Glomerular Disease

All Things Complement
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Abstract
The complement (C) cascade is an ancient system of proteins whose primary role is to initiate and modulate immune responses. During C activation, circulating proteins are cleaved and nascent cleavage fragments participate in a broad range of downstream innate and adaptive immune functions. Although the majority of these functions are either homeostatic or protective, a large body of experimental and clinical evidence also highlights a central role for the C system in the pathogenesis of many types of glomerular disease. From classic pathway activation in lupus nephritis to alternative pathway dysregulation in C3 glomerulopathy, our understanding of the spectrum of C involvement in kidney disease has expanded greatly in recent years. However, the characteristics that make the glomerulus so uniquely susceptible to C-mediated injury are not fully understood, and this remains an area of ongoing investigation. Several C inhibitors have been approved for clinical use, and additional C inhibitory drugs are in development. The use of these drugs in patients with kidney disease will expand our understanding of the benefits and limitations of C inhibition.


Introduction
The complement (C) system is a complex group of interacting proteins that play a critical role in host immune surveillance and defense. C activation results in the generation of multiple protein fragments that mediate biologic processes integral to both health and disease (Table 1) (1). In addition to the well described homeostatic functions of C, a pathologic role for C activation has emerged for a number of the glomerular diseases (Table 2). In many of these diseases, immune-complexes (ICs) trigger activation of the classic pathway (CP). Other diseases, such as atypical hemolytic uremic syndrome (aHUS) and C3 glomerulopathy (C3G), are associated with defective regulation and/or enhanced activation of the alternative pathway (AP). Additional details regarding the role of C in membranous nephropathy, antiglomerular basement membrane (GBM) disease, and antineutrophil cytoplasmic antibody (ANCA)–associated vasculitis were discussed in a recent Clinical Journal of the American Society of Nephrology educational review (2). Other glomerular diseases in which C activation likely plays a critical role will be reviewed in subsequent articles in this series.

Although the mechanisms of C activation and C-mediated injury may vary, the critical role of the C system in glomerular diseases of such diverse etiologies indicates a unique susceptibility of the kidney to the injurious effects of C. In this review we present an overview of the C cascade and evidence that C plays a role in glomerular disease, and we discuss recent advances in C biology that inform the treatment of glomerular disease.

Overview of the C Cascade
C Activation Pathways
The C cascade is comprised of >30 circulating proteins that are activated through one of three initiation pathways (Figure 1) (3). Multiple different molecules can trigger C activation. IgG (particularly the IgG3 and IgG1 isotypes), IgM, C-reactive protein, and serum amyloid P activate the CP. The mannose binding lectin pathway is activated when circulating lectin binds to sugar moieties on pathogens. C3b is deposited on tissues after activation of the CP or mannose binding lectin pathway, and it composes part of the CP C3-convertase (Figure 2).

The CP is not simply a downstream effector of tissue injury in IC-mediated diseases, but it also functions to prevent the development of autoimmunity. In fact, congenital deficiencies of the early CP proteins (C1q, C2, and C4) are among the strongest risk factors for developing lupus (4). This association may be due to impaired phagocytosis of apoptotic cells and nuclear debris in the absence of the CP (Table 1) (5). The risk of developing autoimmunity to these nuclear antigens increases because of their prolonged presence outside of cells (5). Opsonization of antigens with C also lowers the activation threshold of B cells, and C deficiencies may hinder the elimination of autoreactive B cells (5). Thus, the CP reduces the risk of autoimmunity, but also mediates tissue injury caused by autoantibodies.

Some molecules, such as IgA, can directly activate the AP (6), but this pathway does not require specific protein–protein interactions and is constitutively active as a result of spontaneous hydrolysis of C3. Additional details of the molecular interactions involved in C activation are described in an excellent recent review (2).

Activation through any of the C pathways leads to the proteolytic cleavage and activation of C3 (3). Activated C3, referred to as C3b, is central to all three pathways. C3b contains a thioester bond that can
The initiation pathways rapidly self-amplify (Figures 1 and 2). As a result, C activation through any of the AP C3-convertase, an enzyme that cleaves additional C3, can form covalent amide or ester bonds with amino and hydroxyl groups on cell surfaces. C3b is also a key component of the AP C3-convertase, an enzyme that cleaves additional C3 (Figures 1 and 2). As a result, C activation through any of the initiation pathways rapidly self-amplifies as C3b fosters the generation of yet more C3b, and millions of the molecules can be formed and deposited on target surfaces within minutes (7). The addition of a C3b molecule to the C3-convertases generates C5-convertases, enzymes that cleave the protein C5.

