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Triggers of Inflammation after Renal Ischemia/Reperfusion

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Abstract

Renal ischemia/reperfusion (I/R) is a common cause of acute renal failure (ARF). Ischemic ARF is associated with tubulointerstitial inflammation, and studies using animal models have demonstrated that the inflammatory response to I/R exacerbates the resultant renal injury. Ischemic ARF involves complement activation, the generation of cytokines and chemokines within the kidney, and infiltration of the kidney by leukocytes. Recent work has revealed some of the events and signals that trigger the inflammatory response to aseptic, hypoxic injury of the kidney. In many ways, the inflammatory reaction to this injury resembles that seen during ascending urinary infection, and it may represent a general response of the tubular epithelial cells (TECs) to stress or injury. A greater understanding of the signals that trigger the inflammatory response may permit the development of effective therapies to ameliorate ischemic ARF.

Introduction

Renal I/R is one of the most common causes of ARF and places a significant burden on the health care system. It is associated with increased morbidity, prolonged hospitalizations, and increased mortality [1-4]. The renal tubules are susceptible to hypoxic injury due to a number of factors [5], but they are also capable of rapid regeneration and functional recovery. A large body of work in animal models as well as some pathologic analysis of human biopsies demonstrate that ischemic ARF is marked by a robust inflammatory response [6,7]. Furthermore, rodent studies have demonstrated that the inflammatory response to hypoxia contributes to the resultant tissue injury. Therapies that target specific inflammatory cell types or effector proteins such as complement proteins [8-11], chemokines [12,13], or adhesion molecules [14-17] can ameliorate ischemic ARF in animal models. As with most inflammatory diseases, then, the systemic response to injury may be as important as the initial insult.

The nephron is lined by polarized tubular epithelial cells (TECs) that are highly specialized to control the excretion of water, electrolytes, other organic solutes, and maintain the body's acid-base balance. In addition to their role in controlling the content of urine, the TECs play an active role in the defense against ascending urinary infection. In response to pathogens they generate complement components, cytokines, chemokines such as IL-8, and β -defensins [18, 19], some of which will be discussed below in relation to I/R. Severe I/R can cause TEC necrosis and apoptosis. ARF caused by ischemic or toxic insults is often referred to as acute tubular necrosis (ATN), a term derived from the histologic appearance of the kidney [1]. Frank necrosis is usually a minor component of the histology in this disease, however. The profound renal dysfunction which occurs with ATN is more likely caused by altered cellular organization

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in sublethally injured TECs, changes in blood flow and vascular obstruction, obstruction of the renal tubules by detached tubules and debris, and the inflammatory response to hypoxic injury [5].

It is important to understand the signals that initiate inflammation in response to I/R. Targeting the factors that initiate inflammation may be more effective than targeting downstream effectors which often serve redundant functions. Furthermore, cells or factors that cause tissue injury in one stage of the inflammatory response may be important for the termination of inflammation and for tissue repair at later stages. The complement system, for example, is an important initiator of the immune response, but it is also involved in the clearance of injured tissue [20] and sometimes in tissue regeneration [21]. Cellular components of the inflammatory response, such as neutrophils [22] and macrophages [23], may also contribute to tissue destruction during one stage of injury, but later provide necessary signals for the resolution of injury. Because of this, inhibition of a given pathway early in the course of injury may be beneficial, but harmful if performed later in the resolution phase of injury.

Ischemic acute renal failure is an inflammatory disease

In addition to the direct cytotoxic effects of hypoxia, renal I/R induces an inflammatory reaction within the renal parenchyma [7]. I/R causes renal synthesis of pro-inflammatory cytokines such as IL-1, IL-6, and TNF- α [14,24,25]. Chemokines are also rapidly generated in the kidney after I/R [12], and Keratinocyte-derived chemokine (KC, a mouse analog of human IL-8) is an early biomarker of ischemic acute renal failure [26]. Ischemia also causes infiltration of the kidney by leukocytes. Although the role and kinetics of neutrophil infiltration in ischemic ARF is controversial [27,28], neutrophil infiltration of the kidney seems to be an early finding in mice [29] and biopsies from patients with early ATN also demonstrate neutrophils in the vasa recta [6,30]. Macrophages and T cells infiltrate later in the course of the disease, and persist well into the recovery phase [28].

