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Ischemic acute renal failure: An inflammatory disease?

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Ischemic acute renal failure: An inflammatory disease? Inflammation plays a major role in the pathophysiology of acute renal failure resulting from ischemia. In this review, we discuss the contribution of endothelial and epithelial cells and leukocytes to this inflammatory response. The roles of cytokines/chemokines in the injury and recovery phase are reviewed. The ability of the mouse kidney to be protected by prior exposure to ischemia or urinary tract obstruction is discussed as a potential model to emulate as we search for pharmacologic agents that will serve to protect the kidney against injury. Understanding the inflammatory response prevalent in ischemic kidney injury will facilitate identification of molecular targets for therapeutic intervention.

Ischemic acute renal failure (ARF) is a syndrome that develops following a sudden transient drop in total or regional blood flow to the kidney [1–3]. Despite advances in preventative strategies and support measures, this disease continues to be associated with significant morbidity and mortality [4]. In this review, the pathogenesis of ARF is discussed with an emphasis on the growing body of evidence that ARF is an inflammatory disease. The contributions of endothelial injury, leukocyte infiltration, and generation of inflammatory mediators by tubule cells are emphasized. The response of the tubular epithelium to survive is also reviewed and is supplemented with recent findings on the protective effects of ischemic preconditioning.

THE INFLAMMATORY RESPONSE

Endothelial injury and leukocyte infiltration

In renal ischemia/reperfusion (I/R) injury, the inflammatory response results in endothelial activation and injury, enhanced endothelial cell-leukocyte adhesion, leukocyte entrapment, and a compromise in microvascular blood flow (Fig. 1) [5, 6]. These leukocyte-endothelial interactions impact the outer medulla to a greater extent than the cortex, indicated by the marked vascular congestion typically seen in this region of the kidney.

The concept that endothelial cells are a target of postischemic injury was suggested as early as 1972 by Flores et al [7] when they described endothelial swelling and narrowing of the blood vessel lumen as important features of postischemic injury. Recently, evidence for endothelial dysfunction in the cortex has been described in studies that demonstrated retrograde blood flow through peritubular capillaries upon reperfusion following ischemia [8]. With reperfusion, a partial transient compromise of the patency of the peritubular capillaries also was noted. When human umbilical vein endothelial cells or human embryonic kidneys cells expressing endothelial nitric oxide synthase (NOS) were administered either intravenously or into the renal artery following ischemia, cells implanted into the kidney, leading to partial functional protection against injury [8]. In addition, following prolonged ischemia (60 minutes) in the rat, peritubular capillaries suffer permanent damage [9]. The number of microvessels in the inner stripe of the outer medulla declines and is associated with tubulointerstitial fibrosis and altered concentrating ability.

In addition to endothelial cell damage, I/R up-regulates adhesion molecules to promote endothelial-leukocyte interactions. These adhesion molecules include integrins, selectins, and members of the immunoglobulin superfamily, including intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule (VCAM), and P-selectin. The role of ICAM-1 in renal injury was demonstrated by experiments in which the administration of ICAM-1 antibody was found to be protective [10] and the kidneys of ICAM-1 knockout mice were protected against ischemic injury [11]. Activation of endothelial cells with up-regulation of adhesion molecules, as well as injury to some cells leading to cell swelling and decreased vessel patency, will potentiate interactions with leukocytes and platelets, possibly leading to mechanical obstruction of small blood vessels [2].

There are several mechanisms by which leukocytes potentiate renal injury. Leukocytes are activated by

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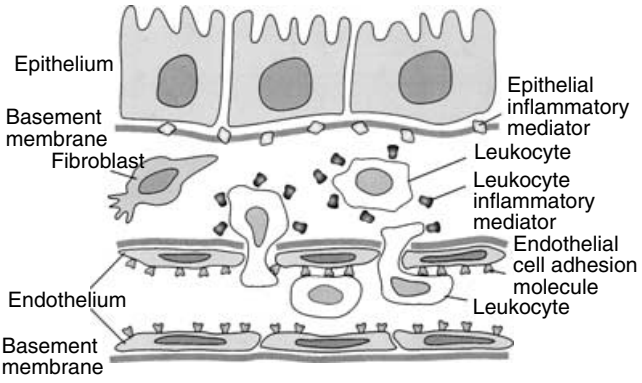