C activation causes kidney injury through direct effects on renal cells and through interactions with cells of the innate and adaptive immune systems. Receptors for C3b are expressed on erythrocytes, leukocytes, and nonhematologic cells, and engagement of these receptors mediates cell activation and phagocytosis (2). Small soluble peptides referred to as anaphylotoxins (C3a and C5a) are also generated during C activation. C3a and C5a receptors are expressed on leukocytes, endothelial cells, mesangial cells, and tubular epithelial cells (2). These fragments trigger systemic inflammatory responses through their receptors, including vascular changes and the chemotaxis of immune cells. C3a directly induces mesangial cells to proliferate and secrete extracellular matrix (8), and it causes tubular epithelial cells to produce proinflammatory chemokines (9). Finally, full activation of the C pathways generates C5b-9 (the membrane attack complex) a multimeric structure that forms pores in the membranes of target cells, causing cell activation and lysis. C5b-9 causes endothelial cells to release mitogens that promote mesangial proliferation (10). When C5b-9 is generated directly on the surface of mesangial cells it triggers production of prostaglandins, cytokines, and reactive oxygen species (11,12). Formation of C5b-9 on podocytes is an important cause of glomerular injury in membranous disease (13).

**C Regulatory Proteins**

To protect host cells from uncontrolled C activation, several C regulatory proteins are expressed on the surface of cells and also circulate in plasma (14). These proteins inactivate the C convertases by dissociating them or by cleaving C3b to form iC3b (Figure 2; see also Matern and Heeger [2]). Further degradation of iC3b yields C3d, which remains covalently attached to target surfaces. Like C3b, the C4b protein has an internal thioester bond that can form covalent bonds with tissue surfaces (15). Inactivation (cleavage) of C4b by regulatory proteins yields C4d, which also remains attached to tissues (Figure 2).

Factor H is a soluble regulator of the AP that is particularly important for protecting the kidney. Although it is only one of several C regulators that function in the glomerulus, a large body of evidence indicates that defects in the function of factor H are sufficient to cause glomerular injury (16). Factor H is comprised of 20 repeating structural domains (short consensus repeats, or SCRs). The C regulatory function of factor H is performed by the first four SCRs, whereas several other regions of the protein mediate binding of the protein to tissue surfaces. The carboxy terminus of factor H (SCRs 19–20) binds to C3b and glycosaminoglycans (17) and confers the ability to bind and protect endothelial cells. Five C factor H–related proteins (CFHRs) arose through gene reduplication and share structural similarities to factor H (18). These proteins each contain the SCR 19–20 binding domain, but they do not contain the SCR 1–4 regulatory domain. The function of the CFHRs is controversial. Experiments have shown that they can regulate C activation in some settings, but the binding domains may also competitively inhibit factor H from functioning on some surfaces (14,18).

The degree to which C is activated in a given tissue is a dynamic process and depends on a number of factors. Cell injury may reduce the surface expression of regulatory proteins (19) and stimulate local production of C proteins by renal cells (20). Both of these cellular responses promote C activation. Other biologic systems also interact with the C cascade. Notable examples include the activation of C5 by thrombin (21), and local impairment of factor H by the protein annexin A2 (22).

**Evidence That the C System Is Involved in Glomerular Diseases**

As early as the 1960s it was understood that the C system was involved in antibody-mediated glomerular injury (23), and by the 1970s C proteins were detected in renal biopsy specimens from patients with GN (24). Experimental evidence in both animal models and in patients links the C system with a large number of glomerular diseases (Table 2).

