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The Role of the Complement System in Acute Kidney Injury

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Summary

Acute kidney injury is a common and severe clinical problem. Patients who develop acute kidney injury are at increased risk of death in spite of supportive measures such as hemodialysis.

Research in recent years has revealed that tissue inflammation is central to the pathogenesis of renal injury, even after non-immune insults such as ischemia/reperfusion and toxins. Examination of clinical samples and pre-clinical models demonstrate that activation of the complement system is a critical cause of acute kidney injury. Furthermore, complement activation within the injured kidney is a proximal trigger of many downstream inflammatory events within the renal parenchyma that exacerbate injury to the kidney. Complement activation may also account for the systemic inflammatory events that contribute to remote organ injury and patient mortality.

Complement inhibitory drugs have now entered clinical use and may provide an important new therapeutic approach for patients suffering from or at high risk of developing acute kidney injury.

Keywords

Acute kidney injury; complement; ischemia/reperfusion

INTRODUCTION

Acute kidney injury (AKI) is a common clinical syndrome caused by a wide range of hemodynamic, ischemic, metabolic, inflammatory, and toxic insults to the kidney. AKI is associated with prolonged hospital stays, and patients who develop AKI have an increased risk of death in spite of supportive care. Currently there is no specific treatment for AKI. A growing body of experimental evidence, however, now indicates that complement activation contributes to the pathogenesis of AKI. Complement inhibitory therapies may represent an effective strategy for attenuating or preventing AKI and its complications.

It has long been known that the complement system is activated in immune complex glomerulonephritis. It has also become clear in recent years that the complement system is involved in a diverse array of other renal diseases. The involvement of this immune system in the pathogenesis of so many diseases of the kidney suggests that the kidney may be intrinsically susceptible to complement-mediated injury. As will be discussed below, complement activation in the kidney is often due to impaired or inadequate control of the

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complement system by the body's endogenous complement regulatory proteins. An important clinical question is whether complement activation is a common final pathway of renal damage in AKI of different etiologies.

ACUTE KIDNEY INJURY

AKI is a severe and common clinical condition. AKI is defined as a loss of renal function over a matter of days. Clinically, AKI is diagnosed by a rise in the serum creatinine, reflecting an acute decline in the glomerular filtration rate (GFR). This can be due to the rapid progression of an underlying renal disease (such as rapidly progressive glomerulonephritis or hemolytic uremic syndrome), or it can be due to renal injury caused by drugs, ischemia, infections, or nephrotoxic metabolites. Ischemia/reperfusion (I/R) is a common cause of AKI in hospitalized patients, and is a major factor in the development of AKI after transplantation, cardiac surgery, and sepsis.^{1, 2} A full discussion of the causes and adverse consequences of AKI is beyond the scope of this review, and interested readers are referred to several excellent publications on the subject.²⁻⁴

The conditions that cause AKI can target specific anatomic structures within the kidney, including the glomerulus, tubulointerstitium, and/or vasculature. Complement activation is an important mechanism of renal injury in diseases affecting each of these compartments. Uncontrolled alternative pathway activation within the microvasculature, for example, is the primary cause of atypical hemolytic uremic syndrome (aHUS).⁵ The complement system is also an important mediator of injury in ANCA associated vasculitis⁶ and anti-glomerular basement membrane disease.⁷ The proximal tubule is the primary site of injury after renal I/R, and complement activation on the ischemic tubule is an important cause of ischemic AKI.⁸ The mechanisms that cause a decline in GFR during AKI are complex and likely vary among patients with AKI caused by different etiologies. It remains to be determined whether engagement of the complement system in all of these different diverse forms of AKI represents independent phenomena, or whether complement activation is a generalizable response of the kidney to injury.

Many different components of both the innate and adaptive immune systems have been implicated in the pathogenesis of AKI. Ischemic renal injury, for example, leads to a robust inflammatory response within the kidney. In response to hypoxic injury, tubular epithelial cells produce numerous inflammatory cytokines,⁹⁻¹² and neutrophils, macrophages, and T cells infiltrate the kidney.^{9, 13, 14} The complement system and the toll-like receptors (TLRs) are early sensors of tissue injury. Blockade of complement activation, TLR2, or TLR4 prevents many of the downstream inflammatory manifestations of AKI.^{8, 15-18} While AKI is predominantly defined by kidney dysfunction, extra-renal complications are also important.^{11, 19} The role of complement activation in these extra-renal manifestations has not been examined in AKI, but studies have linked complement activation to remote organ injury after ischemia of other organs such as the intestine and skeletal muscle.^{20, 21} Regarded together, these studies indicate that complement activation within the kidney after I/R is an important trigger of the subsequent inflammatory response both within the kidney and possibly in remote organs.