Studies using mice with targeted deletions of genes involved in the immune response or with animals treated with specific immune system antagonists have demonstrated that the inflammatory response to hypoxia contributes to the development of tissue injury. These studies are too numerous to review in detail, but they have implicated the C-X-C chemokines macrophage inflammatory protein-2 (MIP-2) and KC [12,13], adhesion molecules [15-17], the complement system [8,9,31], neutrophils [15,16], B cells [32], and T cells [33,34] in the development of ischemic ARF. Some inflammatory events may be markers of injury, but the net effect of many of the systems tested is aggravation of the injury.

The inflammatory response is certainly more complex in human patients who often develop ATN in the context of severe systemic illness or due to multifactorial causes [1]. Patients with diseases such as sepsis have a high risk of developing ARF, and their systemic illness likely has a direct influence on renal inflammation [35]. Furthermore, renal failure may contribute to dysregulation of the immune system [36]. Therefore, renal failure in critically ill patients likely involves a complex host-kidney interaction in which the inflammatory state of the host contributes to the development of renal failure, and injury of the kidney further modulates the inflammatory state of the host.

Potential triggers of inflammation

How does the innate immune system become activated after renal I/R? Simplistically, one can categorize the initiating signals as falling into four categories (Figure 1). Cellular factors may be released or exposed after hypoxic cellular injury. Factors may be actively synthesized by renal cells in response to hypoxia or reperfusion. The immune system may target the tissue due

to structural alterations in proteins or cell surfaces. Finally, the production of necessary anti-inflammatory factors may be impaired after hypoxia. There are similarities in the inflammatory response to I/R of many organs, but inflammatory signals may be generated in a tissue specific fashion. I/R of organs such as the intestine, for example, may cause concurrent bacteremia due to transmigration across an injured epithelium [37], whereas this is not likely to contribute to ischemic injury of the heart or kidney.

Release of factors by necrotic or injured cells

ATP depletion causes TECs to undergo apoptosis or necrosis *in vitro* [38], and both apoptotic and necrotic TECs may be seen in ischemic ARF [39]. Necrosis of cells causes the release of a number of phlogistic factors [23]. High mobility group 1 protein, for example, is a nuclear factor that is released by necrotic cells and promotes inflammation [40]. When released, it stimulates TNF- α production and leukocyte infiltration [40,41]. Mitochondrial proteins may also stimulate neutrophil chemotaxis [42]. Heat shock proteins are generated in TECs in response to hypoxia [43], and are also released by necrotic cells [44]. Heat shock proteins are felt to be cytoprotective, and to mediate cytoskeletal repair of post-ischemic TECs as well as resistance to recurrent hypoxia [45,46]. They may also be ligands for toll like receptors (TLRs) when released from the cells [44], and they may bind and stabilize peptides a number of peptides in the extracellular environment [47]. This may serve to modulate the immune response to factors released by cells [44].

Ischemia induces the kidney to produce a number of inflammatory factors. It is difficult to distinguish inflammatory signals generated by renal cells in response to hypoxia (proximal initiators of inflammation) and downstream factors produced after the inflammatory process has begun. Generation of IL-1, for example, may subsequently stimulate TECs to produce TNF- α and IL-6 [48]. Hypoxia does, however, directly induce a transcriptional response. Renal ischemia activates the transcription factors heat shock factor-1 and hypoxia-inducible factor-1 [49,50]. These same factors were also induced in renal epithelial cells *in vitro* in response to ATP depletion and hypoxia respectively [49]. The specific role of these factor in ischemic ARF remains to be determined, but they demonstrate the active response of TECs to hypoxic stress.

