Molecules Great and Small: The Complement System

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Abstract
The complement cascade, traditionally considered an effector arm of innate immunity required for host defense against pathogens, is now recognized as a crucial pathogenic mediator of various kidney diseases. Complement components produced by the liver and circulating in the plasma undergo activation through the classical and/or mannose-binding lectin pathways to mediate anti-HLA antibody-initiated kidney transplant rejection and autoantibody-initiated GN, the latter including membranous glomerulopathy, antiglomerular basement membrane disease, and lupus nephritis. Inherited and/or acquired abnormalities of complement regulators, which requisite limit restraint on alternative pathway complement activation, contribute to the pathogenesis of the C3 nephropathies and atypical hemolytic uremic syndrome. Increasing evidence links complement produced by endothelial cells and/or tubular cells to the pathogenesis of kidney ischemia-reperfusion injury and progressive kidney fibrosis. Data emerging since the mid-2000s additionally show that immune cells, including T cells and antigen-presenting cells, produce alternative pathway complement components during cognate interactions. The subsequent local complement activation yields production of the anaphylatoxins C3a and C5a, which bind to their respective receptors (C3aR and C5aR) on both partners to augment effector T-cell proliferation and survival, while simultaneously inhibiting regulatory T-cell induction and function. This immune cell–derived complement enhances pathogenic alloreactive T-cell immunity that results in transplant rejection and likely contributes to the pathogenesis of other T cell–mediated kidney diseases. C5a/C5aR ligations on neutrophils have additionally been shown to contribute to vascular inflammation in models of ANCA-mediated renal vasculitis. New translational immunology efforts along with the development of pharmacologic agents that block human complement components and receptors now permit testing of the intriguing concept that targeting complement in patients with an assortment of kidney diseases has the potential to abrogate disease progression and improve patient health.

Introduction
The complement system, traditionally considered a component of innate immunity required for protection from invading pathogens, has been implicated in the pathogenesis of autoimmune kidney disease since the 1960s (1). Fifty years later, the detailed complexities of complement’s role in kidney injury are still being unraveled. Building on early work indicating that macrophages and tubular cells produce complement (2,3), studies performed since the 2000s have altered former paradigms by showing that tissue-derived complement and immune cell–derived complement can each mediate local inflammation and that complement acts as a bridge between innate and adaptive immunity in an array of kidney diseases. Herein, we will review the physiology of the complement system, provide a framework for understanding complement’s varied roles in kidney disease pathogenesis, and highlight potential therapeutic targets.

Biology of the Complement System
The complement system is comprised of >30 soluble and surface-expressed proteins, many of which are zymogens (inactive precursors that require cleavage to become active enzymes). In the latest nomenclature, the smaller cleavage fragments are designated as a (e.g., C3a), and the larger cleavage fragments are denoted as b (e.g., C3b). After they are activated, individual enzymes have the ability to repeatedly cleave their substrates, yielding a self-amplifying cascade. The various components can be considered as principally involved in (1) initiating complement activation, (2) amplifying complement activation, (3) performing effector functions, and/or (4) regulating the cascade (Figure 1).

Activation
Complement activation can be initiated through three pathways (Figure 1) (reviewed in ref. 4). The classical pathway is activated when the hexameric C1q, as part of a C1qrs complex containing two C1r molecules and two C1s molecules, binds to the Fc regions of IgG or IgM. Complement activation through the classical pathway is optimally activated by a hexameric organization of antigen-bound antibodies, a configuration that increases the avidity between C1q and the Fc regions by 20-fold (5). After an induced conformational change, the C1s component cleaves C4 to C4a+C4b and then cleaves C2 to C2a+C2b. C4b can bind to cell surfaces by a thio-ester bond, after which C2b is recruited to form the C4b2b classical pathway C3 convertase capable of cleaving C3 into C3a (an anaphylatoxin) plus C3b.
In the lectin pathway, hexamers of mannose-binding lectins (MBLs) bind to bacterial carbohydrate motifs (including mannose). MBL-associated serine proteases (MASPs) function similarly to C1r and C1s to cleave C4 and then C2, generating the C4bC2b C3 convertase.

