Complement Recognition Pathways in Renal Transplantation

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ABSTRACT

The complement system, consisting of soluble and cell membrane-bound components of the innate immune system, has defined roles in the pathophysiology of renal allograft rejection. Notably, the unavoidable ischemia-reperfusion injury inherent to transplantation is mediated through the terminal complement activation products C5a and C5b-9. Furthermore, biologically active fragments C3a and C5a, produced during complement activation, can modulate both antigen presentation and T cell priming, ultimately leading to allograft rejection. Earlier work identified renal tubule cell synthesis of C3, rather than hepatic synthesis of C3, as the primary source of C3 driving these effects. Recent efforts have focused on identifying the local triggers of complement activation. Collectin-11, a soluble C-type lectin expressed in renal tissue, has been implicated as an important trigger of complement activation in renal tissue. In particular, collectin-11 has been shown to engage L-fucose at sites of ischemic stress, activating the lectin complement pathway and directing the innate immune response to the distressed renal tubule. The interface between collectin-11 and L-fucose, in both the recipient and the allograft, is an attractive target for therapies intended to curtail renal inflammation in the acute phase.


The innate immune system, composed of humoral, cellular, and physical barrier defenses, plays an essential role in the immediate and nonspecific response to invading pathogens. A vital subset of innate immunity is the complement system. Complement defends against invading organisms, clears immune complexes and cell debris, and provides an interface between innate and adaptive immunity. Specifically, complement consists of interactive soluble proteins, membrane-bound receptors, and regulatory proteins. For many years, the focus of immune modulation in organ transplantation has been on the adaptive immune system. However, it has been within the last 15 years that several new aspects of complement biology with respect to solid organ transplantation have emerged. We now know that complement helps direct the alloimmune response to solid organ transplants not only through contributing to damage associated with ischemia-reperfusion injury but also through a significant role in the augmentation of T cell– and B cell–mediated immunity. Indeed, not only has the role of complement as an effector of Antibody-Mediated Rejection (ABMR) been updated, but it is also required to prime effective antibody production against donor tissue. In addition, recent evidence points to complement activation as a significant factor in the progression of chronic native kidney disease, which is likely to have generic application relevant to the transplanted organ. How these observations will be translated to the clinical realm remains to be identified, as any intervention will need to weigh its effect on organ injury and rejection with dampening the antimicrobial functions of the complement system, such as opsonisation, cell lysis, and recruitment of neutrophils and other inflammatory cells. It is our belief that therapeutic strategies can be designed to specifically target the key initiators of complement activation at the relevant location, such as complement-binding anti-HLA antibodies in the vascular compartment or the initiator(s) of local complement activation in the extravascular compartment of the allograft itself. In this article, we will discuss the roles of various complement recognition pathways in ischemia-reperfusion injury and allograft rejection with particular focus on the role of carbohydrate both as a danger signal and a potential target underpinning complement activation after ischemic insult and likely downstream adaptive immune recognition.

Our review will cover (1) The Complement System, (2) Complement in the Development of Adaptive Immunity, (3) Role of Complement in ABMR, (4) The Lectin Pathway (LP) as a Mediator of Organ Injury, (5) Role of mannan-binding lectin (MBL) in Tissue Injury, (6) Role of...
Ficolins in Tissue Injury, and (7) Collectin-11 as a Mediator of Renal Epithelial Inflammation, followed by our concluding remarks.

THE COMPLEMENT SYSTEM

Complement is activated on the surface of pathogens as well as damaged or infected cells via one of three main pathways: the classical, alternative, or lectin pathway.8 These distinct pathways converge at the formation of C3, which is then cleaved to form C5 convertase, with the subsequent production of the terminal pathway complement components, comprising C5a and the membrane attack complex (C5b–9) (Figure 1). Classical pathway activation typically occurs through antibody-antigen complex binding of C1q to the Fc portion of IgM or IgG,1,9,10 which forms part of the C1 complex with classical pathway–specific serine proteases C1r and C1s. The alternative pathway, activated by hydrolysis of plasma C3 and enhanced in some circumstances by an absence of complement inhibitors on cell membranes, also functions as an amplification pathway after the generation of C3b by the classical or lectin pathways.11,12 In contrast, LP initiation takes place through pattern-recognition molecules such as MBL, ficolins, surfactant proteins, and the recently identified C-type lectin, Collectin-11 (CL-11; CL-K1), all of which bind to carbohydrate motifs.

