediates continued ischemia, even when cortical blood flow might have returned to near-normal levels. Endothelial cell injury and dysfunction are primarily responsible for this phenomenon, known as the extension phase of AKI.²⁵ Understanding that ischemic injury can be localized to specific microvascular domains rather than throughout the kidney is important,

as quantifying total renal blood flow as a measure of effective blood flow could be misleading.

The other major epithelial cells of the nephron involved in the pathophysiology of ischemic AKI are those of the medullary thick ascending limb located distally. Apoptotic changes have been detected in human AKI, as shown in distal nephron segments in nephrotoxic acute tubular necrosis. Apoptosis of distal tubular cells also occurs in donor biopsies before engraftment, which was predictive in one study of delayed graft function.²⁶

In an *ex vivo* model of hypoxic AKI, administration of FG-4497 (a specific prolyl-hydroxylase inhibitor that activates hypoxia-inducible factor) in the isolated perfused kidney led to decreased selective outer medullary distal tubular injury.²⁷ Proximal tubular cell injury and dysfunction during ischemia or sepsis leads to afferent arteriolar vasoconstriction mediated by tubular glomerular feedback, proximal tubular luminal obstruction, and backleak of filtrate across injured proximal tubular cells, resulting in ineffective glomerular filtration and a profound drop in GFR (Figure 1).^{28,29}

Morphological changes

A hallmark of ischemic cell injury is loss of the apical brush border of proximal tubular cells. Disruption of microvilli and their detachment from the apical cell surface leads to formation of membrane-bound ‘blebs’ early following ischemia that are released into the tubule lumen. Detachment and loss of tubular cells exposes areas of denuded tubular basement membrane, resulting in focal areas of proximal tubular dilatation, as well as formation of distal tubule casts.³⁰ The sloughed tubular cells, brush-border vesicle remnants, and cellular debris in combination with uromodulin form these granular casts, which have the potential to obstruct the tubule lumen, leading to no GFR in that functional unit.³¹ Necrotic cell death is rare and restricted to the highly susceptible outer medullary regions, whereas features of apoptosis are commonly seen in both proximal and distal tubular cells (see below).³²

Injured glomerular epithelial cells following ischemia or septic injury are not usually seen on histological stains, although studies have shown reversible podocyte-specific molecular and cellular changes. Wagner *et al.*³³ demonstrated in a rat model that renal ischemia induced podocyte effacement with loss of slit diaphragm and proteinuria owing to rapid loss of interactions between the tight junction proteins Neph1 and ZO-1. Cell culture models using human podocytes further showed that ATP depletion resulted in rapid loss of Neph1 and ZO-1 binding, and redistribution of Neph1 and ZO-1 from the cell membrane to cytoplasm; ATP recovery increased phosphorylation of Neph1 and restored Neph1 and ZO-1 binding and their localization at the cell membrane.³³

Cytoskeletal and structural changes

The actin cytoskeleton has an integral role in maintaining cell structure and function, polarity, endocytosis, signal transduction, motility, movement of organelles, exocytosis, cell division, migration, barrier function of

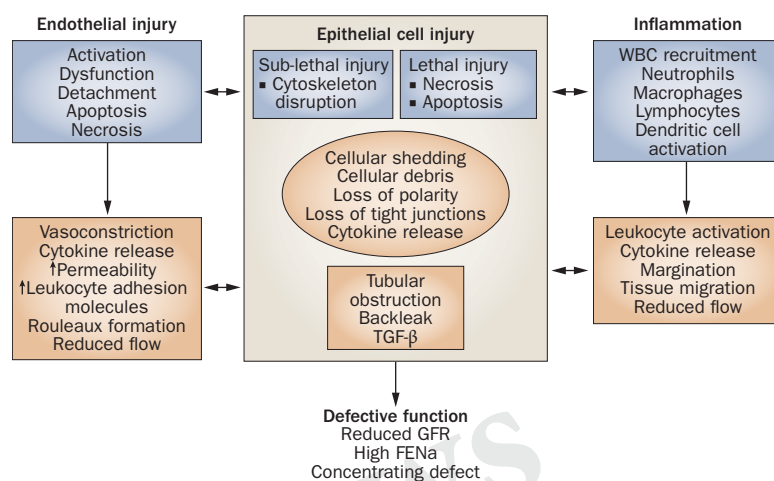


Figure 1 | Pathogenesis of ischemic AKI. The major pathways of GFR impairment in ischemic acute tubular injury are caused by ATP depletion in vascular and tubular cells. Numerous interactions exist between endothelial cells, WBCs and epithelial cells in the pathophysiology of ischemic AKI. These interactions are bidirectional between the cells involved, and result in specific functional and structural alterations. Inflammatory mediators released from proximal tubular cells influence endothelial cell processes (e.g. increase expression of cell adhesion molecules, vasoconstriction) that in turn influence the interactions between WBCs and endothelial cell, leading to reduced microvascular flow and continued hypoxia within the local environment. Additional functional changes, such as a marked reduction in erythropoietin production and 25-hydroxylation of vitamin D, occur. Electrolyte accumulation can rapidly lead to requirement of renal replacement therapy. Metabolic acidosis, as a consequence of AKI, must also be carefully monitored. Abbreviations: AKI, acute kidney injury; FENa, fractional excretion of sodium; GFR, glomerular filtration rate; TGF-β, transforming growth factor β; WBC, white blood cell.