In the alternative pathway, complement activation occurs spontaneously and continuously at a low rate (referred to as tickover). The mechanism involves C3 associating with a water molecule to form C3b(H2O), which recruits factor B (fB) and factor D (fD). fD enzymatically cleaves fB, yielding Bb, the active serine esterase that cleaves C3 to C3a+C5b. C5b recruits C6, C7, C8, and 10–16 C9 molecules to generate the terminal membrane attack complex (MAC), which inserts pores into cell membranes to induce lysis. C3a and C5a are potent signaling molecules, which through their G protein–coupled receptors C3aR and C5aR, respectively, can promote inflammation, chemotaxis and chemokine release. They also mediate neutrophil and macrophage chemotaxis, maintain macrophages to promote intracellular killing of engulfed organisms, and contribute to T-cell and antigen-presenting cell (APC) activation, expansion, and survival (see below) (10–13).

C3b and other bound cleavage products bind to various surface-expressed receptors, including complement receptor 1 (CR1), CR2, CR3, and CR4, functioning as opsonins.

### Amplification

The C3 convertases repeatedly cleave C3 molecules, yielding multiple C3b products, each of which can interact with fB to form more C3 convertases. As consequences, C3 cleavage is the central amplification step of the cascade, and regardless of the initial activation pathway, amplification at the C3 convertase step occurs through the alternative pathway. Regulation of the C3 convertase amplification step is crucial to restrain complement activation so as to prevent pathologic consequences (see below).

### Effector Functions

C4b2b and C3bBb form multimeric complexes with additional C3b molecules, yielding the C5 convertases C4b2bC3b and C3bBbC3b. These enzymes cleave C5 to C5a (an anaphylatoxin) plus C5b, the latter of which binds to C6 and subsequently facilitates binding of C7 and C8 plus 10–16 C9 molecules to form the C5b–9 membrane attack complex (MAC) (Figure 1). The MAC forms a pore in cell membranes, which promotes lysis of non-nucleated cells (e.g., bacteria and human red blood cells [RBCs]). Insertion of MACs into nucleated host cells generally does not result in lysis but can induce cellular activation (8) and/or promote tissue injury (9).

Various complement cleavage products have other effector functions (Figure 2, Table 1). C3a and C5a ligate their seven transmembrane-spanning G protein–coupled receptors C3aR and C5aR, respectively, transmitting proinflammatory signals that induce vasodilation and cytokine and chemokine release. They also mediate neutrophil and macrophage chemotaxis, maintain macrophages to promote intracellular killing of engulfed organisms, and contribute to T-cell and antigen-presenting cell (APC) activation, expansion, and survival (see below) (10–13).

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### Regulation

Complement activation must be physiologically restrained to limit damage to self-cells (4). Complement regulation occurs at multiple steps through distinct mechanisms (Figure 3). Regulation of C3 convertase activity is accomplished by multiple molecules with overlapping but discrete functions. Decay accelerating factor (DAF; CD55) is a glycosphatidylinositol (GPI)-anchored, membrane-bound regulator that accelerates the decay of cell surface–assembled classical and alternative pathway C3 and C5 convertases (facilitating disassociation of Bb from C3bBb and C2b from C4b, while also competitively inhibiting their reformation) (14), thereby preventing amplification, downstream cleavage events, and formation of the MAC (15). This decay accelerating activity functions intrinsically (i.e., restraining complement activation only on the cell surface on which DAF is expressed).

Membrane Cofactor Protein (MCP; CD46) and its murine homolog Crry are surfaced-expressed regulators with cofactor activity (16) functioning as cofactors for serum factor I (fI), which cleaves C3b to iC3b, thereby irreversibly preventing reassembly of the C3 convertase. Crry also exhibits decay accelerating activity (17). The cleavage product iC3b (an opsonin) can be further broken down to C3c and C3dg.
(through fl- and cofactor-dependent cleavage processes) (reviewed in ref. 18), the latter of which interacts with CR2 on B cells to facilitate B-cell activation (19).

Factor H (fH) is a plasma protein that also regulates complement activation at the C3 convertase step (reviewed in ref. 20). The carboxy terminus of this protein binds surface-deposited C3b and surface-expressed polyanionic glycosaminoglycans, including sialic acid residues. After they are bound, the N-terminal domains of fH exhibit decay accelerating and cofactor activities (Figure 3). fH restrains complement activation on host surfaces that do not express other complement regulators, including exposed basement membranes in the glomerulus (which express glycosaminoglycans), explaining, in part, the association between mutations in fH or fl and various C3 nephropathies (see below).

Additional complement regulators (Figure 3) include the GPI-anchored and surfaced-expressed protein protectin (CD59), which blocks formation of the MAC, the surface-expressed CR1, which exhibits decay accelerating activity and cofactor activity for fl, and C1 inhibitor, a serine protease that irreversibly binds to and inactivates C1r, C1s, MASP-1, and MASP-2, thereby limiting classical and MBL pathway activation. Ubiquitously expressed carboxypeptidases rapidly inactivate the anaphylatoxins C3a and C5a (reviewed in ref. 4).