Hypoxia rapidly activates the NF- κ B system in the rat kidney and elicits production of inflammatory factors [51,52]. In one of the studies cited, NF- κ B activation occurred during ischemia and peaked after 15 minutes of reperfusion [52], suggesting that this could be a very proximal signal for the TECs to release inflammatory mediators.

Ischemia also induces iNOS in the kidney [53], and there is experimental evidence that iNOS mediates tubular injury in ischemic ARF [5]. *In vitro* studies using isolated tubules demonstrate that nitric oxide increases in the tubules in response to hypoxia [54], demonstrating that this is a direct response of the TECs to hypoxia. It has been shown that integrin mediated adhesion of T cells to TECs *in vitro* is increased by hypoxia [33]. Thus, TECs respond directly to hypoxia by actively generating cytokines, adhesion molecules, and nitric oxide, factors which have all been demonstrated to mediate renal injury after I/R.

Recognition of injury by the immune system

The immune system has several mechanisms of recognizing harmful pathogens. Pattern recognition receptors such as the TLRs and the specific receptors of the adaptive immune system can initiate responses to rapidly eliminate invasive pathogens. These same immune receptors can recognize injured host cells or endogenous ligands [44,55]. After I/R they can respond to proteins whose structure is altered by ischemic injury or factors that are ordinarily sequestered but are exposed in response to ischemia. The immune system can also respond to surfaces that lack surface proteins such as the MHC or complement inhibitors, and altered/

decreased expression of these inhibitory proteins could permit an immune response. Other mechanisms of immune cell recognition, such as that mediated by T cell receptors, may be important in the pathogenesis of ischemic ARF [33,56]. Because T cell involvement seems to occur late in the evolution of ischemic ARF [28], however, they seem unlikely to be early initiators of the inflammatory response.

Toll-like receptors

The TLRs are pattern recognition receptors that are capable of triggering inflammation in response to conserved molecular patterns usually associated with pathogens [57]. In response to ligand binding these receptors induce a number of innate immune responses, including NF- κ B activation, cell activation, and the production of pro-inflammatory cytokines [57]. These receptors may also respond to endogenous ligands. HSP-60 and HSP-70, for example, are ligands for both TLR-2 and TLR-4 [44]. TECs express both TLR-2 and TLR-4, and expression is increased in response to I/R [58,59]. Mice with a targeted deletion of TLR-2 or that have been treated with TLR-2 antisense oligonucleotides are protected from renal I/R when compared with wild-type controls [60]. In this study, TLR-2 appeared to mediate production of several chemokines and cytokines including KC, MCP-1, IL-1 β , and IL-6. Although this study did not identify the TLR-2 ligand, the HSPs are released by necrotic cells and ischemia is associated with tubular HSP70 expression [59].

The complement system

The complement system is activated in the kidney after I/R, resulting in C3 deposition along the tubular basement membrane and increased circulating C3a [11]. Studies have demonstrated that renal injury is attenuated in complement deficient mice [9], and activation may occur via the mannose binding lectin (MBL) pathway [61] as well as through the alternative pathway [31].

The MBL pathway of complement activation is triggered by pattern recognition receptors (MBL and ficolin [62]) which bind to carbohydrates expressed on the surface of many pathogens. Like the TLRs, the MBL system contributes to the powerful ability of the innate immune system to identify pathogens, but it also has a number of endogenous ligands including apoptotic and necrotic cells, phospholipids, and nucleic acids [62]. These are moieties that are certainly generated and exposed in the post-ischemic kidney. MBL also binds to cytokeratin exposed on the surface of hypoxic endothelial cells [55]. Two studies have demonstrated binding of MBL within the post-ischemic kidney [61,63], and C3 deposition after I/R was decreased in MBL deficient mice compared with wild-type controls [61]. These studies indicate that renal I/R generates or reveals ligands for the MBL system, and binding of MBL to these ligands activates the complement system within the kidney.