Fig. 1. Schematic illustration of the inflammatory mediators produced by tubular epithelial cells and activated leukocytes in renal ischemia/reperfusion (I/R) injury. Tubular epithelia produce TNF-α, IL-1, IL-6, IL-8, TGF-β, MCP-1, ENA-78, RANTES, and fractalkines, whereas leukocytes produce TNF-α, IL-1, IL-8, MCP-1, ROS, and eicosanoids. The release of these chemokines and cytokines serve as effectors for a positive feedback pathway enhancing inflammation and cell injury.

inflammatory mediators, including cytokines, reactive oxygen species (ROS), and eicosanoids, up-regulating adhesion molecules to engage counter-receptors on the activated endothelium. In addition, leukocytes are recruited by chemokines, which are up-regulated by ROS [12], and by the proinflammatory cytokines interleukin-1 (IL-1) and tumor necrosis factor-alpha (TNF-α) [13, 14]. Exposure of leukocytes to circulating cytokines also reduces their deformability and enhances their tendency to be sequestered [15]. Sequestered leukocytes can then potentiate injury by further generating more ROS and eicosanoids, enhancing inflammation and vascular tone. Activation of coagulation pathways, including complement, may also potentiate injury [16–18].

Leukocyte subgroups are likely to contribute in different ways to I/R injury [5, 19, 20]. Myeloperoxidase activity is elevated soon after the ischemic insult and may originate from macrophages and/or neutrophils [21]. If neutrophil accumulation is prevented, however, tissue injury is ameliorated [10, 22]. It is possible that neutrophil depletion models, however, may not adequately differentiate involvement of neutrophils from T lymphocytes and macrophages [21]. Later phases of ARF are characterized by infiltration of macrophages and T lymphocytes which predominate over neutrophils [20, 23]. There are also species differences with neutrophil accumulation more readily apparent postischemia in the mouse than in the rat [3]. Knockout mice lacking CD4+/CD8+, cell adhesion receptors on T lymphocytes, are protected from I/R injury [24], suggesting a causal role for T lymphocytes in mediating injury. In addition, blockade of T-cell CD28-B7 costimulation protects against ischemic injury in rats and significantly inhibits T-cell and macrophage infiltration and activation in situ [23]. It has been reported that B7-1 plays a key role in leukocyte-endothelial interactions along the ascending vasa recta that lead to functional consequences after I/R. Anti-B7-1 antibodies block adherence of CD28-expressing T cells to the B7-1 expressing endothelial cells of these postcapillary venules and this is associated with much less postischemic vascular congestion [25].

The role of the T cell, however, has been recently questioned. Mice deficient in recombination-activating gene (RAG)-1 lack T and B cells and do not produce immunoglobulins or T-cell receptor proteins. In the absence of these cells and their receptors, RAG-1–deficient mice are not protected from ARF induced by ischemia. Tubular necrosis and neutrophil infiltration are present to a comparable degree, as in wild-type mice [26]. These mice have, however, an increased population of natural killer T cells which express CD28. Hence, it is possible that the CD28/B7-1 interaction at the level of the vasa recta can still occur and may explain the lack of a functional difference between the RAG-1–deficient and wild-type mice [27, 28].

Generation of inflammatory mediators by tubule cells

In addition to the contribution of leukocytes and endothelial cells to the inflammatory response following ischemic injury, the renal tubular epithelium also generates mediators that potentiate inflammation (Fig. 1) [29]. These include proinflammatory cytokines such as TNF-α, IL-6, IL-1β, and transforming growth factor-β (TGF-β). Chemotactic cytokines, also known as chemokines, are also produced. These include monocyte chemoattractant protein-1 (MCP-1), IL-8, regulated upon activation, normal T-cell expressed and secreted (RANTES), and epithelial neutrophil-activating protein 78 (ENA-78) [30–32]. MCP-1 and IL-8 are produced by mouse cortical proximal tubular cells following adenosine triphosphate (ATP) depletion in vitro [33]. Bone morphogenetic protein-7 (BMP-7), a member of the TGF-β superfamily, has been reported to protect against ischemic injury by acting on proximal tubular epithelia to reduce basal and TNF-α stimulated expression of MCP-1 and IL-8 [34]; BMP-7 also reduces levels of the proinflammatory cytokines IL-6 and IL-1β [34]. The reduction in macrophage infiltration that follows may explain its protective effect. Fractalkines are also produced by renal epithelial cells [35]. Fractalkines are members of the chemokine superfamily that are directly tethered to the plasma membrane via a long mucin stalk. They have a combined function of a chemokine and an adhesion molecule. On kidney epithelial cells, fractalkines induce migration and adhesion of leukocytes, facilitating monocyte-induced cell injury [35]. α-Melanocyte-stimulating hormone (MSH), an endogenous anti-inflammatory cytokine, protects against injury associated with ischemic ARF [36]. MSH acts directly on renal tubules where it binds to melanocortin receptor to

inhibit activation of genes that cause inflammatory and cytotoxic renal injury [36].