**Sources of Complement**

Liver-derived plasma complement is essential for protection from pathogens and contributes to antibody-initiated, complement-mediated autoimmune injury. Complement components can be produced by tissue-resident (e.g., tubular cells in the kidney [21]) and migratory/immune cells, including T cells and APCs (22–24). A thorough understanding of complement-mediated kidney disease requires consideration of the source of complement production, the site of complement activation, the specific complement components involved, and the role of alternative pathway regulatory mechanisms.
binding to B cell involves antigen-bound C3dg (an iC3b cleavage product) and impairs antibody production (25). The mechanism involves complement regulators in each situation. The effector components and receptors involved, and the function of complement regulators in each situation.

### Links between Complement and Adaptive Immunity

It has been known for decades that complement depletion impairs antibody production (25). The mechanism involves antigen-bound C3dg (an iC3b cleavage product) binding to B cell-expressed CR2 (CD21), which facilitates antigen presentation to B cells and lowers the threshold for B-cell activation (26) (Figure 2).

Work published since the early 2000s uncovered an unexpected role for complement as a regulator of T-cell immunity. During cognate interactions between T cells and APCs, both partners upregulate and secrete alternative pathway complement components C3, fB, and fD, produce C5, and upregulate surface expression of C3aR and C5aR (23,24) (Figure 2). These changes are a consequence of costimulatory molecule signaling by CD28/CD80/CD86 and CD154 (Figure 2). These changes are a consequence of costimulatory molecule signaling by CD28/CD80/CD86 and CD154 that simultaneously and transiently reduces C3aR/C5aR signal transduction in nTreg cells augments Treg function (11). Genetic and pharmacologic blockade of C3aR/C5aR and that signaling through these receptors inhibits Treg induction, function, and stability (12,30) (Figure 2). Our group showed that peripheral, murine-induced regulatory T cell (iTreg) generation, tolerance induction and maintenance in rodents and associated with improved long-term transplant outcomes in humans (29). Data published in 2013 indicate that complement also regulates Treg induction, function, and stability (12,30) (Figure 2). Our group showed that peripheral, murine-induced regulatory T cells (tTregs) express C3aR and C5aR and that signaling through these receptors inhibits Treg function (11). Genetic and pharmacologic blockade of C3aR/C5aR signal transduction in nTreg cells augments their in vitro and in vivo suppressive activity. Mechanisms involve C3a/C5a-induced phosphorylation of AKT and, as a consequence, phosphorylation of the transcription factor Foxo1, which results in lowered nTreg Foxp3 expression. Two additional sets of data showed that genetic deficiency or pharmacologic blockade of C3aR/C5aR signaling augments murine-induced regulatory T cell (iTreg) generation, stabilizes Foxp3 expression, and resists iTreg conversion to IFN-γ/TNF-α–producing effector T cells (12,30). Pharmacologic antagonists to human C3aR and C5aR also augment in vitro generation and stability of human iTreg from naïve precursors (12,30). These new results build on previously published evidence that coengagement of the T-cell receptor

<table>
<thead>
<tr>
<th>Complement Receptor</th>
<th>Alternative Names</th>
<th>Ligand</th>
<th>Effector Functions</th>
<th>Cell Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>CR1</td>
<td>CD35, immune adherence receptor</td>
<td>C3b, iC3b, C4b, C1q</td>
<td>Clearance of immune complexes, enhancement of phagocytosis, and regulation of C3 breakdown</td>
<td>Many nucleated cells and RBCs, B cells, leukocytes, monocytes, and follicular dendritic cells</td>
</tr>
<tr>
<td>CR2</td>
<td>CD21, Epstein–Barr virus receptor</td>
<td>C3dg, C3d, iC3b</td>
<td>Regulation of B-cell function, B-cell coreceptor, and retention of C3d-tagged immune complexes</td>
<td>B and T cells and follicular dendritic cells</td>
</tr>
<tr>
<td>CR3</td>
<td>MAC1, CD11b-CD18, αMβ2 integrin</td>
<td>iC3b, factor H</td>
<td>iC3b enhances contact of opsonized targets, resulting in phagocytosis</td>
<td>Monocytes, macrophages, neutrophils, NK cells, eosinophils, myeloid cells, follicular dendritic cells, and CD4+ and CD8+ T cells</td>
</tr>
<tr>
<td>CR4</td>
<td>CD11c-CD18, αXβ2 integrin</td>
<td>iC3b</td>
<td>iC3b-mediated phagocytosis</td>
<td>Monocytes and macrophages</td>
</tr>
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Modified from reference 130, with permission. CR1, complement receptor 1; RBC, red blood cell; MAC1, membrane attack complex 1.
and the complement regulator CD46 promotes regulatory IL-10 production (31) to delineate a crucial role for complement in modulating the balance between pathogenic and protective adaptive T-cell responses.