**Clinical Biomarkers of C Activation**

**Tissue Staining.** Activation of the C cascade generates several soluble and tissue bound protein fragments, and these fragments can be employed as biomarkers of tissue inflammation. Kidney biopsies are routinely immunostained for deposits of C3 and C4 fragments. Tissue staining for C3 is usually performed using an antibody to C3c, which detects tissue-bound C3b and iC3b (Figure 2). Because C3b is deposited on target tissues by all three activation pathways, detection of these fragments is interpreted as a catch-all marker of C activation. The C4d fragment remains attached to surfaces after C4 inactivation. Detection of C4d is now routinely used as a marker of antibody-mediated rejection, but is also an indicator of active disease in IC-mediated GN (25). Biopsies are also sometimes immunostained for C1q, which is another marker of CP activation. In IC-mediated GN, Ig, C3 fragments, and C4

<table>
<thead>
<tr>
<th>Adaptive Functions of C</th>
<th>Maladaptive Functions of C</th>
</tr>
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<tbody>
<tr>
<td>Opsonize target cells (pathogens and injured cells)</td>
<td>Promote tissue inflammation</td>
</tr>
<tr>
<td>Trigger vascular changes and inflammatory cell chemotaxis</td>
<td>Directly cause injury to host cells</td>
</tr>
<tr>
<td>Lyse pathogens</td>
<td>Mediate cancer cell immune-evasion</td>
</tr>
<tr>
<td>Provide costimulation and proliferation signals for immune cells</td>
<td></td>
</tr>
<tr>
<td>Remove immune-complexes and cellular debris</td>
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</tbody>
</table>

**Table 1. Functions of the C cascade**
fragments generally colocalize (26). The detection of C3 in the absence of Ig or C4 is a sign of an AP-mediated process (Figure 1), and is the basis of the diagnosis of C3G (27).

**C Protein Levels.** Decreased levels of plasma C3 and C4 have long been associated with active inflammation in patients with autoimmune and systemic inflammatory diseases (28). The levels of these proteins are determined by their rate of synthesis (primarily in the liver, but also by renal cells) and the rate of consumption. They are sensitive indicators of activity in some diseases, such as diffuse proliferative lupus nephritis (28). Conditions in which hepatic production of C proteins is altered can confound the interpretation of low C levels. Production of the C proteins increases in pregnancy, for example, whereas it decreases in patients with cirrhosis (28). Furthermore, the levels remain normal in many C-mediated diseases. This may be because local C activation can cause significant tissue injury without depleting the plasma pool of C3, or that activation is driven by locally produced C3 (20). In contrast, it was reported that C3 levels remained depressed in a patient with membranoproliferative GN who underwent bilateral nephrectomy, demonstrating that the consumption of C3 can occur in the plasma or tissues outside of the kidneys (29).

Several clinical studies have measured C activation fragments in plasma and urine as biomarkers of active kidney disease. C activation fragments may be useful for predicting disease flares in patients with lupus, particularly if the level of intact C3 is confounded by pregnancy or other clinical conditions (30). Urinary C3d and C5b-9 are biomarkers of immunologic glomerular injury in patients

### Table 2. Evidence that the C system is involved in glomerular diseases

<table>
<thead>
<tr>
<th>Disease</th>
<th>C Protein Depositsa</th>
<th>Plasma Complement Protein Level</th>
<th>Autoantibodies to Complement Proteins</th>
<th>C Gene Variants Associated with Disease</th>
<th>Clinical Use of Complement Inhibitors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atypical hemolytic uremic syndrome</td>
<td>C3 and C5b-9 deposits have been reported (94)</td>
<td>↓C3, ↑C5a, ↑sC5b-9</td>
<td>Factor H</td>
<td>Factor H Factor I</td>
<td>✓</td>
</tr>
<tr>
<td>C3 Glomerulopathy</td>
<td>Dominant C3 two orders of magnitude greater than that for Ig</td>
<td>↓C3, ↓factor B, ↓properdin, ↓C5, ↓C7, ↓Ba, ↓Bb, ↓C3d, ↓C5a, ↑sC5b-9</td>
<td>C3Nefs (&gt;75%)</td>
<td>Factor H Factor B</td>
<td>✓</td>
</tr>
<tr>
<td>Lupus nephritis</td>
<td>C3, C4, C1q</td>
<td>↓C3, ↓C4, ↓C1q</td>
<td>C1q, C3</td>
<td>Factor H Factor I C3 Factor B CD46 CHFR1 CFHR3 CFHR5 Thrombomodulin</td>
<td>✓</td>
</tr>
<tr>
<td>Membranoproliferative GN</td>
<td>C3, C4, IgG, IgM</td>
<td>↓C3, ↓C4</td>
<td>C1q</td>
<td>Clq, C1r/s, C2, C4</td>
<td>✓</td>
</tr>
<tr>
<td>Catastrophic antiphospholipid antibody syndrome</td>
<td>C3, properdin, ± C4, ± MBL</td>
<td>↑C3a, ↑C3d</td>
<td>C3Nefs</td>
<td>Factor H Factor I CD46</td>
<td>✓</td>
</tr>
<tr>
<td>IgA nephropathy</td>
<td>C3, properdin, ±C4, ± MBL</td>
<td>↑C3a, ↑C3d</td>
<td>C3Nefs</td>
<td>Factor H CFHR1 CFHR3</td>
<td>✓</td>
</tr>
<tr>
<td>ANCA associated vasculitis</td>
<td>C3 and Ig can be seen (95)</td>
<td>↑C3a, ↑C5a, ↑sC5b-9, ↑Bb, ↑C3d, ↑C5, ↓properdin</td>
<td>Factor H</td>
<td>CFHR1 CFHR3</td>
<td>✓</td>
</tr>
<tr>
<td>Postinfectious GN</td>
<td>C3, ± C4</td>
<td>↑C3d, ↑C5, ↓properdin</td>
<td>C3Nefs</td>
<td>Factor H</td>
<td>✓</td>
</tr>
<tr>
<td>Tubulointerstitial diseases</td>
<td>C3 on brush border</td>
<td>Urine iC3b, Bb, C5b-9</td>
<td>C3Nefs</td>
<td>Factor H</td>
<td>✓</td>
</tr>
</tbody>
</table>