COMPLEMENT ACTIVATION AFTER RENAL ISCHEMIA/REPERFUSION

Intra-renal complement activation is primarily detected and characterized by immunostaining renal tissue for C3 activation products. Studies in murine^{8, 22} and rat²³ models demonstrate increased deposition of C3 along the tubular basement membrane after I/R. Deposition of C3 is not seen in peri-tubular capillaries or within the glomeruli.²⁴ Biopsies of human kidneys with histologic evidence of acute tubular necrosis (ATN) also demonstrated C3 deposits along the tubular basement membrane.²² Similar to what is seen

in rodents, patchy tubulointerstitial C3 was seen in histologically normal kidneys, but the extent of deposition was increased in kidneys with ATN.

MECHANISMS OF COMPLEMENT ACTIVATION IN THE ISCHEMIC KIDNEY

AKI is episodic and is frequently caused by clinically apparent factors (e.g. hypotension or nephrotoxins). Thus, complement activation within the kidney is likely a secondary event in the development of injury. On the other hand, not all individuals develop AKI after exposure to the same renal insults, and patient factors (such as variation in the genes for the complement regulatory proteins) likely contribute to an individual's risk of developing kidney injury after exposure to a particular renal insult. Most of the pre-clinical studies examining the role of complement in AKI have employed models of I/R. The histologic changes in the kidney vary in location between different types of insults, so it is possible that activation of the complement system will differ among causes of AKI that target different locations within the kidney.

What causes complement activation in the injured tubulointerstitium? Conditions in the kidney may favor alternative pathway activation at baseline (Figure 1). Complement proteins in plasma, for example, are concentrated by filtration of protein-free plasma at the glomerulus. Alternative pathway activation on tubular epithelial cells is also promoted by synthesis of ammonia which can non-enzymatically form an amide linkage with the thioester bond in C3.²⁵ This nucleophilic modification of C3 forms a molecule with C3b-like properties that can form an alternative pathway convertase.^{25, 26} An acidic environment also promotes alternative pathway activation on tubular epithelial cells.²⁷ There is compelling evidence that extra-hepatic production of complement proteins, particularly by renal tubular epithelial cells, can promote local complement activation and injury. Pathogens or cellular microparticles can increase alternative pathway activation in the bloodstream, and when these entities enter the renal microvasculature they may further increase activation of the alternative pathway in plasma.

There are several mechanisms by which the complement system can be directly activated on injured host cells. Circulating molecules may bind, or “recognize”, molecular signatures that are generated or displayed on damaged tissues. Natural antibodies, for example, bind to neo-epitopes displayed on injured tissue.²⁸ Immune molecules such as natural antibodies, lectins, and c-reactive protein (CRP) can recognize injury markers and then activate the complement system. All cells of the body express complement regulatory proteins, and the expression of these proteins can also be disrupted by tissue injury.²⁹ Along these lines, the apical-basolateral organization of the tubular epithelial cell is disrupted by ischemia, and proteins that are ordinarily restricted to the apical side of the cell may gain access to the basolateral surface. All of these events may occur simultaneously, creating a micro-environment that that favors complement activation in an acutely injured kidney.

The Classical Pathway

The classical pathway of complement is activated by immune-complexes that deposit or form within the glomeruli in diseases such as lupus nephritis and MPGN type I. The classical pathway can also be activated by antibody that binds to tissues in diseases that are not traditionally thought of as “auto-immune.” As mentioned above, neo-epitopes are generated or exposed in tissues during inflammation and non-immune injury.^{30, 31} Immunoglobulin specific for these epitopes can then activate the classical pathway, exacerbating injury. Antibody mediated tissue injury has been observed after ischemic injury of the intestine³² and skeletal muscle.³³ Numerous studies have examined the classical pathway in murine models of renal I/R, however none has identified a role for this pathway in AKI.^{24, 34-36}

The MBL Pathway

Similar to the classical pathway, the MBL pathway is activated by recognition molecules that bind to specific target molecules and then activate serine proteases. The recognition molecules of the MBL pathway include mannose binding lectins or ficolins. These molecules bind to carbohydrate motifs displayed on the surface of bacteria, and also on the surface of some injured cells. These recognition molecules also bind to injury-associated proteins, including cytokeratin.³⁷

Several studies indicate that the MBL system is engaged within the ischemic kidney. Examination of mouse tissues demonstrated that MBLs-A and -C deposited on the renal tubules and vasculature of mice subjected to prolonged ischemic times (30–45 minutes).³⁸ MBL deposits were also seen in the peri-tubular capillaries and tubulointerstitium in a pig model of renal I/R.³⁹ Recent work suggests that activation of MASP-2 in ischemic tissues can cause downstream cleavage of C3 in a C4-independent fashion.⁴⁰ Such a bypass mechanism may help to reconcile the observation that MBL binds to the ischemic kidney but that C4 is not required for complement activation in the kidney after I/R and is not seen in the tubulointerstitium of mice subjected to renal I/R (Figure 2). Mice with targeted deletion of the MBL proteins were protected in one model of renal I/R⁴¹, although they were not protected compared to wild-type mice in another study.²⁴ The role of the MBL pathway in AKI, therefore, is not clear at this point.