**Complement and Kidney Disease**

**Antibody-Initiated Activation of Serum Complement**

Autoantibodies reactive to kidney-expressed self-antigens and/or antibody/antigen complexes deposited in the kidney are considered causative of various human kidney diseases. Increasingly available evidence links the pathogenesis of many of these antibody-initiated kidney pathologies to complement-derived effector mechanisms, in which plasma complement is activated through the classical and mannose-binding lectin (MBL) pathways (Figure 4).

**Membranous Nephropathy.** Membranous nephropathy (MN), a common cause of nephrotic syndrome in adults, is characterized by a fine granular deposit of IgG with C3 in the peripheral capillary loops (32,33). IgG4 reactive to the
M-type phospholipase A2 receptor, a transmembrane glycoprotein expressed on the glomerular podocyte, is present in 70%–98% of patients with MN (34,35). Although IgG4 does not efficiently activate complement through the classical pathway, deposition of C4d, a breakdown product of C4b, is detectable in essentially 100% of patients with primary MN (36,37). Together with the observations that MBL and hypogalactosylated IgG (including IgG4) can be detected in subepithelial deposits in primary MN and that hypogalactosylated IgG can bind to MBL and activate complement through the MBL pathway, the data suggest that MBL-initiated complement activation is pathogenic in MN. MAC is detectable in the urine of patients with MN and considered a dynamic marker of ongoing injury, supporting an integral role for complement in MN (reviewed in ref. 38).

Mechanistic studies in animal models, including Heymann Nephritis, indicate that antipodocyte antibodies lead to insertion of MAC into podocytes and that blocking MAC formation prevents phenotypic expression of disease (39). The resultant sublytic podocyte activation alters cytoskeletal structure crucial for foot process and slit diaphragm integrity and function, leading to proteinuria (40,41). Associated promotion of extracellular matrix results in the characteristic thickened glomerular basement membranes (GBMs) observed in this disease (42).

Although one report in abstract form (G. Appel et al., unpublished data) suggested that anti-C5 mAb had no effect on proteinuria in patients with MN, additional studies are needed to determine if targeting complement is an effective therapy for this disease.

**Anti-GBM Disease.** Autoantibodies targeting the NC1 domain of type IV collagen are pathogenic mediators of anti-GBM disease (43). The proliferative GN observed in anti-GBM disease is characterized by linear deposition of IgG and various complement components along the GBM (44). The classical and alternative pathways are implicated, because MBL, C1q, fB, properdin, C3d/C4d, and C5b-9 have been detected in GBM. An observed correlation between intensity of fB deposition and glomerular crescent formation supports a pathogenic link (45). Evidence indicates that local complement activation results in C3a- and C5a-mediated inflammation as well as MAC-dependent sublytic activation of cells within the glomerulus, which together promote nephropathy and extracellular matrix formation (46). Together, these mechanistic findings support the need to test whether complement inhibition positively affects outcomes in patients with anti-GBM disease.

**Immune Complex-Initiated Glomerular Diseases.** Circulating immune complexes that are deposited in the subepithelial or subendothelial compartments of the glomerulus can also mediate complement-dependent glomerular injury, including GN associated with streptococcal infection, cryoglobulinemia, and lupus. These disease processes are commonly characterized by neutrophils and C3 deposits in glomeruli and systemic C3 depletion, suggestive of a
pathogenic role of complement-mediated inflammation and immune cell recruitment (47).

Animal model data supporting complement activation as pathogenic in lupus nephritis include the observation that fH-deficient MRL/lpr mice died with severe, diffuse GN (48). Conversely, administration of a CR2-Crry fusion protein that targets complement regulation to C3b deposits (CR2 binds C3b) prevented expression of disease (49). In MRL/lpr mice, C5aR blockade decreased glomerular inflammation (50). Anti-C5 mAb ameliorated GN in the murine NZB/W(F1) lupus model (51), indicating a role for terminal complement. A phase 1 human trial with eculizumab (anti-C5) suggested preliminary efficacy, but the treatment period was too brief to draw definitive conclusions (52). Despite these observations, complement is not the sole pathogenic mediator of lupus nephritis, because FcR deficiency but not C3 deficiency prevented phenotypic expression of disease in one model (53).