the junctional complexes, and cell–matrix adhesion.³⁴ Maintaining the integrity of the cytoskeleton is especially important for proximal tubular cells in which amplification of the apical membrane by microvilli is essential for normal cell function. Depletion of cellular ATP leads to rapid disruption of apical F-actin by depolymerization mediated in part by cofilin, and redistribution of the cytoskeletal F-actin core. This disruption causes instability of the surface membrane and formation of membrane-bound extracellular vesicles or blebs that are either exfoliated into the tubular lumen or internalized to potentially be recycled.^{35–39} Other proteins involved in the depolymerization process are tropomyosin and ezrin. During ischemia, ezrin becomes dephosphorylated and the attachment between the microvillar F-actin core and plasma membrane is lost.⁴⁰ Similarly, tropomyosins bind to and stabilize the F-actin microfilament core in the terminal web by preventing access to cofilin. Following ischemia, there is dissociation of tropomyosins from the microfilament core, which enables access of the microfilaments in the terminal web to the binding, severing, and depolymerizing actions of cofilin.⁴¹

Another important consequence of disruption of the actin cytoskeleton is the loss of tight junctions and adherens junctions. These junctional complexes actively participate in numerous functions, such as paracellular transport, cell polarity, and cell morphology. Early ischemic injury results in opening of these tight junctions,

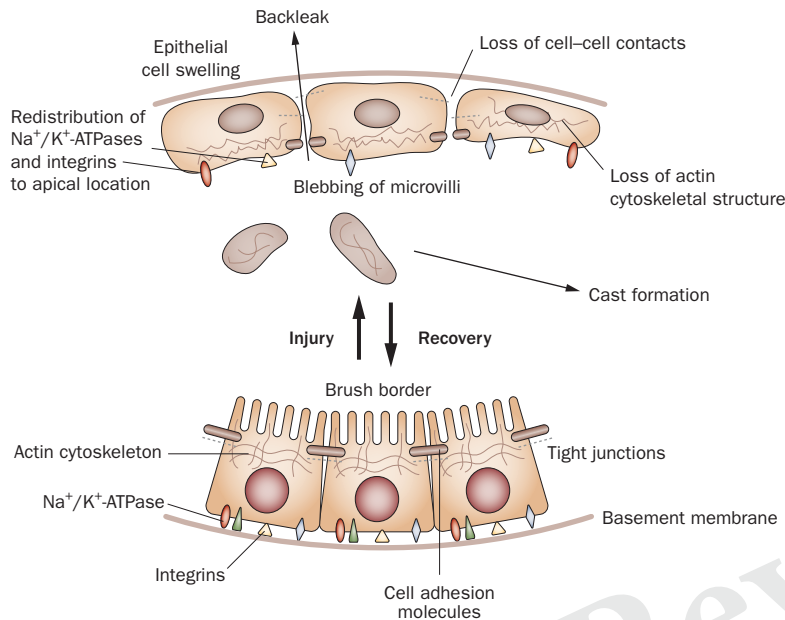


Figure 2 | Effects of sub-lethal injury to tubular cells and their recovery. Damage to epithelial cells occurs early during ischemia and involves alterations to the cytoskeleton and in surface membrane polarity. ATP depletion induces rapid disorganization of the actin cytoskeleton structure, which disrupts tight junctions, and in turn leads to backleak of tubular filtrate. Loss of cell-cell contacts and cell adhesion molecules results in flattened nonpolarized epithelial cells that have denuded basement membranes and expression of mesenchymal markers. Na^+/K^+ -ATPase pumps normally located at the basolateral membrane and tethered by the actin-spectrin cytoskeleton, redistribute to the apical membrane of the proximal tubule and are internalized into the cytosol during ischemic injury. Morphologically, proximal tubular cells can undergo swelling, lose their brush borders, and undergo blebbing of microvilli during injury, leading to cast formation. Severely injured proximal tubular cells undergo mesenchymal differentiation and subsequent re-epithelization. Recovery of proximal tubular cells begins with integrin reattachment, actin cytoskeletal reassembly, repolarization of the surface membranes and subsequent normalization of function. In addition, the sodium pumps revert back to their basolateral location.

leading to increased paracellular permeability and backleak of the glomerular filtrate into the interstitium.⁴² During ischemia, epithelial cells also lose their attachment to the underlying extracellular matrix owing to disruption of integrins. Depletion of ATP results in relocalization of β -integrins from the basal membrane to the apical membrane, with subsequent detachment of viable cells from the tubular basement membrane.⁴³ The exfoliated cells then bind to each other and form cellular casts within the tubular lumen.

Actin cytoskeletal alterations and dysfunction during ischemia result in changes in cell polarity and function (Figure 2). Basolateral Na^+/K^+ -ATPase pumps redistribute to the apical membrane as early as within 10 min following disruption of the spectrin-actin cytoskeleton, which is responsible for attaching the pumps to the membrane.⁴⁴ Redistribution of the pumps results in bidirectional transport of sodium and water across the apical as well as the basolateral epithelial cell membrane, with cellular sodium being transported back into the tubular lumen. This process is one of the major mechanisms of the high fractional excretion of sodium seen in

patients with acute tubular necrosis, and the inefficient use of cellular ATP, as ischemic conditions uncouples ATP use and effective transcellular sodium transport.⁴⁵ High filtrate sodium reaching the distal tubule leads to a reduction in GFR by activation of tubular glomerular feedback, with stimulation of the macula densa mediating afferent arteriolar vasoconstriction.

Apoptosis and necrosis

The fate of the epithelial cell following an ischemic event ultimately depends on the extent of injury. Cells undergoing sub-lethal or less-severe injury have the capability of functional and structural recovery if the insult is interrupted. Cells that suffer a more-severe or lethal injury undergo apoptosis or necrosis, leading to cell death. Apoptosis is an energy-dependent, programmed cell death that results in condensation of nuclear and cytoplasmic material to form apoptotic bodies. These membrane-bound apoptotic bodies are rapidly phagocytosed by macrophages and neighboring epithelial cells. During necrosis, there is cellular and organelle swelling, loss of plasma membrane integrity, and rapid release of cytoplasmic and nuclear material into the lumen or interstitium.⁴⁶ Secondary necrosis occurs when cells undergoing apoptosis do not have adequate cellular levels of ATP to support the staged demise of the cell.