The alternative pathway of complement is functionally similar to a pattern recognition receptor. Rather than responding to a specific pathogen associated ligand, it responds to activation of the other pathways (through their production of C3b), or to the absence of inhibitors on biologic surfaces [64]. Mice deficient in factor B, a necessary component of the alternative pathway, have little detectable deposition of C3 after renal I/R [31], indicating that the activation of complement after renal I/R involves this pathway. In human ATN, C3 is deposited in a pattern similar to that seen in mice, and there is no evident C4 [65]. This suggests that in humans too, complement activation after renal ischemia does not involve the classical pathway. Alternative pathway activation is favored in a microenvironment with high concentrations of its components and with a paucity of complement inhibitors [64]. Renal ischemia in mice produces both of these conditions, causing the tubules to alter their basolateral expression of the complement inhibitor Crry [66], a rodent analog of membrane cofactor protein which is a complement inhibitor expressed by human tubules [67]. I/R also stimulates TECs to produce

C3, further favoring alternative pathway activation [66,68]. Thus, there is both active synthesis of factors that favor complement activation and decreased expression of factors that inhibit this activation (Figure 1).

Once activated, the complement system generates a number of inflammatory signals. Systemic levels of the anaphylatoxin C3a increase after renal I/R [11]. Complement activation after renal I/R, and C5a in particular, has also been linked to generation of C-X-C chemokines such as MIP-2 [8]. We have also found that C3a induces TECs to produce both MIP-2 and KC (unpublished findings). *In vitro* experiments have also demonstrated that MAC deposition on TECs induces them to produce TNF- α and IL-6 [69]. Renal ischemia therefore appears to alter the TECs so as to favor alternative pathway activation upon their surface. Activation of this system directly generates the pro-inflammatory anaphylatoxins and may indirectly induce TECs to generate C-X-C chemokines and pro-inflammatory cytokines such as TNF- α and IL-6. Thus, in effect, the complement system detects hypoxic injury of TECs and then generates a number of inflammatory signals.

Mice deficient in decay accelerating factor (DAF; CD55) are more susceptible to renal I/R than wild-type controls, and mice deficient in both DAF and CD59 are even more sensitive to injury [70]. Activation of the complement system in the *DAF*^{-/-}*CD59*^{-/-} mice subjected to I/R is primarily evident in the peritubular capillaries, not along the tubules, and complement deposition is not detectable at this location in wild-type mice after I/R [70]. This suggests that renal I/R induces changes in endothelial cells that favor complement activation, but that expression of DAF and CD59 by these cells successfully controls the complement system in wild-type mice. Thus, hypoxia alters the way in which both endothelial and epithelial cells interact with the complement system. The changes in the endothelial cells are not sufficient to overcome the inhibitory function of surface DAF and CD59 whereas the cell surface expression of Crry by the epithelial cells is altered sufficiently to permit alternative pathway activation on the basolateral surface.

B cells and natural antibody

Natural antibodies are immunoglobulins that arise without specific antigenic stimulation [71]. They are produced primarily by B-1 cells and have a limited antigenic repertoire that includes self antigens [72,73]. Natural IgM has been implicated in the development of I/R injury in the intestine [74,75], primarily by activation of complement on tissues to which it is bound. The baseline repertoire of circulating natural antibody presumably can bind to antigens that are generated or exposed by I/R. *Mu* chain deficient mice (that lack mature B cells) are protected from renal I/R injury [32], suggesting that immunoglobulin may also be an important initiator of injury in this model too. Protection was not seen, though, in RAG-1 deficient mice (which are deficient in both mature B cells and mature T cells) [76]. Although IgG is not seen deposited in the post-ischemic kidney [76], it is possible that IgM natural antibody is an important initiator of injury. If recognition of neoepitopes by IgM is a mechanism of immune mediated injury, however, it remains to be determined why the *Mu*^{-/-} mice do not show decreased C3 deposition, why C4 is not essential to the development of injury [9], or why B cell reconstitution (which restored circulating IgM) did not restore injury in the cited study [32].