The complexities of complement’s effects on lupus are illustrated by the seemingly paradoxical observation that genetic absence of C4 or C1q in mice (54) and humans (55) increases the risk of developing lupus nephritis. The mechanism is likely related to an absence of complement-derived opsonins, preventing clearance of immune complexes that deposit in the glomerulus and promote FcR-dependent inflammation.

ANCA-Induced Vasculitis. ANCA contribute to small vessel vasculitis, which is characterized by paucity of Ig deposits (56) but with complement component deposition (Bb, C3d, C5c, and C5b-9) at sites of acute vascular and glomerular inflammation (57). Cytokine-primed neutrophils display ANCA-binding antigens (myeloperoxidase [MPO] and proteinase 3) on their surfaces and participate in vascular injury. Complement depletion protected anti-MPO-treated mice from developing necrotizing crescentic GN (58). FB deficiency was protective, but C4 deficiency was not protective, implicating the alternative pathway. Whereas C5 deficiency or blocking anti-C5 mAb was protective (59), C6 deficiency was not protective (60), indicating that C5 cleavage but not MAC formation is pathogenic. Additional animal studies showed that ANCA stimulate neutrophils to produce and release C5, and blockade or deficiency of C5aR (60) prevented disease expression. Together, the data suggest that pathology is mediated by ANCA-induced, neutrophil-derived complement release and leads to C5a/C5aR-induced proinflammatory signaling, particularly in neutrophils and neutrophil-associated vasculature (61), rather than by MAC formation. A small molecule C5aR inhibitor limited expression of a murine model of anti–MPO-induced kidney disease (60), and C5aR antagonism is being tested as a therapy for patients with ANCA vasculitis (European Union Clinical Trials Register ID EUCTR2011–001222015–GB).

Other Glomerular Diseases. IgA nephropathy, characterized by recurrent bouts of GN, focal mesangial cell expansion, and IgA deposition, is likely mediated by MBL-dependent and/or alternative pathway-dependent, antibody-initiated complement activation (62). Deposits of C3 and C5b-9 are detectable in the diseased glomeruli and correlate with disease severity and prognosis. Experimental evidence suggests that sublytic MAC activates mesangial cells, yielding mesangial proliferation and matrix expansion (62).

The pathogenesis of FSGS remains unclear, but IgM and C3 deposits are commonly observed in the affected glomeruli (63). Mutations in fH and C3 have been described in cases of FSGS (64), and a murine model of IgG-initiated FSGS in DAF-deficient mice (65) supports a role for complement dysregulation in some cases. Complement inhibition has not been carefully studied as a therapy for FSGS.

Postinfection GN, classically after *Streptococcus pyogenes* infection, is characterized by proliferative GN and deposition of C3 with or without IgG (reviewed in ref. 66). Although the majority of patients achieve complete remission of the associated nephritic syndrome, some experience delayed resolution or chronic GN, resulting in ESRD. A recent study published on 11 patients at the Mayo Clinic found multiple underlying causes of alternative pathway dysregulation in these chronic patients, including mutations in fH or CFHR5 and/or the presence of C3 nephritic factors (67).

Monoclonal gammopathy has also been associated with the activation of the alternative pathway and subsequent induction of membranoproliferative GN. Circulating A-lightchain dimers were found to bind to fH and inhibit its control function, thus lifting restraint over alternative pathway regulation, but they differed from C3 nephritic factor in that the dimers were unable to bind and stabilize the alternative pathway C3 convertase (68).

**Antibody-Initiated Injury to a Kidney Transplant.** Donor-reactive anti-HLA antibodies are considered pathogenic mediators of acute and chronic transplant injury (69). They can bind to donor tissue and mediate damage through multiple mechanisms, including complement activation (70,71).

A mechanistic link between antibody-mediated injury and terminal complement activation was documented through experiments performed in rodents: whereas heart allografts transplanted into sensitized (with preexisting antidonor antibodies) wild-type rats were rejected in 6–7 days, graft survival was prolonged to >30 days in sensitized C6γ−/− recipients (72). Documentation of impaired MAC complex (C5b-9) formation in the C6γ−/− recipients (73) supports the conclusion that terminal complement is a key effector mechanism. In work by others, anti-C5 mAb (inhibits C5 cleavage) plus cyclosporin and short-term cyclophosphamide resulted in prolonged heart allograft survival in presensitized mice, despite persistent antidonor IgG in the sera and the graft (74).