Apoptotic mechanisms are complex with various factors affecting a number of pathways. The caspase family of proteases is an important initiator and effector of apoptosis.^{47,48} Both intrinsic (mitochondrial) and extrinsic (death receptor) apoptotic pathways are activated in AKI. Specifically, activation of procaspase-9 primarily depends on intrinsic mitochondrial pathways regulated by the Bcl-2 family of proteins, whereas procaspase-8 activation results from extrinsic signaling via cell surface death receptors, such as Fas and Fas-associated protein with death domain.^{47,48} Considerable crosstalk also exists between the intrinsic and extrinsic pathways. Caspase-3, caspase-6, and caspase-7 are effector caspases that are abundant and catalytically robust, cleaving many cellular proteins, which result in the classic apoptotic phenotype. Inhibition of caspase activity has been shown to be protective against injury *in vitro* and in renal epithelial tubular AKI *in vivo*.^{49,50}

Several apoptotic pathways, including the intrinsic (Bcl-2 family, cytochrome c, caspase-9), extrinsic (Fas, FADD, caspase-8) and regulatory (p53 and nuclear factor κB), seem to be activated during ischemic renal tubular cell injury. The balance between cell survival and apoptotic cell death also depends on the relative concentrations of the proapoptotic (Bax, Bad, and Bid) and antiapoptotic (Bcl-2 and Bcl-xl) members of the Bcl-2 family of proteins. Overexpression of proapoptotic or relative deficiency of antiapoptotic proteins can lead to formation of mitochondrial pores.⁵¹⁻⁵³ Other proteins that have an important role in the apoptotic pathways include proximal acting nuclear factor κB and p53.^{54,55} The central proapoptotic transcription factor p53 can be activated by hypoxia, via hypoxia-inducible factor, as well as other noxious stimuli such as certain drugs.

Kinases are responsible for mediating cellular responses involved in apoptosis, survival, and repair through their interaction with signals from growth factors, including hepatocyte growth factor, insulin-like growth factor I, epidermal growth factor, and vascular endothelial growth factor (VEGF).^{56,57} These mechanisms, which can be activated independently via nonischemic pathways in other types of injury, inhibit proapoptotic proteins and activate the antiapoptotic transcription of cyclic AMP response element binding factors. Knock down of p53 in proximal tubular cells using short interfering RNA led to a dose-dependent attenuation of apoptotic signaling and kidney injury in clamp ischemia and transplant models, indicating a potential therapeutic approach for ischemic and nephrotoxic kidney injury.^{58,59}

Necrosis of epithelial cells results from increased intracellular calcium and the activation of membrane phospholipases and calpain.^{60,61} Necrotic cells do not, therefore, exhibit the nuclear fragmentation or chromatin condensation seen in apoptosis, and neither do they form apoptotic bodies. Functionally, severe ATP depletion results first in mitochondrial injury with subsequent arrest of oxidative phosphorylation causing further depletion of energy stores, and robust formation of reactive oxygen species. Reactive oxygen species, such as the hydroxyl radical, peroxynitrite, and hyperchlorous acid, are generated by catalytic conversion in epithelial cells during ischemic injury. They damage cells in a variety of ways, including peroxidation of lipids in the plasma membrane and intracellular membranes, and destabilization of cytoskeletal proteins and integrins required to maintain cell–cell adhesion as well as interactions between cells and the extracellular matrix. Reactive oxygen species can also have vasoconstrictive effects by scavenging nitric oxide.⁶²

Autophagy, which is the process involved in degradation of a cell's own components through the lysosomal machinery, is now increasingly recognized as perhaps the most frequent cell-death pathway for injured epithelium. Li *et al.*⁶³ demonstrated the important role of autophagy in renal epithelial cells in obstructive uropathy models, while Koesters *et al.*⁶⁴ showed that transforming growth factor β expression leads to excessive autophagy in injured tubules.

Endothelial dysfunction

Endothelial cells contribute to vascular tone, regulation of blood flow to local tissue beds, modulation of coagulation and inflammation, and vascular permeability. Both ischemia and sepsis have profound effects on the renal endothelium, resulting in microvascular dysregulation and continued ischemia and further injury, especially in the outer stripe of the kidney. Histopathologically these effects are seen as vascular congestion, formation of edema, diminished microvascular blood flow, and margination and adhesion of inflammatory cells to the endothelium, leading to the extension phase of AKI.³⁴ Although a marked decrease in total kidney perfusion results in global ischemia, decreased regional perfusion can extend ischemic injury locally without affecting

global perfusion. The complexity in vascular beds within the kidney makes interpretation of total kidney blood flow challenging following ischemic or septic injury.