Proximal tubular epithelia may also modulate T lymphocyte activity [33, 37]. CD40 is expressed by epithelial cells of the proximal tubule and serves as a receptor for CD154, its ligand typically present on T cells. When human proximal tubular epithelia are exposed to CD154, ligation of CD40, in turn, engages TNF receptor-activating factor 6 (TRAF6). CD40 and TRAF6 then translocate from separate membrane microdomains to associate with one another in the cytoplasmic compartment, where TRAF6 in turn activates phosphorylation of the jun-kinase (JNK) and p38 mitogen-activated protein kinase (MAPK) pathways, which, in turn, stimulate IL-8 and MCP-1 production by these cells [33]. CD40 also induces RANTES production by human renal tubular epithelia, an effect which is amplified by production of IL-4 and IL-13 by Th2 cells, a subpopulation of T cells [37].

PROXIMAL TUBULE CELL INJURY

In most animal models of ARF, it is the S3 segment of the proximal tubule in the outer stripe of the outer medulla and not the medullary thick ascending limb (MTAL) that is most susceptible to ischemic injury [38–40]. This is likely due to ischemia-related reductions in microvascular blood flow and oxygen delivery to the outer medulla at a time when cortical blood flow is returned to near normal levels. In addition, unlike the MTAL, S3 segments have a limited capacity to undergo anaerobic metabolism (i.e., glycolysis). Although compared to the proximal tubule, there are fewer histologic changes in the MTAL, ischemia modifies the expression of a large number of genes, and a number of cytokines are produced and secreted by this segment [41, 42]. These cytokines, in addition to those produced by the S3 segment, likely impact the microvasculature, serving as effectors for a positive feedback pathway enhancing inflammation and vascular obstruction.

Sublethal injury

Sublethal injury of the proximal tubular epithelium as a result of ATP depletion leads to early changes in the actin cytoskeleton, loss of junctional complexes, including tight junctions and adherens junctions, as well as a change in localization of cell adhesion molecules and polarized membrane proteins (Fig. 2) [4, 43–45]. Following I/R, intracellular calcium increases and calpain, a protease, and NOS are activated. Severing of actin filaments and digestion of actin binding proteins follow, which, in turn, disrupt the cortical actin cytoskeleton [43, 46].

In proximal tubular epithelia, tight junctions at apicolateral borders are disrupted, either in response to loss of the cortical actin cytoskeleton or in response to inflammatory mediators. The tight junction proteins,

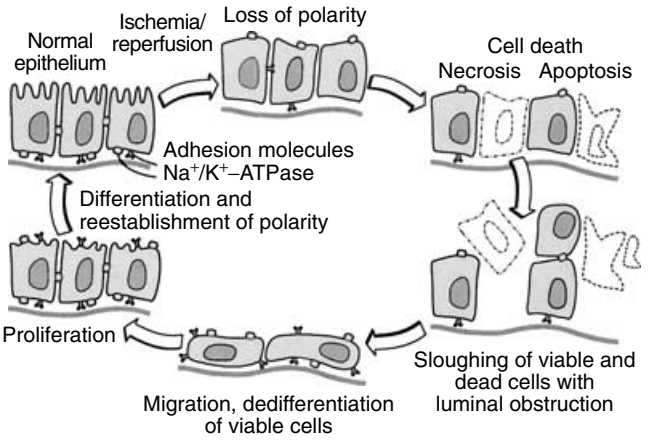

Fig. 2. Schematic illustration of the cycle of tubular epithelial cell injury and repair following renal ischemia/reperfusion. Tubular epithelia are typically cuboidal in shape and apically-basally polarized; the Na^+/K^+-ATPase localizes to basolateral plasma membranes, whereas cell adhesion molecules, such as integrins localize basally. In response to ischemia reperfusion, the Na^+/K^+-ATPase appears apically, and integrins are detected on lateral and basal plasma membranes [62]. Some of the injured epithelial cells undergo necrosis and/or apoptosis detaching from the underlying basement membrane into the tubular space where they contribute to tubular occlusion. Viable cells that remain attached, dedifferentiate, spread, and migrate to repopulate the denuded basement membrane. With cell proliferation, cell-cell and cell-matrix contacts are restored, and the epithelium redifferentiates and repolarizes, forming a functional, normal epithelium.