In 2013, studies in a humanized mouse model (8) showed that antidonor HLA antibodies bind to human aortic endothelium to initiate complement activation, resulting in MAC insertion into aortic endothelial cells. This induced endothelial cell activation, characterized by noncanonical NF-κB activation and upregulated production of chemokines, cytokines, and adhesion molecule expression, which in turn, facilitated T cell–mediated injury to the aortic allograft (8). The findings support the conclusion that complement bridges pathogenic humoral and cellular alloimmunity to mediate tissue damage.

Clinical studies have begun to test the efficacy of complement-targeted strategies to treat human antibody-mediated transplant rejection. Anti-human C5 mAb plus plasma exchange reduced the incidence of antibody-mediated rejection in 26 sensitized recipients of kidney transplants (75) and successfully reversed established antibody-mediated rejection in a small cohort (76).
Complement-Based Diagnostics Relevant to Transplantation. The recognition that complement participates in antibody-initiated allograft rejection suggested that identifying serum anti-HLA antibodies capable of binding C1q would enhance their prognostic use after kidney transplantation (77). A 2013 paper, indeed, suggests that, among patients with serum anti-HLA antibodies, those binding to C1q+ had the worst kidney graft survival (78). In another example of complement-based diagnostics, C4d staining of kidney transplant tissue is currently considered one key criterion for diagnosing antibody-mediated allograft rejection (79).

Kidney Injury Mediated by Serum Complement in the Absence of Antibody

Emerging evidence suggests that unrestrained C3 convertase activity underlies the pathogenesis of several diseases involving the kidney, including paroxysmal nocturnal hemoglobinuria (PNH), forms of C3 nephropathy, and atypical hemolytic uremic syndrome (aHUS) (Figure 5).

PNH. PNH is a hematologic disorder characterized by bone marrow failure, thrombocytopenia, complement-mediated intravascular hemolysis, and hemoglobinuria. It is caused by a clonal expansion of RBC precursors that contain a mutation in the X-linked phosphatidylinositol glycan anchor biosynthesis, class A gene (PIGA) that encodes for a protein involved in GPI anchor synthesis (80). DAF and CD59 are GPI-anchored molecules that require posttranslational addition of GPI anchors to guide them to cell surfaces. The PIGA mutation prevents DAF and CD59 surface expression on the affected RBCs. The inability to regulate alternative complement amplification/amplification at the C3 convertase step (absent DAF) and prevent MAC formation (absent CD59) causes spontaneous lysis of the mutant RBCs. Therapeutically inhibiting MAC formation with an anti-C5 antibody that prevents C5 cleavage reduces morbidity and increases quality of life for patients with PNH (81). Although effective in preventing lysis, the anti-C5 mAb does not affect the unregulated upstream production and deposition of C3b on RBC surfaces resulting from the absence of DAF. Evidence indicates that this C3b deposition functions as an opsonin, causing macrophage-dependent RBC destruction in the liver/spleen, despite anti-C5 therapy (82). Alternative treatment strategies, including those targeting C3 activation (83), require additional study.

C3 Nephropathies. C3 nephropathies are nephritic kidney diseases characterized by low serum C3 and glomerular C3 deposits without IgG. Subsets of C3 nephropathies are associated with serum C3 nephritic factors (Figure 5): acquired autoantibodies that impair complement regulation by binding directly to the C3bBb C3 convertase or its components, enhancing properdin-mediated stabilization of the complex, and/or inhibiting fH-mediated C3b degradation (84).

C3 nephropathies can also occur in association with genetic mutations in complement components and/or regulators that result in impaired complement regulation (Figure 5) (84). Loss-of-function mutations in fH, gain-of-function mutations in C3 (conferring resistance to fH-mediated cleavage), and gain-of-function mutations in complement fH regulatory proteins (which compete with fH for binding to C3b and impair the function of fH) have been described (84).

Regardless of the specific molecular basis for the complement dysregulation, the resultant complement activation likely predominantly causes glomerular disease, because the glomerulus contains fenestrated endothelium with exposed GBM that requires functional fH and fI to prevent local complement activation. Supporting this concept, fH-deficient mice develop membranoproliferative GN with low serum C3 levels (85), and recombinant fH restores plasma C3 levels with resolution of C3 deposition in the GBM (86). Mice deficient in both fH and fB do not develop disease, confirming a pathogenic role for alternative pathway activation (85). fH<sup>-/-</sup>/C5<sup>-/-</sup> mice and fH<sup>-/-</sup> mice treated with anti-C5 mAb developed less severe disease, whereas C6-deficient mice were not protected, inferring a role for C5a/C5aR in immune cell recruitment (87).