Vascular tone

Conger *et al.*^{65,66} were among the first to demonstrate that postischemic rat kidneys displayed vasoconstriction in response to decreased renal perfusion pressure and, therefore, could not autoregulate blood flow, even when total renal blood flow had returned to baseline values up to 1 week after injury. Two groups demonstrated that this response could be blocked by Ca^{2+} antagonists, and loss of endothelial NO synthase (eNOS) function was owing to a loss of vasodilator responses to acetylcholine and bradykinin.^{67,68} Selective inhibition, depletion, or deletion of inducible NOS (iNOS) have clearly shown renoprotective effects during ischemia.^{67,68} Overall, therefore, there is an imbalance of eNOS and iNOS in ischemic AKI. Thus, it is also been proposed that owing to a relative decrease in eNOS, secondary to endothelial dysfunction and damage, there is a loss of antithrombotic properties of the endothelium leading to increased susceptibility to microvascular thrombosis.⁶⁹

Administration of L-arginine, the NO-donor monodamine, or the eNOS cofactor saporpterin can preserve medullary perfusion and attenuate AKI induced by ischemia or reperfusion; conversely the administration of N-nitro-L-arginine methyl ester, an NO blocker, has been reported to aggravate the course of AKI following ischemic or reperfusion injury.⁷⁰ Although clearly important, these pharmacological studies continue to assess the contribution of eNOS impairment in the overall course of reduced renal function following ischemia or reperfusion injury.^{71,72}

Permeability

The endothelial barrier separates the lumen of the blood vessel from the surrounding tissue, and controls the exchange of cells and fluids between the two compartments. The endothelium is defined by transcellular and paracellular pathways, the latter being a major contributor to endothelial dysfunction induced by inflammation. Sutton *et al.*⁷³ studied the role of endothelial cells in AKI by utilizing fluorescent dextrans and two-photon intravital imaging in a series of experiments. The increased microvascular permeability observed in AKI is likely to be caused by a combination of factors, such as disruption of the endothelial monolayer and actin cytoskeleton, breakdown of perivascular matrix, alterations in contacts between endothelial cells, upregulated leukocyte–endothelial interactions, and severe alterations in the integrity of the adherens junctions of the renal microvasculature. *In vivo* two-photon imaging demonstrated a loss of capillary barrier function within 2–4 h of reperfusion, with maximal effects seen at 24 h after injury.⁷³

Breakdown of the barrier function provided by the endothelium might also be owing to activation of matrix metalloproteinase-2 or matrix metalloproteinase-9, which temporally correlates with an increase in microvascular permeability.^{25,74} Minocycline, a broad-spectrum

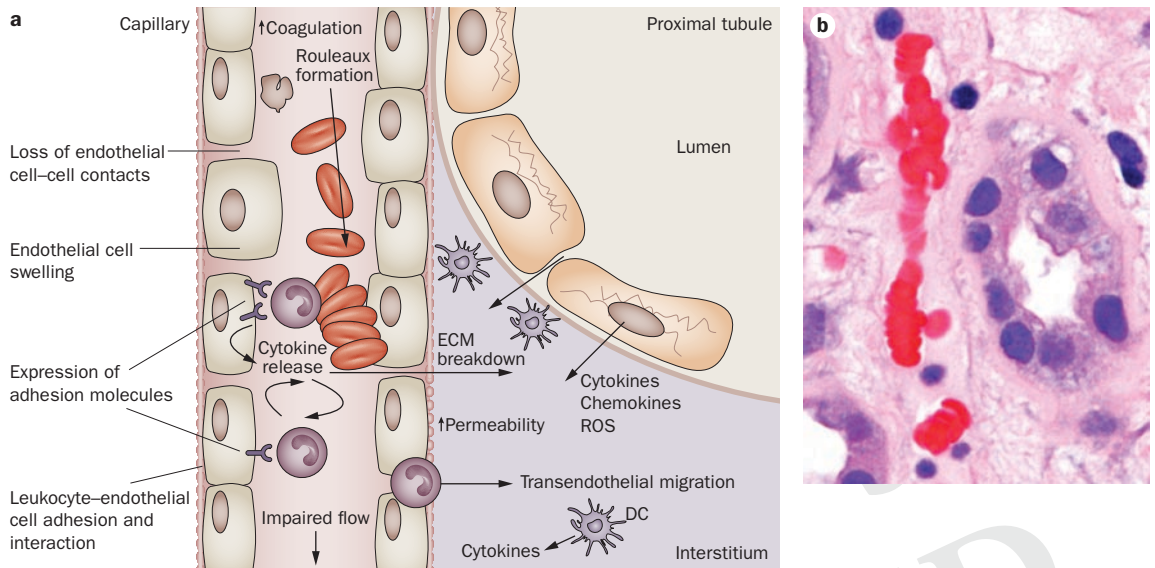


Figure 3 | Events in endothelial cell activation, injury and reduced microvascular flow. **a** | Ischemia causes upregulation and expression of genes encoding various cell surface proteins, such as E-selectin, P-selectin, vascular cell adhesion protein 1 and intercellular adhesion molecule 1, and downregulates the expression of thrombomodulin. Activated leukocytes bind to endothelial cells through these adhesion molecules. Endothelial injury increases the production of endothelin-1 and decreases endothelium-derived nitric oxide synthase, which induces vasoconstriction and platelet aggregation, promoting a hypercoagulable environment. The combination of leukocyte adhesion and activation, platelet aggregation, and endothelial injury serves as the basis for vascular congestion of the cortical and medullary microvasculature. Permeability defects between endothelial cells occur as a result of alterations in tight junctions and adherens junctions. The close proximity and crosstalk between the epithelial proximal tubular cells and microvascular endothelial cells, as well as release of cytokines and chemokines, further increase inflammation. Dendritic cells also have a role in this inflammatory cascade, and amplify inflammatory signals between endothelial cells and epithelial cells. **b** | Hematoxylin and eosin stain of a human kidney biopsy from a patient with AKI following ischemic injury. Abbreviations: AKI, acute kidney injury; DC, dendritic cell; ECM, extracellular matrix; ROS, reactive oxygen species.

inhibitor of matrix metalloproteinases, and the gelatinase inhibitor ABT-518, both ameliorated the increase in microvascular permeability in a rat model of ischemic renal injury.⁷⁴