Alternative Pathway Activation

The alternative pathway provides an amplification loop that augments activation through the classical and MBL pathways. It is not always appreciated, however, that the alternative pathway is an important initiator of complement activation. Pre-clinical and clinical studies have revealed that activation of the alternative pathway is central to the pathogenesis of many forms of AKI, including ischemic AKI and aHUS.⁴² As outlined above, the classical and MBL pathways are initiated by specific “recognition” molecules (e.g. immunoglobulin and the MBLs, respectively). The alternative pathway, on the other hand, is continually activated in plasma through a process called “tickover”.^{43, 44} Tickover refers to a reaction whereby the internal thioester of C3 is hydrolyzed by water. Similar to C3b, the hydrolyzed C3 [C3(H₂O)] can bind to factor B and form a C3-convertase [C3(H₂O)Bb]. This convertase is not as efficient at C3 cleavage as is C3bBb⁴⁵, but it continually generates C3b which can initiate alternative pathway activation on nearby surfaces.

When C3b is generated by any of the activation pathways, including the tickover process, it can form a thioester bond with hydroxyl and primary amine groups on nearby surfaces.^{46, 47} This covalently linked C3b can combine with factor B to form the alternative pathway C3-convertase unless nearby complement regulatory proteins inactivate the convertase. Complement regulatory proteins work through two mechanisms. They can accelerate the disassociation of the C3bBb complex (“decay acceleration”). Some regulatory proteins enable a serine protease called factor I to cleave and inactivate C3b and C4b, which are essential parts of the alternative and classical C3 convertase, respectively. This regulatory function is referred to as “cofactor” activity. Given the ability of the alternative pathway to auto-activate and self-amplify, this pathway rapidly activates on surfaces that lack complement regulators, including invasive pathogens, the GBM, and injured cells. Continuous, effective control of the alternative pathway on host cells by the regulatory proteins is necessary in order to prevent spontaneous alternative pathway activation. Indeed, host tissues may have an intrinsic potential for activating or inhibiting the alternative pathway, and this balance may be altered during states of illness, injury, or cellular activation.

Because the alternative pathway so readily amplifies complement activation on biologic and artificial surfaces, it is not surprising that this pathway contributes to tissue injury in many different diseases and after a wide range of ischemic, mechanical, hemodynamic, and metabolic insults. It is noteworthy, however, that the kidney is a particularly common target of alternative pathway activation. Indeed, in patients with congenital mutations that impair their ability to control the alternative pathway – such as defects in factor H function – the kidney is the most common target of complement-mediated injury. The vulnerability of the kidney to alternative pathway mediated injury is probably due to the factors mentioned above. Clearly, regulation of the alternative pathway within the kidney is inadequate to prevent injury or becomes impaired by the injury process in many clinical settings.

Targeted deletion of the gene for factor B prevents C3 deposition in the tubulointerstitium of mice after I/R⁸, demonstrating that activation in this compartment requires an intact alternative pathway. Complement activation in this model is not affected by deficiency of C1q, C4, or MBL-A/C.²⁴ These other pathways – the classical and the MBL – may still contribute to initiation of complement activation in the tubulointerstitium, but they are evidently dispensable in the murine model. These experiments do not test whether there could be multiple initiating pathways, since the knockout mouse strains studied were deficient in isolated complement proteins.

Interestingly, complement is activated via different pathways in the glomeruli and the tubulointerstitium. IgM and C4 are co-deposited in the mesangium of healthy mice²⁴ (figure 2), and are also detected in the glomeruli of healthy human kidneys.⁴⁸⁻⁵⁰ Complement activation by the IgM does not, however, cause C3 deposition. We interpret this as reflecting effective complement regulation at this location, so mesangial IgM effectively engages the classical [or possibly the MBL pathway⁵¹⁻⁵³], but does not proceed to the level of C3 cleavage (Figure 2). Restricted complement activation has also been described at other sites and is determined by the combination of complement regulatory protein on the specific surface.^{29, 54-56} In contrast to glomerular complement activation, complement activation in the tubulointerstitium does not cause C4 deposition but it does cause fixation of C3 fragments to the tubules. Complement activation in the glomeruli and the tubulointerstitium are thus initiated by different mechanisms, and proceeds to a different extent based upon the efficacy of local regulatory proteins.