Effective therapy for C3 nephropathies remains enigmatic, but limited studies in animal models and patients have suggested that restoration of alternative pathway regulation may prove effective. Infusions of fresh frozen plasma containing fH may benefit patients deficient in fH (88), and therapy targeting C5 showed some success in patients with forms of C3 nephropathies (89,90).

aHUS. The current concept is that aHUS, characterized by hemolysis and renal failure associated with kidney endothelial cell injury, typically in the absence of detectable complement deposition in the glomerulus (91), is also a result of complement dysregulation. Inherited loss-of-function mutations in fI, MCP, and fH as well as acquired, blocking, anti-fH antibodies have been associated with cases of aHUS (92). Gain-of-function mutations in C3 and/or fB, which promote accumulation of the C3bBb convertase and overwhelm/resist complement regulation, have been described (93).

Additional insights derived from work using mice with a mutated fH were that they lack the ability to bind to cell surfaces but maintain its complement-regulatory capacity (Figure 5). Although complement activation in the plasma was controlled, the animals developed C5-dependent features of thrombotic microangiopathy (94,95), indicating that fH must regulate complement activation while bound to surfaces in the kidney to prevent disease.

Most humans with inherited mutations associated with aHUS or C3 nephropathy are heterozygotes. Current concepts are that common allelic variants in complement regulators present in the general population confer a complotype that predisposes to disease (96) and that additional immune insults unmask complement regulatory deficiencies. As one illustration supporting this concept, poststreptococcal GN, generally a self-limited disease, resulted in progressive C3 nephropathy in a patient with a complement regulator mutation (97).

Control of complement activation using eculizumab (anti-C5) has revolutionized treatment of aHUS. Approximately 85% of treated patients have achieved remission (98). Those refractory to eculizumab may have disease driven by C3 cleavage products (which would not be affected by anti-C5 mAb), raising the possibility that targeting C3 and/or C3a/C5aR may ultimately prove to be more effective.

Kidney-Derived Complement and Disease

Ischemia-Reperfusion Injury. Ischemia-reperfusion (IR) injury results from tissue hypoxia, mitochondrial damage,
and ATP depletion followed by the generation of free oxygen radicals on reperfusion, which initially damage endothelium (99). Ensuing inflammation is driven by Toll-like receptor signaling, and cytokines, chemokines, and complement amplify the inflammation, resulting in tubular injury and kidney dysfunction (Figure 6).

To summarize findings reviewed elsewhere (100), complement deposition and loss of membrane-bound complement regulators occur during murine kidney IR injury, and overexpression of Crry (murine homolog of MCP) ameliorated IR injury. IR injury was dampened in complement-depleted mice and C3-deficient, fB-deficient, or C5-deficient mice. Conversely, DAF-, Crry-, or fH-deficient mice are more susceptible to IR injury. Reciprocal transplant studies showed that donor kidney–derived C3 and not systemic recipient C3 is the predominant mediator of IR injury (101). Using C3aR-, C5aR-, or C3aR/C5aR-deficient mice (102), investigators observed that deficiency of either or
both of these receptors protected mice from kidney IR injury and that their expression on either renal tubular epithelial cells or circulating leukocytes contributes to the pathogenesis. Together, the data indicate that IR injury upregulates production of complement components by kidney endothelial and tubular cells and infiltrating immune cells. Local activation through the alternative pathway yields C3a/C5a, which amplifies local inflammation through autocrine/paracrine ligations with their kidney cell–expressed receptors (102). Confirmatory evidence in humans includes detection of soluble C5b-9 after reperfusion of deceased donor but not living donor kidneys (103) and higher expression of complement genes in deceased versus living donor kidneys on reperfusion (104).

An analog of the human complement-regulatory protein CD35 (CR1; blocks C3 convertase) was conjugated to a myristoylated peptidyl tail, such that, when administered by intravenous perfusion of the harvested organ ex vivo, it will self-insert into the lipid bilayer of the endothelial cell membranes. This reagent was effective in preventing post-transplant kidney IR injury in rats (105). The human reagent, mirococept (APT070), is being studied in a clinical trial for prevention of DGF (100). Eculizumab is also being tested for efficacy in preventing post-transplant DGF (NCT01403389 and NCT01919346).