Coagulation

Endothelial cells have a central role in coagulation through their interaction with protein C and thrombomodulin. Protein C is activated by thrombin-mediated cleavage and the rate of this reaction is augmented 1,000-fold when thrombin binds to the endothelial cell-surface receptor thrombomodulin.⁷⁵ The activation rate of protein C is further increased by approximately 10-fold when endothelial cell protein C receptor (EPCR) binds protein C and presents it to the thrombin–thrombomodulin complex. Activated protein C acquires antithrombotic and profibrinolytic properties, and participates in numerous anti-inflammatory and cytoprotective pathways to restore normal homeostasis.⁷⁶ Activated protein C is also an agonist of protease activated receptor-1.⁷⁷

During an inflammatory response, many of the natural anticoagulants, including protein C, are degraded, or their production is decreased together with downregulation of EPCR and thrombomodulin expression, which decreases the anticoagulant and anti-inflammatory effects of the protein C pathway. Damaged endothelial cells undergo apoptosis, which amplifies the coagulation cascade further by providing a procoagulant surface.⁷⁸ Continued

activation of the inflammation and coagulation pathways leads to increased microvascular coagulation and further endothelial cell dysfunction. Ultimately, microvascular function is compromised, resulting in disseminated intravascular coagulation and microvascular thrombosis, decreased local tissue perfusion, and organ dysfunction or failure. Treatment with thrombomodulin both before and after injury attenuates damage, with minimization of vascular permeability defects, and improved renal blood flow.⁷⁹

Activation of leukocytes and their release of cytokines require signals from chemokines circulating in the bloodstream, or through direct contact with the endothelium (Figure 3). Rolling leukocytes can be activated by chemoattractants, such as complement C5a and platelet activating factor. Once activated, integrins on the leukocytes bind to endothelial ligands to promote firm adhesion, with integrin $\beta 2$ being the most important.⁸⁰ These interactions with the endothelium are mediated through endothelial adhesion molecules that are upregulated during ischemic conditions.

Singbartl *et al.*⁸¹ found that P-selectin on platelets, but not on endothelial cells, was the main determinant in neutrophil-mediated ischemic AKI. Blockade of the common ligand for E-selectin, P-selectin, and L-selectin provided protection from both ischemic injury and mortality, which seemed to depend on the presence of a key fucosyl sugar on the selectin ligand.^{82,83} In a cecal

ligation and puncture (CLP) model of septic azotemia, mice engineered to be deficient for E-selectin, P-selectin, or both, were completely protected against injury.^{84,85}

Long-term effects of endothelial injury

Injury to endothelial cells could contribute to chronic disease. Basile *et al.*⁸⁶ documented a considerable decrease in the density of blood vessels following acute ischemic injury, which led to the phenomenon of ‘vascular dropout’. This phenomenon was verified by Hörbelt *et al.*⁸⁷ who found that vascular density was reduced by almost 45% at 4 weeks after an ischemic insult. This observation indicates that, unlike renal epithelial tubular cells, the renal vascular system lacks comparable regenerative potential. Whether apoptosis and necrosis contribute to vascular cell dropout is not yet clear. Ischemia has been shown to inhibit VEGF, while inducing the VEGF inhibitor ADAMTS1.⁸⁸ The lack of vascular repair was postulated to be due to the reduction in VEGF expression, as administration of VEGF to postischemic rats preserved microvascular density.⁸⁹ Vascular dropout might mediate increases in the expression of hypoxia inducible factor and fibrosis, and alter proper hemodynamics, leading to hypertension. Basile and co-workers have shown that the poor regenerative potential of endothelial cells and transformation into fibroblasts is in large part owing to the lack of VEGF expression,⁹⁰ which could accelerate the progression of CKD following initial recovery from ischemia or reperfusion-induced AKI.^{86,91} Vascular dropout could predispose individuals to recurrent ischemic events and AKI.⁹

Leukocytes and inflammation

Inflammation and recruitment of leukocytes during epithelial injury are now recognized as major mediators of all phases of endothelial and tubular cell injury (Figure 3). Early inflammation is classically characterized by margination of leukocytes to the activated vascular endothelium via interactions between selectins and ligands that enable firm adhesion, followed by transmigration into the interstitium. A number of potent mediators are generated by the injured epithelial proximal tubular cell, including proinflammatory cytokines, such as tumor necrosis factor (TNF), interleukin (IL)-6, IL-1 β , IL-8, C-C motif chemokine 2, transforming growth factor β , and C-C motif chemokine 5.⁹² Toll-like receptor (TLR) 2 is an important mediator of endothelial ischemic injury, while TLR4 has been shown in animal models of both ischemic and septic injury to have a similar role,⁹³ especially in proximal tubular cells.⁹⁴

Neutrophils are the first cells to accumulate at the site of ischemic injury.⁹⁴ Blockade of neutrophil function or neutrophil depletion provides only partial protection against injury, indicating that other leukocytes also mediate injury. These inflammatory mediators include macrophages, B cells, and T cells.⁹⁵ Selective deletion of these cells in knockout mouse models, and through antibody-mediated blockade, shows that these cells mediate tubular injury at various phases of the process, and there are synergistic interactions between different

cell types.⁹⁶ Furthermore, expression of the complement component C5a is markedly upregulated on proximal tubular epithelial cells as well as interstitial macrophages, and is a powerful chemoattractant with procoagulant properties.⁹⁷ Complement cascades are activated during sepsis, and C5a has been found to be elevated in rodent models of sepsis.⁹⁸ Blocking C5a or its receptor could improve survival with sepsis.^{98,99}