Other Local Factors that May Contribute to Alternative Pathway Activation in AKI

Molecular activators of the alternative pathway in AKI—The protein properdin has long been known to stabilize the alternative pathway convertase once it has formed, but early work indicated that it was not required to initiate alternative pathway activation.⁵⁷ There is a growing appreciation, however, that properdin can bind to selected surfaces and serve as an initiator of alternative pathway activation.^{58, 59} Filtered properdin binds to the apical surface of tubular epithelial cells in proteinuric states and positively regulates alternative pathway activation on the cells.⁶⁰ A recent study also demonstrated that properdin binds to the peritubular capillaries after renal I/R.⁶¹ Thus study used mice deficient in decay accelerating factor (DAF, CD55) and CD59 (a regulator of membrane attack complex formation) in order to amplify the role of complement activation, but it did not examine whether properdin binds to the peritubular capillaries in wild-type mice or whether bound properdin causes complement activation in the presence of intact complement regulation by DAF and CD59. Whether properdin is required for activation or enhances activation of the alternative pathway on the basolateral surface of tubular epithelial cells in AKI has not yet been explored.

Local production of complement proteins in renal ischemia/reperfusion—

Tubular epithelial cells can synthesize C3, C4, and factor B.⁶²⁻⁶⁴ C3 synthesis is rapidly upregulated within the kidney after warm and cold ischemia.⁶⁵ A series of elegant studies by Sacks and colleagues demonstrated that production of C3 by the renal epithelium promotes local complement activation in the transplanted kidney, and this local synthesis of complement proteins is an important cause of acute renal injury.^{66, 67} Epithelial production of these complement proteins can be stimulated by inflammation and cytokines, and C3 production is also directly induced by ischemia.⁶⁶

In an unbiased gene expression array study of renal transplants at the time of implantation (e.g. prior to reperfusion), complement genes (C1q, C1r, C1s, C2, C3, C4, C6, and factor B) were the functional group of genes most strongly upregulated in deceased donor kidneys compared to kidneys from living donors.⁶⁸ The expression level of several of the complement genes, including the gene for C3, was associated with the cold ischemia time. Complement gene expression was also inversely correlated with early graft function (days 2 and 3) and also with late graft function (years 2 and 3).⁶⁸ A second study also reported that renal mRNA for C3 was increased in brain dead donors at the time the organ was harvested, although the level of C3 expression was not correlated with the cold ischemia time in this study.⁶⁹ Although these studies do not confirm a pathogenic role for C3 produced by the renal allograft, they demonstrate that renal expression of C3 in humans can be upregulated in response to ischemia or other inflammatory signals similar to what is seen in rodents.

Extra-renal complement activation—The complement system is activated when plasma comes in contact with artificial surfaces, including dialysis membranes. Early studies demonstrated that polyacrylonitrile dialysis membranes caused less complement activation and sequestration of leukocytes than cuprophane dialysis membranes.⁷⁰ In a rat model of AKI, activation of complement by exposing the rats' blood to complement activating surfaces, including cuprophane, delayed their recovery.⁷¹ In patients with AKI, dialysis using polyacrylonitrile dialysis membranes was also associated with better outcomes than use of cuprophane membranes.⁷² These studies indicate that extra-renal complement activation (through dialysis, exposure of blood to other biomaterials, or infections) may exacerbate AKI. Furthermore, although the newer, more biocompatible dialysis membranes are not as complement activating as cuprophane dialysis membranes, C3a is still generated to a measureable degree when blood comes in contact with these membranes.^{73, 74}

THE ROLE OF COMPLEMENT REGULATORY PROTEINS IN AKI

As discussed above, the important role of the alternative pathway in AKI suggests that local complement regulation within the tubulointerstitium is either inadequate to control the activating factors or regulation is disrupted during the development of AKI. Complement activation within tissues is determined by the local concentrations of complement proteins and activating molecules as balanced by complement regulatory proteins. Activation on host tissue indicates that the balance of these factors favors activation. Complement regulatory proteins are expressed on glomerular cells (endothelial, mesangial, and epithelial), tubular epithelial cells, and microvascular endothelial cells.^{75, 76} In addition to the cell surface proteins, soluble complement regulatory proteins are present in plasma: factor H is a soluble alternative pathway regulator and C4-binding protein is a soluble classical pathway regulator.⁷⁷ Furthermore, factor H contains several binding domains that enable it to bind to anionic molecules, providing complement regulation on cell and membrane surfaces.⁷⁸ The importance of factor H for controlling the alternative pathway within the kidney is illustrated by the strong association of renal disease with mutations or autoantibodies to factor H.⁷⁹

Some renal cells, such as mesangial cells, express several different complement regulatory proteins.⁸⁰ However, tubular epithelial cells only express membrane cofactor protein (MCP or CD46). MCP is a transmembrane protein that regulates complement activation by serving as a necessary “cofactor” for factor I mediated cleavage of C3b and C4b.⁸¹ Tubular epithelial cells may also express low levels of CD59 on their apical surface.⁷⁵ In rodents, Crry (a homologue of MCP) is the only complement regulatory protein expressed by tubular epithelial cells.^{82, 83} The expression of MCP in humans and Crry in rodents is restricted to the basolateral aspect of the tubular epithelial cells^{75, 82, 83}, the site of C3 deposition in the post-ischemic kidney. Experiments in mice reveal, however, that the expression of Crry on epithelial cells decreases after ischemia. In vitro and in vivo experiments demonstrate that a decrease in complement regulation by Crry on the surface of tubular epithelial cells is sufficient to permit alternative pathway activation at this location.⁸²⁻⁸⁵ Thus, complement regulation on the basolateral surface of tubular epithelial cells in mice is critically dependent upon the protein Crry, and this protection is disrupted by ischemic injury of the cells.