**Chronic Kidney Injury and Fibrosis.**
Mechanisms of kidney fibrosis, including late kidney transplant failure, involve immune and nonimmune mechanisms (106). The functional and structural changes of chronic renal allograft failure share similarities with those observed in other forms of chronic progressive kidney disease, in which decline of functioning nephron mass has been considered the key event (107). Emerging evidence suggests that intragraft complement activation contributes to this progressive kidney injury (108). C3 is implicated in the activation of the renin-angiotensin system and the epithelial-to-mesenchymal transition (109,110). Together with observations that absence/blockade of C5/C5aR (but not blocking MAC formation) limited kidney fibrosis in several animal models (111,112), the data suggest that kidney-derived complement participates in fibrosis of native and transplanted kidneys (Figure 7).

Associative evidence linking complement to progressive human kidney transplant injury derives from studies of complement gene polymorphisms and transplant outcomes. Specific C5 polymorphisms in both the donor and recipient have been associated with worse late graft function but not risk of acute rejection (113). Although controversial, donor kidney expression of a specific polymorphic variant of C3 is associated with worse post-transplant outcomes (114,115). Additionally, proteomic studies of kidney allograft tissue revealed strong associations between chronic injury and alternative pathway but not classical pathway complement components (116). An ongoing study of chronic anti-C5 mAb therapy in kidney transplant recipients (NCT01327573) could potentially provide additional insight into the role of complement as a mediator of progressive graft dysfunction and interstitial fibrosis and tubular atrophy.
Complement and T Cell–Mediated Transplant Rejection

Extending from the fundamental discoveries that absence of donor C3 prevents murine kidney transplant rejection (117) and that immune cell–derived complement augments effector T-cell differentiation and survival (23,24), studies performed in transplant models revealed that wild-type mice reject DAF-deficient heart allografts with accelerated kinetics (118). The accelerated rejection is caused by a complement-dependent augmentation of antidonor T-cell immunity. Donor or recipient DAF deficiency accelerated skin graft rejection (23), bypassed the requirement for CD4 help in murine heart transplant rejection (119), and overcame immune privilege of the eye to cause rapid corneal transplant rejection (120). Local complement production and C5a/C5aR interactions also influence effector CD8+ T-cell responses to allogeneic vascular endothelial cells (121) in in vitro culture systems and in vivo in response to a heart transplant (8,121) as well as T cell–dependent kidney transplant rejection in rodents (122). Together with the findings that anti-C5 mAb synergizes with CTLA4-Ig to prevent T-cell priming, limits T-cell trafficking to an allograft, and prolongs transplant survival in mice (123), the work supports the conclusion that complement is a physiologic regulator of pathogenic T-cell immunity that causes allograft rejection (as well as various autoimmune diseases) in animal models.

Confirmatory human experiments published in 2013 show that C3a and C5a are generated during in vitro cultures of human T cells responding to allogeneic dendritic cells (DCs) (27). Both partners express the receptors for C3a and C5a (124–127), and C3aR- and C5aR-antagonists inhibit human T-cell proliferation, whereas recombinant C3a/C5a promotes alloreactive human CD4+ T-cell expansion. Various subsets of human DCs produce complement, and C5aR/C3aR signaling regulates DC activation and function (27). Pharmacologic C5aR blockade reduced human anti-mouse graft-versus-host disease scores, inhibited T-cell responses in NOD/SCID/γcnull mouse recipients of human PBMCs, and enhanced stability of iTReg, verifying that the human C3aR-dependent effects on human T cells apply in vivo (12,27). In further support of a role for local complement in human transplantation, the quantity of RNA message for alternative pathway complement components and receptors is higher in human allograft biopsies with histologic evidence of rejection compared with noninjured control tissue (122,128). Together, these translational findings provide proof-of-concept that C3a/C3aR and C5a/C5aR ligations are viable targets for facilitating prolonged survival of human transplants.

Conclusion

The complement system is a pathophysiologic mediator of kidney disease in humans. Building on the notion that plasma complement functions as an effector mechanism of antibody-initiated kidney injury, the recognition that kidney-derived and immune cell–derived complement participates in innate and adaptive immune responses and the appreciation that disorders of complement regulation underlie multiple kidney diseases have altered fundamental paradigms of complement biology. Ongoing translational immunology efforts along with the development of pharmacologic agents that block human complement components and receptors (129) now permit testing of the intriguing concept that targeting complement in patients with an assortment of kidney diseases has the potential to abrogate disease progression and improve patient health.

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