Thurman *et al.*^{100,101} showed that C3a was required for CXC chemokine production by epithelial cells, and Crry, a complement inhibitor localized to the basolateral membrane of epithelial cells, was decreased following ischemic injury. Knock down of C3a and caspase-3 using RNA interference also protected renal function in a transplant model.¹⁰² Numerous cytokines, whether released from the endothelium or epithelial cells, work together to augment the inflammatory response following ischemic or septic injury.¹⁰³ Furthermore, cultured mouse tubular cells stimulated with lipopolysaccharide led to an upregulation of TLR2, TLR3, and TLR4 and secretion of C-C chemokines, such as C-C motif chemokine 2 and C-C motif chemokine 5. These data indicate expression of tubular TLR might be involved in mediating interstitial leukocyte infiltration and tubular injury during bacterial sepsis.¹⁰⁴

TLR2 and TLR4 are constitutively expressed on renal epithelium, and their expression is increased following renal ischemia or reperfusion injury. El-Achkar *et al.*⁵¹ have shown that in a CLP rat model of sepsis, TLR4 expression increased markedly in all tubules (proximal and distal), glomeruli, and the renal vasculature. Furthermore, this group demonstrated that sepsis led to a TLR4-dependent increase in the expression of the proinflammatory mediator Cox-2; this protein was mostly restricted to cortical and medullary thick ascending loops of Henle, which characteristically express and secrete uromodulin.⁵² Uromodulin could stabilize the outer medulla during injury by decreasing inflammation, possibly through an effect on TLR4.⁵³ Genetic deletion of either *TLR2* or *TLR4* protects against renal ischemia or reperfusion injury,^{94,105} thus indicating the prominent role of TLRs in AKI.

Macrophages produce proinflammatory cytokines that can stimulate the activity of other leukocytes. Day *et al.*¹⁰⁶ showed that depletion of macrophages in the kidney and spleen using liposomal clodronate before renal ischemia reperfusion injury prevented AKI, whereas adoptive transfer of macrophages reconstituted AKI. This group also demonstrated that agonists of sphingosine-1-phosphate induced lymphopenia, which had a protective effect.¹⁰⁷ However, studies have also shown a lymphocyte independent role of the sphingosine-1 phosphate receptor (S1PR) in maintaining structural integrity after AKI, as S1PRs in the proximal tubule are necessary for stress-induced cell survival, and agonists of this receptor are renoprotective via direct effects on tubular cells.¹⁰⁸ Dendritic cells are also thought to have a role in AKI. Dong *et al.*¹⁰⁹ demonstrated that after AKI, renal dendritic cells produce the proinflammatory cytokines TNF, IL-6, C-C motif chemokine 2, and C-C motif

chemokine 5, and that depletion of dendritic cells before ischemia substantially reduced the levels of TNF produced in the kidney.

T-regulatory (T_{REG}) cells also have a role in ischemic AKI. Gandolfo *et al.*¹¹⁰ showed in a murine model of ischemic AKI that T_{REG} cell trafficking into the kidneys was increased after 3 days and 10 days. Postischemic kidneys had increased numbers of T-cell receptor (TCR) β^+CD4^+ and $TCR\beta^+CD8^+$ T cells, with increased production of proinflammatory cytokines. The researchers also noted that depletion of T_{REG} cells using anti-CD25 antibodies 1 day after ischemic injury increased renal tubular damage, reduced tubular proliferation, increased the production of cytokines from infiltrating T cells at 3 days, and TNF generation by $TCR\beta^+CD4^+$ T cells at 10 days. In a separate study, infusion of $CD4^+CD25^+$ T_{REG} cells 1 day after initial injury reduced interferon γ production by $TCR\beta^+CD4^+$ T cells at 3 days, improved repair and reduced cytokine generation at 10 days.¹¹¹ These studies demonstrate that T_{REG} cells infiltrate reperfused kidneys during the healing process, promoting repair, likely through modulation of proinflammatory cytokine production of other T-cell subsets. Partial depletion of T_{REG} cells with an anti-CD25 monoclonal antibody potentiated kidney damage induced by ischemia reperfusion injury, and reducing the number of T_{REG} cells resulted in more neutrophils, macrophages, and innate cytokine transcription in the kidney after ischemia reperfusion injury.¹¹¹ Furthermore, mice deficient in $FoxP3^+$ T_{REG} cells had a greater accumulation of inflammatory leukocytes after renal ischemia reperfusion injury than mice containing T_{REG} cells; co-transfer of isolated T_{REG} cells and Scurfy lymph node cells attenuated ischemia reperfusion injury-induced renal injury and leukocyte accumulation.¹¹²

Natural killer cells have been reported to infiltrate the postischemic kidney by 4 h of reperfusion. Li *et al.*¹¹³ demonstrated the essential role of natural killer cells and neutrophils in the innate immune response to renal ischemia reperfusion injury by mediating neutrophil infiltration and production of interferon γ . Furthermore, considerable protection from kidney ischemia reperfusion injury was evident in mice deficient in natural killer cells and mice administered an anti-CD1d monoclonal antibody that blocked the interaction between antigen-presenting cells and natural killer cells.¹¹³

The anticoagulant function of antigen-presenting cells is responsible for suppressing lipopolysaccharide-induced stimulation of the proinflammatory mediators angiotensin-converting enzyme 1, IL-6, and IL-18, perhaps accounting for its ability to modulate renal hemodynamics and protecting against septic AKI.¹¹⁴ Taken together, these findings show that suppression of inflammation is a key target towards preventing and limiting AKI.