aDeposits seen in renal biopsy tissue. CFHR, complement factor H related proteins. Check marks represent diseases where patients treated with C inhibitors have been reported.
with membranous nephropathy (31). Given that the drug eculizumab blocks the cleavage of C5, it is logical that detection of sC5b-9 would be a useful guide to eculizumab therapy, but local C activation may not always cause detectable increases in the systemic levels of sC5b-9 (32). In addition, the C proteins are labile, and measurement of the activation fragments is sensitive to sample handling. These issues must be considered when interpreting biomarker findings.

**Animal Models**

Both naturally occurring and laboratory-generated animal models have been instrumental in our understanding...
of the role of C in the glomerular diseases. GN in a strain of Norwegian Yorkshire pigs was caused by congenital deficiency of factor H (33). Mouse models of C deficiency have defined a role for C in both IC-mediated glomerular diseases (34) and in glomerular injury in the absence of Ig (35). More recently, animal models led to the discovery that C activation contributes to diseases such as ANCA-associated vasculitis (36) and FSGS (37), diseases not previously considered to be C-mediated. Animal models have also been instrumental in demonstrating the efficacy of C therapeutics before clinical trials (38,39).

There are several differences between the mouse and human C systems that limit the applicability of mouse models to human disease. Although the general organization of the cascade is the same, structural differences between mouse and human proteins prevent some human therapeutics from working in mice. Eculizumab, for example, does not inhibit mouse C5 (40), and compstatin (a small molecule inhibitor of human C3) does not inhibit mouse C3 (41). Mouse C also has lower lytic activity than that of other species, possibly because murine C4 does not generate an effective CP C5-convertase (42). The role of C5b-9 may therefore be underrepresented in murine models of IC-mediated diseases. The study of IC-mediated glomerular disease is also complicated by differences between rodents and humans in the mechanisms of IC clearance. In humans, C receptor-1 (CR1) on erythrocytes binds to C3b containing ICs and transports them to the liver. In rodents, on the other hand, ICs are transported to the liver by platelets, and adherence of the ICs is mediated by factor H on the platelet surface instead of CR1 (43).

Molecular Studies

A large number of different molecular abnormalities are associated with C dysregulation within the glomerulus (44–46). Rare variants in the genes for factor H, C3, factor B, factor I, membrane cofactor protein (MCP, or CD46), and thrombomodulin have been identified in patients with aHUS (46,47). Studies have also revealed that rare variants and hybrid forms of the CFHR genes are associated with aHUS (18,48–52), and deletion of CFHR1–3 is associated with the development of autoantibodies to factor H, an abnormality that is found in approximately 10% of aHUS patients (53).

C3 nephritic factor (an autoantibody against the AP C3-convertase) is the most common C abnormality found in patients with C3G (54). A number of genetic impairments in AP regulation have also been identified in patients with this disease, including variants in the genes for factor H, factor B, C3, and the CFHRs (27,44,45,55). Experimental analyses of the disease-associated CFHR variants suggest that they impair the ability of factor H to control AP activation on tissue surfaces (18,56).

