Complement and the Kidney

Richard J. Quigg

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The C system contains three activation pathways along with regulators poised at key points throughout. All three pathways can participate in innate immunity because of the discriminatory capacity of the key proteins C1q, mannose-binding lectin (MBL)1 and factor H for microorganisms. C also aids in the development of a directed and optimal acquired immune response. Once this response has formed, C is crucial for the metabolism of immune complexes and is an important effector of immunity, indirectly through generation of C4b, C3a, C3b, iC3b, C3d, and C5a, which interact with cognate receptors on blood and tissue cells, as well as directly through the formation of the C5b-9 membrane attack complex.

Because of the extensive use of rodents to investigate human renal diseases, it is important to consider differences among C systems. Although the activating pathways in rodents and humans are largely conserved, there are some notable differences among C regulators. These include the presence of a gene termed CR1-related gene y (Crry) given its nucleotide similarity to human CR1 (1). The protein product is also known as Crry, and has widespread reactivity throughout the body in rats and mice, including in the kidney (2–4). Unfortunately, Crry has no direct human homologue, so conclusions of human physiology are limited from studies of Crry function, which are comparable to those of CR1 (5, 6). CR1 (CD35) and CR2 (CD21) are products of separate genes in humans, while in mice they arise by alternative splicing from a single C2 gene, which gives rise to products referred to as CR1/CR2 (7). In primates, immune complex metabolism occurs by E-associated CR1 (8, 9), while in rodents immune complexes appear to be processed by platelet-associated factor H (10).

The kidney is a vascular organ with a specialized capillary bed, the glomerulus. Because of certain unique structural features as well as its constant exposure to blood, the glomerulus can be involved in type II and III inflammation, for which C activation is relevant. In contrast, the tubulointerstitium of the kidney is much more restricted in its access to circulating C proteins. Possibly because of exposure to microorganisms ascending through the urinary tract, this area of the kidney has adapted to make its own C proteins, which is applicable to infection, disease states and renal transplantation.

Glomerular disease

Our understanding of how the C system relates to glomerular diseases has evolved considerably. First, the identification of Ig and C components in human renal biopsies provided circumstantial evidence that C was a key player in glomerular diseases (11). Subsequently, the use of strategies to systemically deplete C such as with cobra venom factor (CVF) have been used to show the C dependence of short-term animal models representing a variety of human diseases (12–15). More recently, contemporary molecular biological tools such as the production and use of genetically manipulated mice and recombinant proteins, particularly useful in long-term models of disease, are being employed to more clearly determine how C fits in glomerular diseases. Finally, rationale inhibition of the C system is being applied clinically to human diseases (16, 17).

As an example, consider the glomerular pathology occurring in the autoimmune disease, systemic lupus erythematosus (SLE). SLE is useful to discuss here, as it is a common clinical condition and widely studied by immunologists; as well, the glomerular disease exhibits a spectrum that can recapitulate just about any type of glomerular immunopathology. In human lupus nephritis, there is a large amount and diversity of immune reactants in glomeruli, including IgG, IgM, IgA, C1q, C4, C3, and C5b-9 (18), leading to the designation “full house” pattern. In addition, there is systemic consumption of C and the appearance of C activation products in sera (19). Altogether, these observations would suggest, but not prove, an important role for C in lupus nephritis.

There are a number of mouse strains which spontaneously develop lupus nephritis with immune complex and C deposition in glomeruli similar to the human disease, such as the New Zealand Black/White (NZB/W)F1, and MRL/Mp-Tnfrsf6lpr/lpr (or simply MRL/lpr) lupus strains (20). The latter arises due to deficiency of Fas protein on the autoimmune-prone MRL/Mp background (21). Although observational and therapeutic studies have long been possible (22–24), a more comprehensive understanding for the role of C in lupus nephritis has come recently through the use of specific transgenic and gene-targeted strains (25), and recombinant forms of natural protein and Ab inhibitors of C activation.

Studies by Wang et al. (26) showed that administration of a mouse anti-C5 Ab near the onset of autoimmune disease in

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3 Abbreviations used in this paper: MBL, mannose-binding lectin; Crry, CR1-related gene y; CVF, cobra venom factor; SLE, systemic lupus erythematosus; GN, glomerulonephritis; I/R, ischemia-reperfusion; HUS, hemolytic uremic syndrome; MPGN, membranoproliferative GN; CCP, C control protein.
NZB/W mice prevented development of glomerulonephritis (GN) which translated into improved survival. MRL/lpr mice that were given rCry-Ig from the onset of autoimmune disease at 12 wk of age until it was well advanced at 24 wk of age were protected from renal functional and structural injury (27). Comparable results occurred in transgenic MRL/lpr mice in which Cry was expressed as a soluble protein both systemically and locally in kidney (28). These three studies in accurate murine models of lupus nephritis illustrate a role for C activation in this disease. Because Cry affects C3 convertases and anti-C5 blocks cleavage of C5, these studies still have fallen short of determining which specific mediator(s) of the C pathway beneficial effects could be attributed to. These positive results are the impetus for a planned study using anti-C5 in human lupus nephritis. 