The inability of factor H in serum to prevent alternative pathway activation on the tubular epithelial cells could be due to inadequate concentration of the protein at the site of complement activation or insufficient ability of the protein to regulate the complement system on this particular surface. Experiments using tubular epithelial cells in culture demonstrated that factor H does provide some complement regulation on the apical and the basal surfaces of the cells.⁸² However, full suppression of complement activation on the cells required the addition of supraphysiologic concentrations of factor H.⁸⁶ On the other hand, blockade of factor H function in vivo exacerbated ischemic AKI in mice, and administration of a targeted recombinant form of factor H suppressed complement activation in the kidneys after I/R.⁸⁶ These experiments suggest that factor H provides some protection from complement-mediated injury of the tubules after I/R. However, factor H does not fully control alternative pathway activation on injured tubular epithelial cells or on cells genetically deficient in cell surface complement regulatory proteins (Figure 1).

Congenital defects in factor H and MCP are well-established risk factors for the development of aHUS⁵, a disease believed to involve intravascular activation of the alternative pathway. Although the relevance of this for other forms of AKI is not clear, it is noteworthy that disease recurrence is very high after transplantation.⁸⁷ Ischemia during the transplant procedure is believed to be a causative factor in triggering disease flares.⁸⁸ DAF is another cell surface complement regulatory protein. It is not expressed on the renal tubules, but it is expressed on vascular structures throughout murine and human kidneys.^{75, 83} Although complement activation is not seen within the renal vasculature of wild-type mice after I/R, C3 deposition was seen on the endothelium of DAF deficient mice and these mice developed more severe ischemic AKI than wild-type controls.⁸⁹ Furthermore, mice deficient in both DAF and CD59 had even more severe injury than mice deficient only in DAF. A subsequent study demonstrated that peritubular capillary complement activation in mice deficient in both DAF and CD59 is an alternative pathway-mediated process and requires binding of properdin to the endothelium.⁶¹ Regarded together, these clinical observations and experiments suggest that the vasculature is ordinarily protected from complement-mediated injury after ischemia, but genetic defects in complement regulation can render patients susceptible to intravascular alternative pathway activation after renal insults such as ischemia/reperfusion.

DOWNSTREAM EFFECTORS OF COMPLEMENT-MEDIATED INJURY

Full activation of the complement system generates several biologically active fragments. These complement activation fragments cause tissue inflammation through several

mechanisms (Figure 3). Experiments have directly tested the role of several of these complement activation fragments in the pathogenesis of AKI.

Anaphylotoxins (C3a, C5a)

C3a and C5a are small, soluble peptides that are released during complement activation. These molecules have potent pro-inflammatory effects, although they are rapidly inactivated by the serum enzyme carboxypeptidase N. Massive activation of the complement system by administration of cobra venom factor or the injection of animals with the anaphylatoxins causes systemic inflammation characterized by increased capillary permeability, bronchoconstriction, aggregation of leukocytes in the pulmonary vasculature, and hypotension.^{90, 91} Although the effects of limited, local generation are not as dramatic, the receptors for the anaphylatoxins are expressed on myeloid and renal cells and signaling through these receptors contributes to tissue inflammation in a number of renal disease models.

C5a signals through the C5a receptor (C5aR), a member of the rhodopsin family of seven transmembrane-spanning G protein coupled receptors. C5aR is expressed on neutrophils and monocytes, and also on endothelial and epithelial cells.^{92, 93} C5a is a potent chemotactic agent and promotes adhesion and activation of leukocytes. C5a signaling through C5aR also activates endothelial cell expression of P-selectin and thereby promotes leukocyte adhesion.⁹⁴ Within the kidney, the C5aR is expressed by mesangial cells, proximal tubular epithelial cells, and distal tubular epithelial cells^{92, 95}, and expression by proximal tubular epithelial cells increases after renal I/R.⁹⁶ Studies of proximal tubular cells have not, however, revealed a strong pro-inflammatory synthetic response to C5a.^{15, 95}