Under normal physiologic conditions, complement activation is controlled by surface-bound and soluble proteins that mediate the degradation of complement convertases, ultimately preventing the formation of complement effectors C3a, C3b, C5a, and C5b–9. Fluid phase complement-regulating plasma proteins include C1 esterase inhibitor (C1 INH), C4b binding protein, factor H, and factor I. Cell-membrane regulatory proteins include decay-accelerating factor (DAF; CD55), membrane cofactor protein (MCP; CD46), and CR1 (CD35). These proteins modulate the complement response and protect host cells and tissues from damage related to complement activation.13 During inflammation and cell stress this equilibrium shifts away from regulation and can lead to uncontrolled complement-mediated injury and rejection.14 Indeed, after renal ischemia-reperfusion injury, which is an unavoidable consequence of transplantation, postischemic renal dysfunction is dependent on the local conversion of tubule-derived C3 to its activated form,15 which growing evidence suggests is mediated through triggering of the LP,16,17 discussed in more detail below.

COMPLEMENT IN THE DEVELOPMENT OF ADAPTIVE IMMUNITY

The role of complement in regulating T cell alloimmunity was discovered when it was observed that wild-type mice do not acutely reject renal allografts from C3-deficient donors.18 Further support, implicating a role in regulating B cell alloimmunity, came from a study of C3-deficient mice that were unable to produce high-affinity IgG responses against MHC in skin grafts.5 During interactions between antigen presenting cells and T cells, the alternative pathway complement components C3, factor B, and factor D are released along with the terminal pathway complement component C5.19–21 Additionally, complement receptors C3aR and C5aR are upregulated on T cells in response to the expression of these complement components.22,23 Increased production of complement effector products and corresponding receptor expression, in parallel with reduced cell surface DAF, favors complement activation. With increased local production of C3a and C5a and engagement of their respective receptors, T cells and antigen presenting cells are stimulated (Figure 2).22 Via intracellular AKT signaling, the antiapoptotic Bcl2 protein is upregulated whereas the proapoptotic Fas molecule is downregulated, thereby enhancing T cell proliferation and diminishing T cell apoptosis.22,24 Therefore, complement could indirectly play a role in antibody production through regulation of T cell immunity,18 as well as direct stimulation of B cell priming by opsonized antigen.25,26