All of these molecular defects are associated with uncontrolled activation of the AP, and in some cases a causative relationship between the genetic abnormality and functional C dysregulation has been confirmed experimentally (57). From a diagnostic perspective, however, the large number of possible genetic variants and autoantibodies makes the analysis of individual patients complicated. Nevertheless, testing for genetic variants and autoantibodies can help establish the diagnosis, and identifying autoimmune causes of C dysregulation can guide immunosuppressive treatment (58).

Mechanisms of C Activation in Glomerular Diseases

Pathologic C activation within host tissues involves: (1) molecular events that promote C activation (e.g., IC deposition), and/or (2) local impairments in the regulation of C activation. These mechanisms are not mutually exclusive, as ICs cause CP activation but can also create a microenvironment from which C regulators are excluded (59). All of the resident renal cells express C regulatory proteins (16). Yet, the frequent involvement of the C system in renal disease demonstrates that the regulatory capacity of these proteins can be overwhelmed or subverted.

Vasculature and Glomerular Endothelial Cells

Subendothelial deposition of ICs is common in lupus nephritis and membranoproliferative GN (Figure 3). IgG and IgM containing ICs activate the CP, causing direct injury and inflammation of nearby tissues. Animal models also demonstrate that injury of the glomerulus in IC-mediated diseases involves amplification through the AP (34,60). In aHUS, the AP may be activated directly on glomerular endothelial cells due to impaired regulation. Even in patients with a genetic predisposition to aHUS, however, the majority of flares occur after a systemic illness or stressor (61). A recent study reported that C1q and C4d can be seen in the vasculature of most patients with aHUS (62). It is possible that illness or endothelial damage triggers C activation through the CP, but that uncontrolled AP activation perpetuates microvascular injury in susceptible patients. The AP is also critical for the development of ANCA vasculitis (36), although the exact mechanism and location of AP activation are not yet known.

GBM

In contrast to cells, basement membranes do not intrinsically express regulatory proteins and are dependent on circulating factor H to control AP activation (2). Genetic defects (61), autoantibodies (53), or other proteins can interfere with the function of factor H (22). Factor H deficiency is associated with C activation in the fluid phase and directly on the GBM (63), mechanisms that are believed to cause C3G (Figure 4). Dysregulation of the AP results in C3 deposits in the relative absence of Ig, and this is the diagnostic basis of C3G (27). Dense deposit disease is a subtype of C3G characterized by detection of electron dense deposits in the GBM by electron microscopy (27). The composition of these deposits is not known, but similar deposits are seen in factor H deficient mice (35). Antibodies to the noncollagenous-1 domain of type 4 collagen directly activate C on the GBM (2). Activation is initiated through the CP in that setting, but detection of AP proteins suggests that this pathway is also involved (2).

Podocytes

ICs are often seen in the subepithelial space of patients with membranous disease. IgG binds to the M-type
phospholipase A2 receptor on podocytes in some patients (64), and other podocyte antigens have also been identified (65). Podocytes express CR1, which can regulate the CP and AP, but CR1 is cleaved from the surface of the cells in patients with lupus nephritis (66). The loss of CR1 may permit C activation at this location, or it may be a consequence of C5b-9 formation (66).

Mesangium
ICs deposit in the mesangium in several different types of GN, including lupus nephritis, causing C activation. Patients with C3G can also present with C3 deposits in the mesangium (55), indicating that factor H is functionally important for controlling AP activation at this location even though mesangial cells express cell surface C regulators (16). In IgA nephropathy ICs containing IgA1 deposit in the mesangium, similar to other IC-mediated glomerular diseases (67). Not surprisingly, there is extensive clinical evidence of glomerular C activation in patients with this disease (68). Unbiased genome wide association studies have also identified variants in CFH and the CFHRs that strongly associate with the risk of developing IgA nephropathy (69,70). Thus, the disease may be caused by the formation of IgA1 containing ICs, but injury of the glomerulus may also be determined by an individual’s ability to regulate amplification through the AP.