Investigators have also shown that T cells also have a major role vascular permeability during ischemic injury. Gene microarray analysis showed the production of TNF and interferon γ protein was increased in CD3 and CD4 T cells from the blood and kidney after ischemia. Furthermore, it has also been demonstrated that

in mice deficient in CD3, CD4 and CD8 T cells, there is an attenuated rise in renal vascular permeability after ischemic injury. Therefore, T cells directly contribute to the increased vascular permeability, potentially through T-cell cytokine production.^{115,116}

Another feature noted during inflammation and endothelial cell injury is the phenomenon of erythrocyte trapping with Rouleaux formation, causing obstruction and prolonging the reduction in microvascular blood flow and exacerbating tubular injury.³⁵

Distant organ effects of AKI

AKI is a systemic event that can potentially alter the function of other organs. Kelly *et al.*¹¹⁷ demonstrated the effects of renal ischemia on cardiac tissues as shown by induction of IL-1, TNF, and intercellular adhesion molecule 1 mRNA expression as early as 6 h postischemia. Kramer *et al.*¹¹⁸ showed that renal ischemic injury led to an increase in pulmonary vascular permeability defects, which were mediated through macrophages. Furthermore, this group showed in a rat model of bilateral renal ischemic injury or nephrectomy that expression of lung epithelial sodium channel, Na^+/K^+ -ATPase, and aquaporin-5 was downregulated, which was not the case in unilateral ischemic models, indicating a role for uremic toxins in modulating these effects in the lung.¹¹⁹ Functional changes in the brain have also been shown in the setting of AKI as noted in mice that had increased neuronal pyknosis and microgliosis in the brain.¹²⁰ In addition, extravasation of Evans blue dye into the brain indicated that the blood-brain barrier was disrupted in mice with AKI.¹²⁰

Extrarenal organs might conversely regulate ischemic AKI such as the effect of brain death on renal transplants. Traumatic brain injury elicits a cytokine and inflammatory response that leads to renal inflammation in transplants from brain-dead, but not living donors.¹²¹ The fact that AKI is associated with high mortality and morbidity indicates that multiorgan crosstalk is a major and likely contributor to dysfunction of nonrenal organs.

Molecules that protect against injury

Much of the discussion above has focused on proteins or events that promote injury. However, there are protective mechanisms that provide a defense against numerous stresses. The heat shock protein system is induced during stress conditions; for example, overexpression of heat shock proteins 25, 90, and 72 before injury have been found to have protective effects.^{122–124} These proteins are believed to help restore normal cell function by assisting in the refolding of denatured proteins, as well as aiding in the appropriate folding of newly synthesized proteins. Heat shock proteins also degrade irreparable proteins and toxins to limit their accumulation.

The enzyme heme oxygenase 1 has anti-inflammatory, vasodilatory, cytoprotective, antiapoptotic, and anti-proliferative effects.^{125–127} Mice deficient in this enzyme were shown to have marked exacerbation of glycerol-induced AKI, whereas overexpression of heme oxygenase 1 in cultured renal epithelial cells induced

upregulation of the cell cycle inhibitory protein p21, which conferred resistance to apoptosis.^{125–127} Therefore, the actions of heme oxygenase 1 make it a potentially therapeutic enzyme in the prevention and reduction of AKI. More importantly, the upregulation or over-expression of heme oxygenase 1 might also benefit in the repair and regeneration of tubular cells.

Repair of injury

Renal tubular epithelial cells have the remarkable potential to regenerate after an ischemic or toxic insult. Minimally injured cells are repaired when blood flow is re-established whereas more severely injured cells undergo a dedifferentiated stage in which they appear as flattened cells with an ill-defined brush border.³⁰ Viable cells proliferate and spread across the denuded basement membrane and later regain their differentiated characteristics as tubular epithelial cells. The cytoskeleton is reassembled and cell polarity is restored on ATP repletion. Na⁺/K⁺-ATPase is lost from the apical domain and relocates to the basolateral membranes, and lipid polarity lags behind re-establishment of protein polarity.^{128,129}

Ichimura *et al.*¹³⁰ demonstrated that injured kidney epithelial cells expressing kidney injury molecule-1 (KIM1) could assume the attributes of endogenous phagocytes by internalizing apoptotic bodies. KIM1 was found to be directly responsible for phagocytosis in cultured primary rat tubular epithelial cells, and also in porcine and canine epithelial cell lines. KIM1 specifically recognizes the apoptosis marker phosphatidylserine and oxidized lipoproteins expressed by apoptotic tubular epithelial cells, which leads to remodeling and repair of the tubule.¹³⁰

Another protein, the transmembrane glycoprotein NMB (GPNMB), is upregulated 15-fold following ischemic damage in kidney tissue, more than 10-fold in macrophages, and threefold in surviving epithelial cells after injury.¹³¹ Macrophages and epithelial cells expressing GPNMB contained three times more apoptotic bodies than cells not expressing GPNMB.¹³¹ Mutation of *GPNMB* or ablation of inflammatory macrophages prevents normal repair of the kidney. Kidneys from postischemic *GPNMB*-mutant mice exhibited a fivefold increase in apoptotic cellular debris compared with wildtype mice.¹³¹ GPNMB is, therefore, a phagocytic protein that is necessary for recruitment of the autophagy protein LC3 to the phagosome where these proteins are co-localized, and for lysosomal fusion with the phagosome and degradation of their content. These studies demonstrate that GPNMB is necessary for cross-talk between the macroautophagy degradation pathway and phagocytosis, and an important component of epithelial repair.¹³¹

Macrophages also have an important role in repair and recovery. The Wnt pathway ligand Wnt7b is produced by macrophages to stimulate repair and regeneration. When macrophages are ablated from the injured kidney, the canonical Wnt pathway response in kidney epithelial cells is reduced.¹³² Furthermore, when Wnt7b is somatically deleted in macrophages, repair of injury is greatly diminished. Injection of the Wnt pathway regulator Dkk2 into mice accelerates the repair process and indicates a

therapeutic option for ischemic AKI.¹³² Because Wnt7b is known to stimulate epithelial responses during kidney development, these findings indicate that macrophages are able to rapidly invade an injured tissue and re-establish a developmental program that is beneficial for repair and regeneration.¹³²

Growth factors and signals from injured cells are crucial to promote timely and appropriate regeneration. In animal models, administration of exogenous growth factors, such as epidermal growth factor, insulin-like growth factor I, α -melanocyte stimulating hormone, erythropoietin, hepatocyte growth factor, and bone morphogenic protein-7, have been shown to accelerate renal recovery.^{133–135} All these proteins are likely to increase GFR through direct hemodynamic effects and increase tubular epithelial cell recovery.