ROLE OF COMPLEMENT IN ABMR

ABMR is defined as the development of a donor-specific antibody in conjunction with histologic changes on graft biopsy and deterioration of graft function.27 As an effector of acute ABMR, the role of complement is well established, although this function has recently been extended in the context of chronic rejection. In 1969, Terasaki showed that patients with alloantibody capable of inducing complement-dependent cell lysis were more likely to suffer from hyperacute rejection.28 Donor-reactive anti-HLA antibodies have been described as mediators of acute and chronic transplant injury29 and it has been documented that complement depletion impairs antibody production.30 ABMR occurs via the classical complement pathway when complement component C1q binds to donor-specific anti-HLA antibody (DSA) at the site of antibody attachment to the endothelium (Figure 2). Loupy et al. have generated evidence that the capacity of serum DSA to interact with C1q could determine the cytotoxic potential of these antibodies and therefore be used to risk stratify and diagnose ABMR.4 They reported that the development of a C1q-binding DSA in the first year post-transplantation was an independent risk factor for allograft loss and was associated with a higher risk of ABMR. Furthermore, their results showed that patients who developed complement-binding DSAs had the lowest 5-year rate of graft survival as compared with patients with non-complement-binding DSAs and patients without DSAs.4 On the other hand, Sicard et al., evaluating the ability of DSAs to bind C1q and C3d, a terminal C3 cleavage product, found that C3d was associated with a higher risk of graft loss, whereas C1q did not achieve statistical significance.31 Further studies have
since demonstrated the relevance of C1q-binding DSAs beyond the first year post-transplantation as a potential marker for both adult and pediatric patients at high risk for late ABMR and subsequent graft loss. Lefaucheur et al. extended these initial observations of complement-binding DSAs and concluded that acute ABMR was mainly driven by the IgG3 subtype DSA whereas subclinical ABMR was driven by IgG4 DSA. Furthermore, these antibody subtypes along with C1q-binding DSA were again independently associated with allograft failure. What remains unknown is how durable these correlations are, because the capacity of antibodies to bind complement may change over time. Regardless, it appears that the ability to correlate complement-binding DSAs with distinct patterns of ABMR could be extremely helpful in identifying patients who would benefit from additional immune modulation of the complement system, such as treatment with eculizumab. Initial work on complement system modulation showed that in presensitized mice bearing heart allografts, therapeutic inhibition of complement with anti-C5, in addition to treatment with cyclosporine and cyclophosphamide, prevented acute rejection and prolonged allograft survival. This therapeutic approach was extended to humans with the study of eculizumab, the anti–human C5 mAb. Clinical studies have since demonstrated reduced ABMR. However, the risk of downstream complement inhibition increases susceptibility to sepsis and encapsulated organisms. In an attempt to target upstream complement components, C1 INH is currently being tested in a clinical trial as a treatment of acute ABMR and is showing some promise. It should be noted, however, that C1 INH is a broad protease inhibitor with actions that extend to the alternative and lectin pathways, as well as noncomplement proteases.