Several strategies have been used to examine the role of C5aR in renal I/R. In a murine model of renal I/R, mice were treated with a small molecule inhibitor of the C5aR.⁹⁶ C5aR blockade protected the mice from injury in this study. Treatment of the mice with this agent prevented the generation of the CXC chemokines KC and MIP-2 but did not prevent TNF-generation in the kidney. C5aR blockade also attenuated neutrophil influx in the post-ischemic kidney. Because C5aR-blockade also protected neutrophil-depleted mice from injury, the authors concluded that C5a causes injury through mechanisms beyond its role in neutrophil trafficking. Mice with targeted deletion of the C5a receptor are also protected from ischemic AKI and mRNA levels for several inflammatory cytokines were lower in these animals than in wild-type controls.⁹⁷ Experiments using bone marrow chimeras indicated that expression of the anaphylatoxin receptors on both renal cells and bone marrow derived leukocytes contributed to renal injury after I/R. Furthermore, mice deficient in the C5a receptor seemed to be protected to a greater degree than mice deficient in the C3a receptor. The above studies indicate that C5a signaling likely contributes to the pathogenesis of ischemic AKI through several mechanisms. Some pro-inflammatory effects of C5a generation are likely due to its role in neutrophil chemotaxis, but production of C5a within the kidney may also directly elicit pathogenic responses from resident renal cells.

Injection of animals with C3a elicits many of the same effects as does C5a, although C3a does not appear to be as potent a chemotactic agent. The C3a receptor is expressed on tubular epithelial cells, but does not appear to be expressed on mesangial cells or podocytes.⁹⁸ In vitro stimulation of tubular epithelial cells with C3a induced production of pro-1 type 1 collagen, indicating a synthetic response of the cells to C3a signaling. Gene array analysis of murine tissue from wild-type mice demonstrated that complement activation in the kidney after I/R is associated with the production of the chemokines MIP-2 and KC, two murine homologues of IL-8.¹⁵ In vitro studies demonstrated that it is C3a, not C5a, which induces the tubular epithelial cells to generate these pro-inflammatory chemokines. Studies have shown that C3a receptor antagonists attenuate other forms of renal

injury.^{99, 100} but have not been tested in models of ischemic AKI. As mentioned above, though, mice with targeted genetic deletion of the C3a receptor are protected from ischemic AKI compared to control mice.⁹⁷

The Membrane Attack Complex

The membrane attack complex (MAC; C5b-9) is a multimeric product of complement activation that forms lytic pores in the outer membrane of target surfaces. It can lyse cells, and in sublytic quantities it causes cell activation. Studies using C6 deficient mice, which cannot generate MAC, demonstrated that these mice are protected from renal I/R.³⁴ Given that complement activation is most prominent in the tubulointerstitium, the primary targets of the MAC are likely tubular epithelial cells. MAC formation on the cell surface could contribute to cell necrosis, exacerbate ATP depletion in hypoxic cells¹⁰¹, and trigger intracellular signaling pathways and cell activation.¹⁰² Fewer neutrophils infiltrated the kidneys of C6 deficient mice after I/R³⁴, which may be a direct effect of reduced inflammatory signaling or may be a non-specific result of milder tubulointerstitial injury. When complement is activated on cell or tissue surfaces some of the formed MAC complex remains soluble. Interestingly, soluble (cytolytically inactive) MAC can increase adhesion molecule expression and promote neutrophil infiltration^{103, 104}, providing another potential link between complement activation on the tubular epithelial cells and renal inflammation.

C3b/iC3b/C3dg

During complement activation C3b is covalently fixed to cell surfaces. Subsequent cleavage of C3b by the complement regulatory proteins converts C3b to iC3b and then to C3dg and C3d.¹⁰⁵ These C3 activation fragments are useful biomarkers of complement activation and can be easily detected by immunofluorescence microscopy. They are also ligands for several complement receptors and contribute to the immune response. Complement receptors (CR) 1-4 are expressed on hematologic cells and bind to the C3 fragments.¹⁰⁶ These proteins, particularly CR1, control complement activation, and also mediate leukocyte functions. The specific roles of the complement receptors in AKI are unknown, but these receptors have numerous effects on the innate^{107, 108} and adaptive¹⁰⁹ immune responses and likely contribute to renal inflammation.

COMPLEMENT ACTIVATION IN THE TRANSPLANTED KIDNEY

As highlighted throughout this paper, much of the evidence regarding complement activation in the ischemic human kidney comes from studies of transplanted organs. Inevitably transplanted kidneys are subjected to some degree of ischemia, and complement activation may be an important cause of injury to the allograft and contribute to delayed graft function. Evaluation of complement activation in transplanted kidneys is confounded by possible rejection of the allograft, but some studies examined the kidneys prior to reperfusion.^{68, 69, 110} Complement activation in the transplanted kidney likely contributes to acute injury, but it also influences the adaptive immune response and long term graft survival.⁶⁷ In addition to acute deleterious effects on the allograft, intra-renal complement activation at the time of transplantation may impact other diseases. Recurrence of atypical aHUS, for example, is very common in transplant recipients who do not receive prophylactic plasma exchange or eculizumab, and the rate of recurrence is highest in the peri-transplant period.¹¹¹

THE USE OF COMPLEMENT INHIBITORS FOR THE PREVENTION AND TREATMENT OF ACUTE KIDNEY INJURY

Although eculizumab is the only FDA approved complement inhibitor, there are a large number of complement inhibitory drugs in development.¹¹² Several therapeutic complement inhibitors have been tested in models of AKI. The efficacy of these agents highlights the pathogenic role of complement activation in AKI and supports the potential clinical benefit of treating patients at risk of AKI with complement inhibitors in the future.