ZO-1, ZO-2, and cingulin, are dephosphorylated, forming large insoluble complexes that associate with cytoskeletal elements, including fodrin [47]. Reassembly of tight junctions, in turn, depends on tyrosine kinase activity. Loss of tight junction integrity alters both paracellular permeability and cell polarity with the increase in permeability promoting back-leak of glomerular filtrate. Na^+/K^+-ATPase, which localizes normally to the basolateral plasma membrane, appears after ischemia on the apical plasma membrane [46], thereby reducing the efficiency of transcellular sodium transport and increasing intraluminal sodium delivery to the distal tubule. The enhanced distal sodium delivery may activate tubuloglomerular feedback at the macula densa, resulting in vasoconstriction of preglomerular arterioles and a decline in glomerular filtration rate (GFR) [48]. The mislocalized Na^+/K^+-ATPase may also explain the marked increase in fractional sodium excretion [49] in patients with ischemic ARF as well as the increase in fractional sodium and lithium excretion, normally cotransported by the proximal and distal tubules, in transplant recipients during postischemic injury to the renal allograft [50].

ATP depletion also disrupts the adherens junction which contains transmembrane cadherin proteins that link to the actin cytoskeleton and signaling proteins via catenins [44]. In mouse proximal tubular cells following ATP depletion both β-catenin and plakoglobin are tyrosine phosphorylated [51], possibly through activation of

the tyrosine kinase c-Src [52]; E-cadherin, α-catenin and β-catenin are withdrawn from the plasma membrane as intact cadherin-catenin complexes [53]. With longer durations of ATP depletion in vitro and ischemia of kidney in vivo, E-cadherin is degraded and complexes are partially disrupted [54].

Dissociation of the zonula adherens in response to ATP depletion also induces nuclear translocation of β-catenin and T-cell factor (TCF)/lymphoid enhancer factor-1 (LEF-1), a transcriptional factor with which β-catenin associates [53]. The translocation of the β-catenin-TCF/LEF-1 complex into the nucleus suggests that ATP depletion may activate the *wnt/wingless* signal transduction pathway, known to modulate the expression of genes that regulate cell proliferation, apoptosis, and differentiation [55]. The expression of these genes in mammalian cells encode c-myc, cyclin, and E-cadherin, which likely function in those cell responses necessary for regeneration and repair of the kidney epithelium. It remains unclear, however, whether inflammatory mediators activate *wnt/wingless* signaling through β-catenin. The inflammatory cytokines, TNF-α, IL-1, and interferon-γ (IFN-γ), produce a number of injurious changes in proximal tubular epithelial cells [56]. The actin cytoskeleton is disrupted and E-cadherin is dispersed, suggesting affects on β-catenin signaling via the *wnt/wingless* pathway.

Inflammatory mediators have also been implicated in mediating exfoliation of epithelial cells [56, 57] and thus may add to their role in the pathogenesis of tubular injury. The pro-inflammatory cytokines, TNF-α, IL-1, and IFN-γ, disrupt cell-matrix adhesion dependent on β_1 integrin, inducing cell shedding into the lumen. Integrins anchor epithelial cells to extracellular matrix proteins in the basement membrane which in the proximal tubule include laminin-1, -10, and collagen IV [58]. In the kidney, β_1 integrins are the major receptor family expressed along the nephron. In proximal tubular epithelia, the α_6 and α_2 integrin subunits partner with β_1, forming receptors for laminin and collagen expressed on basal plasma membranes. Following ATP depletion in vitro, the α_3 [59] and β_1 integrin subunits [60] redistribute to apical plasma membranes, suggesting that the loss of cell-matrix adhesion facilitates cell exfoliation into the luminal space [59]. In this location, continued cell-cell interactions via integrin may contribute to occlusion. Furthermore, the appearance of apical integrin on the surviving epithelium may potentiate occlusion by facilitating cell-cell interactions with the obstructive cast [59, 61]. However, in the postischemic kidney in vivo, the β_1 integrin subunit is not detected by immunohistochemistry on apical surfaces of the surviving epithelium or on exfoliated cells in the obstructive cast [62, 63], possibly due to endocytosis and subsequent degradation. What remains unclear is whether other integrin families expressed in the kidney in vivo or induced by ischemic injury, are present in these locations. The presence of other integrin families, particularly those that recognize the arginine, glycine, aspartic (RGD) sequence, may explain the ability of soluble RGD peptides [64] to interfere with cell-cell and cell-fibronectin interactions [62] in the obstructive cast, ameliorating ischemic injury and decreasing intratubular obstruction.