RENAL ISCHEMIA-REPERFUSION INJURY LEADS TO LOCAL, EXTRAVASCULAR COMPLEMENT ACTIVATION THROUGH A C4-INDEPENDENT PATHWAY WITH SUBSEQUENT FORMATION OF C5A AND C5B-9, SUGGESTING A CONTRIBUTION TO CONTINUED INFLAMMATION VIA THE ALTERNATIVE PATHWAY.15 ALTHOUGH A SUBSEQUENT STUDY IN COMPLEMENT FACTOR B–DEFICIENT MICE CONFIRMED A ROLE FOR THE ALTERNATIVE PATHWAY, THE COMPLEMENT RECOGNITION PATHWAY THAT TRIGGERED INJURY WAS NOT CONCLUSIVELY IDENTIFIED AT THIS STAGE.40 MORE RECENTLY, THE LP WAS IMPLICATED AS THE PRIMARY MODE OF RENAL COMPLEMENT ACTIVATION AND SUBSEQUENT TISSUE INJURY AFTER ISCHEMIC INJURY.17,41 COMPLEMENT ACTIVATION VIA THE LP BEGINS WHEN ONE OF A NUMBER OF PATTERN RECOGNITION RECEPTORS (PRRS) BINDS TO A PATHOGEN-ASSOCIATED MOLECULAR PATTERN (PAMP) THAT IS DISPLAYED ON THE SURFACE OF AN INVADING MICROORGANISM, OR WHEN A PRR BINDS TO A DAMAGE-ASSOCIATED MOLECULAR PATTERN (DAMP) DISPLAYED ON ENDGENOUS LIGANDS DURING INFLAMMATION OR CELL STRESS (FIGURE 3).42 THERE ARE A VARIETY OF LP PRRS IN THE COMPLEMENT SYSTEM, WHICH INCLUDE: COLLECTINS, WHICH ARE SOLUBLE COLLAGEN-LIKE LECTINS, SUCH AS MBL AND COLLECTIN-11 (CL-11); SURFACTANT PROTEINS (SP-A AND SP-D); AND FICOLINS (FICOLIN-1, FICOLIN-2, AND FICOLIN-3).44 A COMMON AND KEY FEATURE OF THESE PRRS IS THEIR INTERACTION WITH MBL-ASSOCIATED SERINE PROTEASES, OR MASPS, OF WHICH THERE ARE THREE, NAMED MASP-1, MASP-2, AND MASP-3.45,46 THE LP WAS INITIALLY THOUGHT TO BE ACTIVATED THROUGH THE ACTION OF MASP-1 AND MASP-2,44 WHEREAS MASP-3 HAS BEEN IMPlicated IN ACTIVATION OF THE ALTERNATIVE PATHWAY.47 THE OBSERVATION OF COMPLEMENT ACTIVATION WHEN THE COLLECTIN-MASP COMPLEX BONDS TO LIGANDS OF INVADING PATHOGENS OR ENDGENOUS MATERIAL DEMONSTRATED THAT LP ACTIVATION WAS CRITICALLY ASSOCIATED WITH DONOR ALLOANTIGEN RECOGNITION AND ANTIBODY-MEDIATED REJECTION.5,25,30 A COMMON AND KEY FEATURE OF THESE PRRS IS THEIR INTERACTION WITH MBL-ASSOCIATED SERINE PROTEASES, OR MASPS, OF WHICH THERE ARE THREE, NAMED MASP-1, MASP-2, AND MASP-3.45,46 THE LP WAS INITIALLY THOUGHT TO BE ACTIVATED THROUGH THE ACTION OF MASP-1 AND MASP-2,44 WHEREAS MASP-3 HAS BEEN IMPLICATED IN ACTIVATION OF THE ALTERNATIVE PATHWAY.47 THE OBSERVATION OF COMPLEMENT ACTIVATION WHEN THE COLLECTIN-MASP COMPLEX BONDS TO LIGANDS OF INVADING PATHOGENS OR ENDGENOUS MATERIAL DEMONSTRATED THAT LP ACTIVATION WAS CRITICALLY ASSOCIATED WITH DONOR ALLOANTIGEN RECOGNITION AND ANTIBODY-MEDIATED REJECTION.5,25,30 A COMMON AND KEY FEATURE OF THESE PRRS IS THEIR INTERACTION WITH MBL-ASSOCIATED SERINE PROTEASES, OR MASPS, OF WHICH THERE ARE THREE, NAMED MASP-1, MASP-2, AND MASP-3.45,46 THE LP WAS INITIALLY THOUGHT TO BE ACTIVATED THROUGH THE ACTION OF MASP-1 AND MASP-2,44 WHEREAS MASP-3 HAS BEEN IMPLICATED IN ACTIVATION OF THE ALTERNATIVE PATHWAY.47 THE OBSERVATION OF COMPLEMENT ACTIVATION WHEN THE COLLECTIN-MASP COMPLEX BONDS TO LIGANDS OF INVADING PATHOGENS OR ENDGENOUS MATERIAL DEMONSTRATED THAT LP ACTIVATION WAS CRITICALLY ASSOCIATED WITH DONOR ALLOANTIGEN RECOGNITION AND ANTIBODY-MEDIATED REJECTION.5,25,30
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COLLECTIN-11 AS A MEDIATOR
such as MBL. Models of renal and cardiac
injury in both native and transplanted
16,17,49 Traditionally, LP activation
C4 and C2 leading to formation
of C3 convertase (C4b2a). Recent
work, however, has shown the activity
of a C4-independent bypass lectin activation
pathway.16 In this study, MASP-2 was
found to mediate injury in the absence
of C4 in both cardiac and intestinal ischemia-
reperfusion injury. Further studies in a re-
nal isograft model comparing wild-type
and MASP-2–deficient mice showed that
renal function was preserved with MASP-2
deficiency and the effect of MASP-2 was
independent of C4, implicating the C4 by-
pass route of activation as an important
mediator of tissue injury.17 These observa-
tion help explain why C4-deficient mice
are afforded no protection in both native
renal ischemia15 and cell-mediated allograft
rejection models.50 Studies on human sera
from MASP-2–deficient patients support
the existence of a C4-bypass pathway in
man.16 Thus, there exists a novel mech-
anism of LP activation and a number of
candidate pattern recognition molecules
that could trigger this mechanism. We
will now consider the evidence for the
most relevant of these molecules in the
context of allograft injury, especially of
the kidney.