Treatment of rats and mice with C5a receptor antagonists prevented renal injury in models of renal I/R.^{113, 114} In one of the studies the drug was administered pre-ischemia either intravenously or by oral administration, and mice treated with the agent by either route developed milder injury.¹¹³ A monoclonal antibody to mouse factor B prevented complement activation in the kidneys of mice after I/R, and ameliorated injury.¹¹⁵ Mice treated with the antibody developed less tubular necrosis and fewer apoptotic tubular epithelial cells were observed than in control mice. An antibody to properdin has also been shown to protect mice from ischemic AKI.⁶¹ The anti-factor B and anti-properdin monoclonal antibodies selectively block the alternative pathway, so they prevent the pathologic effects of this complement pathway in AKI while leaving the other complement pathways intact.

Several agents have been developed that incorporate the regulatory regions of endogenous complement regulatory proteins. A soluble form of CR1 was the first of these, and has shown efficacy in several diseases.¹¹⁶ A soluble form of Crry (a murine homolog of CR1) was developed and tested in a model of renal I/R.¹¹⁷ Treatment with Crry reduced C3 deposition within the kidney, but did not reduce renal injury. Interestingly, the abundance of C3 deposition was inversely proportional to the degree of renal injury (as assessed by urea nitrogen levels). One possible explanation is that the recombinant Crry, which is approximately 160 kD in size¹¹⁸, could only reach the site of complement activation in regions of increased capillary permeability. Agents such as this must reach the site of C3b generation in order to limit complement activation.

Several strategies have been used to develop complement inhibitors that target cell membrane or specific tissue sites. The advantage of this approach is that, like the endogenous complement regulators, the targeted agents block complement-mediated injury of host tissues without impairing complement functions elsewhere in the body. Tomlinson's group has developed several different agents that use the C3d-binding region of CR2 to direct complement inhibitory proteins to sites of iC3b and C3d deposits (e.g. tissues in which complement activation has occurred).¹¹⁹⁻¹²¹ Although these proteins can bind to opsonized pathogens, protecting them from eradication, the targeted drugs do not seem to increase susceptibility of treated mice to infection.¹¹⁹ We tested one of these agents in a mouse model of I/R. This agent uses the CR2 fragment to deliver the complement inhibitory region of factor H to sites of complement activation. It reduced interstitial complement activation and reduced the severity of ischemic AKI, although protection from injury was incomplete.⁸⁶

Another targeted complement inhibitor utilizes a myristoyl membrane binding tail in order to affix the complement regulatory region of CR1 to cell surfaces. Renal allografts can be coated with this agent prior to implantation, effectively providing cell surfaces with additional control of the complement system during reperfusion.¹²² This complement inhibitor protected rat kidneys from I/R injury in a syngeneic transplant model in which the kidneys were subjected to 60 minutes of warm ischemia.¹²² Examination of human kidneys that were perfused ex vivo with the agent demonstrated that it had bound to the glomeruli

and tubules.¹²² A Phase II clinical efficacy trial is currently underway that will test whether this agent (APT070) protects allografts from DGF.

As highlighted at the beginning of this review, it is not clear whether complement-mediated renal injury is common to the many different etiologies of AKI. Furthermore, the pre-clinical studies of complement inhibitors in models of AKI leave several important mechanistic questions unanswered. One issue is that of tissue penetration. Complement activation in the ischemic kidney appears to predominantly affect the proximal tubules. Monoclonal antibodies may effectively block complement activation by binding up complement factors in plasma, and small molecules will likely distribute throughout the extracellular space (Figure 4). However, complement inhibitors that incorporate the decay accelerating function or cofactor function of the endogenous complement regulatory proteins will need to reach the site of complement activation in order to access the C3-convertase. Similarly, targeted complement inhibitors must be able to reach the anatomic site of complement activation. Furthermore, in patients with mutations or autoantibodies that promote complement activation, these therapies may not always overcome the patient's particular defect. C3 nephritic factors, for example, may hinder the inactivation of C3b by complement inhibitors just as they hinder the activity of factor H.

As with all therapies for AKI, complement inhibitors may no longer be effective by the time a clinical diagnosis is made. It is not clear to what degree complement inhibition will be protective if administered in patients with established AKI or what the window of efficacy is. For now, therefore, a clinical trial of complement inhibition in AKI is best undertaken as a preventative study in a high-risk clinical setting.¹²³ Such studies could potentially be performed on patients undergoing cardiovascular surgery or kidney transplantation.