Tubulointerstitium
There is minimal expression of the C regulatory proteins on the apical surface of tubular epithelial cells (16). Ordinarily this surface does not come in contact with C proteins, but the AP may cause tubulointerstitial injury in patients with proteinuric kidney diseases due to free passage of C proteins into the tubules (71). Tubular epithelial cells also synthesize C3, and locally produced C3 may be an important cause of acute and chronic tubulointerstitial injury (20,72). Other conditions in the tubulointerstitium also favor AP activation. Ammonia can form an amide linkage with C3 and activate the AP. In settings of reduced nephron mass, the amount of ammonia produced by each nephron increases to maintain acid-base balance, and this adaptation may drive progressive tubulointerstitial inflammation and damage (73).

The Unique Susceptibility of the Glomerulus to C-Mediated Injury
In spite of intensive study, it still is not known why the glomerulus is so uniquely vulnerable to C-mediated injury. Factor H circulates in plasma and should control AP activation throughout the body. Similarly, MCP is expressed on endothelial cells and other cells throughout the body. Why, then, do genetic defects in these proteins primarily cause inflammation within the glomerulus? Other attributes of the microenvironment within the kidney (concentrated plasma proteins, local production of C proteins, and pH) likely contribute to C activation (Figure 3). Nevertheless, further study is needed in order to fully understand all of the mechanisms that cause pathologic C activation in the kidney.
Why Do Some Defects in AP Regulation Cause C3G and Some Cause aHUS?

Impaired AP regulation by factor H is strongly associated with both aHUS (61,74) and C3G (27,45,55), yet these diseases are clinically and pathologically distinct. The majority of the genetic variants in factor H associated with aHUS affect the carboxy terminus of the protein (61,74). As the carboxy terminus facilitates binding to cell surfaces, dysfunction in this region may limit the ability of factor H to protect glomerular endothelial cells from the AP (Figure 4). Autoantibodies to factor H also predominantly affect this region of the protein (75). The defects in MCP, factor I, C3, factor B, and thrombomodulin that are associated with aHUS are also believed to impair regulation of the AP on the endothelial cell surface (61,74).

The autoimmune and genetic defects associated with C3G, on the other hand, tend to affect C regulation by the protein (Figure 4). The most common C defect in C3G is the presence of C3 nephritic factor, an autoantibody that protects the AP C3-convertase from inactivation by factor H (45,54). Genetic variants in C3 and factor B that confer resistance to inactivation of the C3-convertase by factor H (and hence, overactivity of the convertase) have also been found in patients with C3G (45,55). A variety of different histologic patterns and ultrastructural changes are observed in patients with C3G (45,55), but it remains unclear whether these are related to the associated C abnormalities.

The factor H defects that cause aHUS and C3G have been recapitulated in mice. Homozygous factor H deficiency caused C3G-like disease (35), whereas a partial deficiency in the carboxy-terminus of factor H was associated with aHUS-like disease (76). This paradigm does not apply to all patients, however, and some of the genetic variants associated with aHUS have also been identified in patients with C3G (45). Thus, more work is needed to identify the additional environmental, genetic, and epigenetic factors that influence the development and course of these diseases.

C As a Therapeutic Target

In aHUS and C3G, C activation is the primary insult that causes glomerular injury. Consequently, agents that prevent C activation will block the underlying process that causes these diseases. For antibody-mediated diseases, C inhibition may reduce inflammatory effects of ICs deposited within the glomerulus. In addition, C blockade may reduce T cell activation at the antigen presenting cell–T cell interface as well as the B cell response to antigens, thereby reducing the magnitude of the adaptive response to antigens (2).

Eculizumab is a monoclonal antibody that blocks the cleavage of C5, preventing formation of C5a and C5b-9 (40). In phase 2 clinical trials, treatment with eculizumab led to a rapid increase in the platelet count and steady
improvement in renal function (77,78), and it has been approved for treatment of aHUS. These studies have demonstrated the safety and efficacy of C inhibition in patients with glomerular disease, and that inhibition can be maintained chronically.