ROLE OF MBL IN TISSUE INJURY

Early efforts focused on well established
PRRs as potential initiators of the LP,
polymorphisms resulting in low serum
MBL levels were associated with poor graft
outcome.62 To add to the controversy,
however, in a study comparing donor and
recipient gene profiles for MBL-2 and
MASP-2 it was shown that there was no
association with graft outcome.63

ROLE OF FICOLINS IN TISSUE INJURY

Three ficolins have been described in hu-
mans, namely ficolin-1, ficolin-2, and
ficolin-3.42 There have been a handful
of studies that have examined their role
in human allograft rejection. One study
of >1200 donor and recipient pairs in-
vestigating lectin gene profiles, specifi-
cally ficolin-2 gene haplotypes, showed
no association with allograft survival.63
However, another study of common
functional polymorphisms in the fico-
lin-2 gene revealed an association be-
tween this polymorphism and lower
incidence of renal transplant rejec-
tion.64 Furthermore, high pretransplant
levels of ficolin-3, the most abundant
ficolin in serum, showed strong associa-
tion with poor allograft survival after
renal transplantation.65,66

COLLECTIN-11 AS A MEDIATOR
OF RENAL EPITHELIAL
INFLAMMATION

Collectin-11 was first identified over a
decade ago as a component of the in-
native immune system.67 However, it is
only recently that the wider biologic
The significance of this molecule has begun to emerge. The biologically active structure of CL-11 is a trimer, approximately 300 kD. It is composed of monomers with a globular head containing a carbohydrate recognition domain and a collagenous tail that contains MASP binding motifs. Synthesis occurs in many tissues, including the liver, brain, heart, and the kidney. Previous work with CL-11 demonstrated an important role in host defense against bacteria and other pathogens via its carbohydrate recognition domain, which shows a preference for L-fucose in humans.

In patients with disseminated intravascular coagulation, serum levels of CL-11 were typically double those of healthy control subjects. More recently, CL-11 was shown to have strong binding to nucleic acids, thus indicating a potential role in clearance of apoptotic cells after inflammation.

Renal tubule epithelial cells appear to be the main source of CL-11 in normal renal tissue, with increased expression after renal ischemia–reperfusion injury. Additionally, CL-11 was found to colocalize with carbohydrate ligand L-fucose on inflamed proximal tubule cells. A critical observation was that complement component C5d, a marker for complement activation, was present on the basolateral surface of the renal tubule cells in the hypoxia–sensitive zone only where CL-11 and L-fucose engage. Further support for the role of CL-11 in complement-mediated renal injury was the observation that CL-11–deficient mice were resistant to postischemic renal failure and associated complement-mediated damage, indicating the role of local complement activation in the extravascular compartment. The mechanism for this is believed to be through abnormal expression of a fucosylated ligand at the hypoxia-stressed cell surface, thereby allowing CL-11 to bind and subsequently initiate MASP-2–mediated complement activation. Understanding the nature of the fucosylated ligand(s) is critical to the design and application of relevant therapies to block the interaction of these molecules in the setting of renal inflammation.

**CONCLUSION**

As laboratory investigation into complement-binding DSAs as well as the triggering of CL-11–mediated complement activation continues, work is taking place in the clinical realm to further understand and modulate the role of complement-mediated injury in renal transplantation. Clinical trials will aid in determining if complement inhibition is a viable therapeutic option. There is no doubt that therapies such as eculizumab (anti-C5) have shown great promise for certain complement-mediated pathologies such as paroxysmal nocturnal hemoglobinuria and atypical hemolytic uremic syndrome. Indeed, clinical trials continue on the potential use of C1 INH and eculizumab in treating and preventing ABMR of solid organ transplants. Recent bench work has identified various complement components at play in both the vascular and extravascular compartments. It is likely that these components cause allograft injury via separate, although not mutually exclusive complement recognition pathways, which may have a subsequent effect on how future therapies are delivered in the clinic. Examples include specific DSA subtypes as well as the LP as potential earlier targets for intervention rather than the downstream complement components, C3 or C5, to reduce the risk of ABMR or the pathogenic response to ischemia–reperfusion, respectively. Local production of CL-11 at the site of tissue injury may be the distinguishing feature that sets it apart in functional terms from other pattern recognition molecules of the lectin complement pathway. Further understanding of complement-binding DSAs and the initiation of the LP and associated tissue ligands will allow for a clearer picture of the molecular and cellular mechanisms at play and hopefully lead to more precise therapeutic interventions.

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**DISCLOSURES**

None.

**REFERENCES**