The tubular epithelium as a pro-inflammatory tissue

The TECs play an active role in initiating the inflammatory response, and even proximal inflammatory signals are promoted through increased expression of inflammatory factors and receptors (Figure 2). Expression of TLR2 and TLR4 by the cells is increased after I/R [58]. Synthesis of C3 by the TECs contributes to complement mediated injury [66,68], and

expression of the C5a receptor on the cells is increases after I/R [8]. In contrast, surface expression of complement inhibitors is decreased [66]. Signaling through the TLR2 [60] receptor and anaphylatoxin receptors [8] induce TECs to elaborate chemokines, and TECs produce chemokines in direct response to oxidative stress [52]. Tubular epithelium is not merely a passive victim of hypoxic injury, therefore, but actively marshals the inflammatory systems that contribute to even greater injury. The signaling response of the epithelium to aseptic hypoxic injury is strikingly similar to its response to ascending infection [18], and signaling through the TLRs induces production of KC in both settings. There may be unifying mechanisms by which the epithelial cells sense “threat” and assume this pro-inflammatory phenotype. It is tempting to postulate that the TECs have evolved these innate immune responses to protect the host from ascending infection but that the same mechanisms are maladaptive in the setting of aseptic injury. If so, anti-inflammatory therapies aimed at protecting the kidneys from ischemic injury may render the host more susceptible to urinary infection.

The role of anti-inflammatory therapy in ARF

Given the large number of animal studies demonstrating the importance of inflammation in the development of ischemic acute renal failure, as well as clinical correlates demonstrating activation of the same systems in patients with ARF, there is reason to hope that anti-inflammatory agents can ameliorate ischemic ARF. Trials of novel agents in ARF face several difficulties, however, including heterogeneity of the patient population and inadequate power to demonstrate moderate efficacy.

One of the greatest obstacles to effective treatment of ischemic ARF is our inability to diagnose the disease early in its clinical course. Physicians rely upon serum markers such as blood urea nitrogen and creatinine, but these markers may not become elevated until more than 24 hours after the insult [5]. Identifying earlier and more reliable biomarkers of injury has become a major goal of research in ARF. This is particularly important in relation to anti-inflammatory therapy, as the inflammatory response appears to be initiated rapidly, evolves over the course of the injury, and factors that are harmful during one phase of injury may be important for the resolution or recovery during another phase of injury. Once the inflammatory response has been set in motion, anti-inflammatory therapies may be ineffective and could conceivably delay recovery. Appropriate use of anti-inflammatory agents will therefore require early identification of the renal injury and also, possibly, stratification based upon where in the evolution of the inflammatory response the patient is. Even without improved biomarkers, however, there are clinical settings in which the ischemic insult can be anticipated, such as renal transplantation or patients who are scheduled to undergo cardiac surgery. Strategies that prevent the initiation of inflammation by targeting the earliest signals or recognition of the injured tissue may be of particular therapeutic benefit in these settings.

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Figure 1.

Triggers of inflammation after aseptic tissue injury. Tissue injury can initiate inflammation by several mechanisms which may occur simultaneously. Cellular injury can cause the release or exposure of inflammatory factors. Stress or injury can induce the active synthesis of pro-inflammatory signals. Immune system receptors may recognize altered or exposed surface structures. Decreased expression of inhibitory factors may permit uncontrolled activation of inflammatory cells or systems.

Figure 2.

Tubular epithelial cells generate inflammatory signals in response to injury or stress. In response to ischemia, epithelial cells favor complement activation by synthesizing C3 and decreasing their surface expression of Crry. Expression of the C5a receptor and TLRs 2 and 4 increase. Anaphylatoxins and ligation of the TLRs induce the cells to produce C-X-C chemokines.