The proximal tubular epithelium can also undergo apoptosis or necrosis depending on the severity of injury. This topic is covered in detail by Kaushal et al [65] and Dagher [66].

Protection against injury by ischemic preconditioning

An area of increasing interest is the possibility of rendering an organ resistant to subsequent injury by a prior insult or preconditioning maneuver. Ischemic preconditioning of the kidney confers protection against a subsequent ischemic attack [67]. Several candidates that could potentially serve as mediators of preconditioning have been identified (reviewed in [3]). Of these, the NOS pathway is particularly important to mention. Recently, this laboratory demonstrated that inducible NOS (iNOS) is responsible for a component of the long-term protection afforded the kidney by ischemic preconditioning [68]. Thirty minutes of prior ischemia results in a prolonged increase in the expression of iNOS and endothelial NOS (eNOS) as well as heat shock protein 25 (HSP25). In addition, there is increased interstitial expression of alpha-smooth muscle actin (α-SMA), an indicator of long-term renal interstitial changes. Gene deletion of iNOS, but not eNOS, increased kidney susceptibility to ischemia, as did treatment with pharmacologic inhibitors of nitric oxide synthesis, including N-nitro-L-arginine (L-NNA) and L-N6-(1-iminoethyl) lysine (L-NIL), the latter a specific inhibitor of iNOS. When the initial period of ischemia was reduced (15 minutes), there was less protection of the kidney from subsequent ischemia on day 8. Under these conditions, there was no sustained increase in iNOS or eNOS expression, and protection was not abolished by L-NIL treatment, suggesting that the residual protection was not related to iNOS. In addition, renal function was not impaired and expression of interstitial α-SMA did not change. The data indicate that iNOS plays an important role in kidney protection afforded by ischemic preconditioning, resulting in longer periods of ischemia, and that persistent long-term changes in the renal interstitium may be critical in affording this protection by sustaining iNOS synthesis. In addition, this study is the first to demonstrate in any organ system that partial protection persists up to 12 weeks after an initial ischemic event.

MAPKs are also likely to be involved in affording protection following ischemic preconditioning [67, 69]. MAPKs are ubiquitously expressed serine/threonine kinases which in mammalian cells play central roles in

determining if the response to multiple signaling inputs is proliferation, differentiation, or apoptosis. In mammalian cells, three MAPK cascades have been identified. The first, the extracellular-regulated protein kinase (ERK) pathway, is activated by growth factors and many other agonists, including vasoactive peptides; ERKs are involved in cell proliferation and differentiation. JNK, also known as stress-activated protein kinase (SAPK), and p38 are components of two other MAPK cascades. JNK and p38 are activated by inflammatory cytokines (TNF and IL-1) and by cell stress [70]. When the kidney is preconditioned to injury by ischemia, ischemia-induced activation of JNK and p38 is markedly reduced, as is activation of their upstream MAPK kinases (MKK7, MKK4, and MKK3/6). By contrast, activation of ERK1/2 and its upstream MAPK kinase activator, MKK3/4, is unaltered [67]. Thus, the relative ratio of ERK1/2 activation to JNK or p38 activation is enhanced in the preconditioned postischemic kidney. This suggests that activation patterns of MAPKs may explain the endogenous ability of the kidney to protect itself against injury.

CONCLUSION

Inflammation is a significant component of renal I/R injury, playing a considerable role in its pathophysiology. Although significant progress has been made in defining the major components of this process, the complex cross-talk between endothelial cells, inflammatory cells, and the injured epithelium with each generating and often responding to cytokines and chemokines is not well understood. In addition, we have not yet taken full advantage of the large body of data on inflammation in other organ systems. Furthermore, preconditioning the kidney to afford protection to subsequent bouts of ischemia may serve as a useful model challenging us to therapeutically mimic endogenous mechanisms of protection. A better understanding of the pathophysiology underlying ARF with better ways to limit the inflammatory component will allow promising therapies to be identified.

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