Role of stem cells in repair

Interest in the role of progenitor cells, stem cells, and mesenchymal stem cells (MSCs) in tubular epithelial cell injury has been increasing. CD133 progenitor cells with regenerative potential have been identified in the human kidney.¹³⁶ These cells were able to differentiate into both renal epithelium and endothelium *in vitro*. Mice with glycerol-induced AKI injected with these cells showed improved recovery from tubular damage.¹³⁷ MSCs are also present in the kidney and might be derived from the embryonic tissue or bone marrow. Bone marrow cells can migrate to the kidney and participate in normal tubular epithelial cell turnover and repair after AKI.¹³⁸ Lange *et al.*¹³⁹ demonstrated that infusion of MSCs improved recovery of renal function and were predominantly located in glomerular capillaries, whereas tubules showed no iron labeling indicating absent tubular transdifferentiation. In a rat model, knock down of VEGF by short interfering RNA reduced the effectiveness of MSCs in the treatment of ischemic AKI. Animals treated with MSCs depleted of VEGF had reduced microvessel density, indicating that VEGF is important during the early and late phase of renoprotective action of stem cell treatment.¹⁴⁰ With the use of genetic fate-mapping techniques and chimeric mice, Humphreys *et al.*^{141,142} showed that the predominant mechanism of repair following ischemic AKI was regeneration of surviving tubular epithelial cells rather than engraftment of bone marrow stem cells. Therefore, the renotropism exhibited by progenitor cells and stem cells could have a huge impact on therapeutic options in the future once their roles are more fully defined.¹⁴³

Several approaches are available to reduce the effects of endothelial cell injury as well as potentially minimize endothelial cell damage itself. The concept of restoring vascular supply to damaged or ischemic organs for accelerating their regeneration is well-established.¹⁴⁴ One therapeutic strategy based on this concept is the delivery of angiogenic factors to the site of injury. Another strategy could be the use of endothelial progenitor cells. This heterogeneous group of cells originate from hematopoietic stem cells (HSCs) or their angioblastic subpopulation and MSCs. In the bone marrow, these cells are characterized by surface markers, such as CD34,

VEGFR-2, and CD133; moreover, circulating endothelial progenitor cells can express markers such as KIT and cell surface antigen Sc1. Upon further differentiation, these cells lose CD133 and express VE-cadherin and von Willebrand factor.¹⁴⁵ Data indicate that endothelial progenitor cells are mobilized after acute ischemic injury and are recruited to the kidney, where they ameliorate AKI through both paracrine effects as well as repair of the injured microvasculature.¹⁴⁶ Human HSCs administered systemically 24 h after kidney injury were selectively recruited to injured kidneys of immunodeficient mice and localized prominently in and around the vasculature.¹⁴⁶ This recruitment was associated with repair of the kidney microvasculature and tubular epithelial cells, improved functional recovery, and increased survival. HSCs recruited to the kidney expressed markers consistent with circulating endothelial progenitor cells and synthesized high levels of proangiogenic cytokines, which promoted proliferation of both endothelial and epithelial cells. Although purified HSCs acquired endothelial progenitor markers once recruited to the kidney, engraftment of human endothelial cells in the mouse capillary walls was rare, indicating that renal repair by human stem cells is mediated by paracrine mechanisms rather than replacement of the vasculature.¹⁴⁷ Targeting the mechanisms to block these dysfunctional intracellular processes could be of key therapeutic value.

Conclusions

A number of processes resulting in AKI involve ischemia followed by a complex interaction of various cell

types within the kidney. Epithelial cell injury mediates functional alterations through direct failure of the cells to transport ions and molecules, or indirectly by mediating a decrease in GFR. Epithelial cells also influence the function of endothelial cells by releasing chemokines, cytokines, and other soluble mediators. Interactions between endothelial cells and leukocytes contribute to continued hypoxia, inflammation, and further epithelial cell injury and dysfunction. Numerous therapeutic targets have been identified that prevent or limit ongoing injury. Additional approaches to improve repair and minimize fibrosis and vascular dropout will also be critical in limiting the development of CKD and transition from CKD to end-stage renal disease as a consequence of AKI in patients at high risk.

Review criteria

This Review was based on a search of the PubMed and OVID databases using a combination of search terms that included “acute kidney injury”, “acute tubular necrosis”, “apoptosis”, “endothelial cell injury”, “inflammation”, “leukocytes”, “cytokines”, “coagulation”, “stem cells”, “endothelial progenitor cells”, “prerenal azotemia”, “cytoskeletal alterations”, “renal blood flow”, “glomerular filtration rate”, “actin”, “oxidative stress”, “reactive oxygen species”, “heme oxygenase”, “heat shock proteins”, “repair”, “vascular permeability”, “neutrophils”, “T cells”, and “macrophages”. Reference lists of selected articles were searched for further material. Articles were chosen based on their originality and relevance to this Review, and only English-language articles were selected.

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Author contributions

A. A. Sharfuddin and B. A. Molitoris contributed equally to researching data for the article, discussion of the content, writing and reviewing/editing of the manuscript before submission.