Data suggest that eculizumab is superior to plasma exchange/infusion for the treatment of aHUS, although it is not effective in all patients (77,78). The response to treatment does not seem to depend upon identification of a genetic defect in a C-related gene (77,78), although a small subset of patients are resistant to the drug due to a genetic variant in the C5 gene (79). Earlier treatment with eculizumab is associated with better renal outcomes (80), and patients with worse renal function at presentation are less likely to achieve complete remission of thrombotic microangiopathy-related symptoms (77). This may be due to the presence of irreversible renal damage at the time of treatment. It is also possible that C3a and C3b/iC3b/C3d contribute to the pathogenesis of renal injury (9,81,82), and drugs that target the C system at the level of C3 or higher may be advantageous in some patients. Hemolytic uremic syndrome can also be caused by a variety of different systemic stressors, including drugs, autoimmune disease, hypertension, infections, cancer, and pregnancy (83). It is not yet clear whether eculizumab is beneficial in these settings, although there is evidence that pregnancy-associated hemolytic uremic syndrome is usually C-related (84).

Eculizumab has also been used off label for several other renal diseases, including C3G (85), catastrophic antiphospholipid antibody syndrome (86), lupus nephritis (87), and IgA nephropathy (88). Eculizumab only appears to be beneficial in a subset of patients with C3G. Earlier treatment is associated with a better response, so some of the patients may have already had irreversible renal injury by the time they were treated (85). Different underlying C defects may lead to C3 activation in the fluid phase or directly on the GBM (Figure 4), and these differences could affect how important C3 fragments are in the disease. Patients with elevated levels of C5b-9 (i.e., evidence of active terminal C activation) may be those most likely to benefit from eculizumab (85). It is not clear at this time whether the level of C blockade is the key to successful treatment, or whether earlier intervention would suffice.

Many other C inhibitors are currently in development (89). Monoclonal antibodies that target other C proteins have been tested, including antibodies to C1s, factor D, factor B, properdin, C3b, and the mannose associated serine proteases. Small molecules have been developed to block signaling of C3a and C5a at their receptors and can be administered subcutaneously or orally. C5a blockade was protective in a murine model of ANCA vasculitis (90), and the C5a receptor antagonist used in that study is currently being tested in a clinical trial of patients with ANCA vasculitis (ClinicalTrials.gov identifier: NCT02222155). Engineered proteins that specifically block C activation at sites of tissue injury and small interfering RNA agents that knock-down the production of C proteins are also in development. Each of the different therapeutic strategies has advantages as well as limitations, and the utility of these agents relative to eculizumab are discussed in a recent review (91).

Because the C system is an important part of the immune system, the major risk of all therapeutic C inhibitors is likely to be that of infection. Deficiency or blockade of the terminal C proteins is primarily associated with meningococcal infections (92). Patients treated with eculizumab should be immunized against Neisseria meningitides and/or prophylactically treated with antibiotics. In spite of immunization, approximately 1% of treated patients per year develop Neisserial infections (93). It has been suggested that antibodies to the organism are less effective in the setting of C inhibition. Drugs that block the C system higher in the cascade might also increase the risk of infection with encapsulated bacteria (92).

Conclusions and Future Directions

It has been known for >50 years that the C system plays an important role in IC-mediated glomerular injury, yet the field of C biology continues to rapidly change as new discoveries are made. Although aHUS and C3G are rare diseases, they are extreme examples of AP dysregulation that provide crucial insight into the biology of the AP. Careful examination of patients with these diseases has resulted in major advances in our understanding of C activation and C regulation within the glomerulus. These advances were made possible by multicenter clinical trials as well as multinational disease registries. The knowledge gained by these studies has already affected the care of these two diseases, and it has also informed our understanding of more common glomerular diseases. The connection between CFHR1–3 deletion and IgA nephropathy was discovered by a genome wide association study (69), for example, but our understanding of the function of the CFHRs is due to careful study of patients with aHUS and C3G.

There is still a great deal to be learned regarding the mechanisms by which the C system is activated within the glomerulus, the roles of the different C activation fragments in glomerular injury, and the optimal use of C inhibitory drugs in patients with kidney disease. New molecular causes of aHUS and C3G will undoubtedly be discovered that will improve our understanding of these diseases. Genetic studies of patients with other glomerular diseases will also reveal disease-specific as well as generalized mechanisms that increase or decrease the risk of glomerular disease. Given the complexity of the C system and the large number of molecules involved in C activation and regulation, this field may particularly benefit from large scale genomic and proteomic studies of patients with GN. Perhaps most importantly, as additional patients with glomerular diseases are treated with C inhibitory drugs, efforts must be made to gain further insights into the risks and benefits of targeting the C cascade in this group of diseases.

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