The most worrisome potential side effect of complement inhibition is infection. A large percentage of patients with AKI have sepsis, and patients with AKI are at high risk of developing infections. Targeted complement inhibitors are appealing insofar as they correct a defect that causes intra-renal complement activation (e.g. the loss of complement regulation on the tubular basement membrane) without affecting the balance of complement activation and complement regulation elsewhere in the body. Patients treated with eculizumab should be vaccinated against meningococcal infections but this is impractical in AKI. Therefore, as in patients with aHUS, patients treated with complement inhibitors for AKI should receive prophylactic antibiotics unless they have been previously vaccinated.¹²⁴

CONCLUSIONS

The complement system provides a rapid and effective system by which the body eliminates invasive organisms and injured host cells. Although it has long been appreciated that the complement system causes injury to the host in autoimmune diseases of the kidney, recent work has revealed that inappropriate complement activation contributes to the pathogenesis of AKI. Complement activation in the setting of ischemia/reperfusion, sepsis, and chemical injury is fostered by a local imbalance between activating and inhibitory elements. The discovery that tubular epithelial cells generate complement proteins, such as C3, suggests that the kidney has evolved to create a complement-activating environment. Furthermore, reduced expression of complement regulatory proteins on ischemic tubules further promotes local activation of the alternative pathway.

There is abundant pre-clinical and clinical data demonstrating the role of complement activation in the development of AKI. Obstacles to the use of complement inhibitory agents in patients with AKI include early diagnosis of the disease and the risk of infection in patients treated with these drugs. The use of eculizumab in patients with aHUS and other

renal diseases, however, will improve our understanding of the benefits and limitations of these drugs in patients with acute renal disease. Careful examination of the effects of these drugs in patients during acute disease episodes and in the peri-transplant period will be useful for identifying their effects on the course of AKI. Several other complement inhibitory drugs are also under development, and these drugs may also soon become viable therapeutic options for patients with AKI.

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Figure 1.

Control of complement activation within the kidney. The complement system is continually activated in plasma through spontaneous “tickover” of the alternative pathway. The concentration of alternative pathway proteins increases within the kidney due to filtration of protein-free plasma and by local synthesis of the proteins. A low pH and production of ammonia also increase activation. Cellular microparticles and pathogens in the bloodstream may also increase activation within the plasma. Complement activation in the fluid phase deposits C3b on renal surfaces, but factor H in the plasma controls this process. Factor H also controls complement activation on the glomerular basement membrane and on cell surfaces. Renal cells, such as tubular epithelial cells, express cell surface complement regulatory proteins that control amplification of complement activation on the cell surface, but expression of these proteins can be disrupted by cellular injury.

Figure 2.

Two compartments of complement activation in kidneys with ischemic acute kidney injury. Immunofluorescence microscopy was performed on kidneys from (A) sham treated wild-type animals, and (B) wild-type, (C) *fB*^{-/-} (alternative pathway deficient) and (D) *C4*^{-/-} (classical and MBL pathway deficient) animals subjected to renal ischemia/reperfusion. Mesangial C4 (stained red) was seen prominently in the glomeruli of wild-type and *fB*^{-/-} mice, but was not seen in the tubulointerstitium. C3 (stained green) was seen in the tubulointerstitium of wild-type and *C4*^{-/-} mice, but was not seen in the kidneys of *fB*^{-/-} mice. Glomeruli are indicated with arrowheads. These results indicate that there is limited classical or lectin pathway activation in the glomeruli of mice after renal I/R, but that activation does not proceed to the level of C3 cleavage. Activation in the tubulointerstitium does not require C4, indicating that it is an alternative pathway mediated process. Original magnification $\times 400$. Reprinted with permission.²⁴

Figure 3.

Pro-inflammatory effects of complement activation. Complement activation generates several biologically active proteins, including C3a, C5a, C3b, and the membrane attack complex (C5b-9). C3a and C5a cause cellular activation via signaling through cell surface receptors. C3a and C5a can also affect the inflammatory response via chemo-attraction and activation of leukocytes. C5b-9 can cause cellular activation, cellular injury, and the formation of transmembrane pores can cause cell lysis. C3b is covalently fixed to cell surfaces and serves as a ligand for receptors expressed by leukocytes.

Figure 4.

Delivery of complement inhibitory therapeutic agents to the renal tubulointerstitium. Systemically administered drugs will reach the renal tubulointerstitium via the peritubular capillaries. Small molecules are likely to distribute into the tubulointerstitium. Full size monoclonal antibodies may be more confined to the vascular space, but these drugs may still be effective if their target can diffuse between the interstitium and vascular spaces. Proteins that work through decay acceleration or cofactor activity (i.e. catalyze cleavage of C3b to iC3b) need to gain access to the site of complement activation in order to be effective.