MRL/lpr mice deficient in factors B (29) and D (30) were protected from lupus nephritis. Although consistent with the previously mentioned studies with anti-C5 and Cry, these results are somewhat surprising, as traditional thinking has lupus nephritis occurring through immune complex-directed classical pathway activation. In these settings, alternative pathway activation may arise independently of the classical pathway (31, 32) or can be initiated by classical pathway activation.

Interestingly, lupus nephritis in the MRL/lpr model can proceed independently from FcγR (33), which could lead one to believe this is a C-dependent and FcγR-independent disease. This simplistic view does not hold up as C3-deficient MRL/lpr mice developed lupus nephritis comparable to wild-type MRL/lpr mice (34). Like with C3-deficient 129/C57BL/6.lpr/lpr mice (35), C3 deficiency did not affect the autoimmune per se in MRL/lpr mice (34). However, C3-deficient MRL/lpr mice had greater amounts of glomerular IgG-containing immune complexes, consistent with a role for C activation, these results are somewhat surprising.

In conditions in which the glomerular permselectivity barrier is impaired, plasma proteins appear in the urinary space. Progressive glomerular diseases are invariably accompanied by tubulointerstitial damage, the extent of which is closely linked to an adverse renal outcome. The various proteins of the C system are among the proteins appearing in urine in non-selective glomerular proteinuria, and their activation has also been suggested to contribute to this tubulointerstitial damage (59), for which there is a growing amount of experimental evidence (60, 61). There is also the potential for tubular cells to synthesize their own C proteins when stimulated by non-C proteins present in the tubular lumen (62). The Matsuo laboratory (63) used the proteinuric model in rats induced by the aminonucleoside of puromycin. In this study, animals that were C depleted with CVF, or in which C was inhibited with soluble rCR1, had virtual elimination of C activation, significantly less tubulointerstitial damage, and preserved renal blood flow. As noted before, the use of CVF and rCR1 allows one to conclude C is crucial to protect against C activation in the portions of tubular cells that are exposed to urine, and the expression of C regulators is low in the apical regions of tubular cells in both human and rodent kidney (3, 58).

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**Tubulointerstitial disease**

Ischemia-reperfusion (I/R) injury occurs when blood flow to an organ is interrupted for some period followed by reperfusion. In the kidney, I/R can lead to the syndrome of ischemic acute tubular necrosis which occurs most directly in vascular surgery and transplantation, but, can also be the result of hypotension and/or sepsis. The organs studied most carefully in animal models of I/R have been the heart, intestine and kidney. The relevance of C activation to cardiac I/R injury was shown over thirty years ago, and has been confirmed more recently through the use of systemic and targeted forms of human rCR1 (39–41). In I/R of organs other than the kidney, C activation appears to occur primarily via classical and MBL pathways, which appears to be due to the binding of natural Abs or MBL, respectively, to newly expressed Ags on ischemic tissue (42–45). In contrast, Abs are not deposited in the kidney after I/R (46) and data from the Sacks and Holers laboratories (47, 48) are consistent in showing a predominant role for alternative pathway activation in renal I/R injury. Exactly how this alternative pathway activation might occur has not been determined, but must involve an overwhelming of intrinsic C regulation such as by factor H and Cry. Although the definitive role for neutrophils in renal I/R injury remains controversial (49–51), there is a C-dependent neutrophil accumulation in this model involving C5a and C5b-9 (47, 48, 52). Both C5a and C5b-9 have also been shown to contribute to renal injury independent from their role in attracting neutrophils, such as through induction of apoptosis (47, 52–56). Overall, activation of the alternative C pathway in renal I/R seems to play an important role in the inflammatory response and in injury of tubular epithelial cells (47, 48, 52–54).

Under normal circumstances, the nearly three million glomeruli in two human kidneys (57) do a remarkable job of restricting plasma proteins from entering the 170 liters of daily glomerular ultrafiltrate. Hence, the apical portions of tubular cells are exposed to a largely protein-free solution. In the case of the C cascade, the large sizes of nearly all of the member proteins further restrict their passage into urine. As such, it is not crucial to protect against C activation in the portions of tubular cells that are exposed to urine, and the expression of C regulators is low in the apical regions of tubular cells in both human and rodent kidney (3, 58).

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**Transplantation**

The C system has been shown to play a clear role in the rejection that occurs in xenotransplants (reviewed in Refs. 64 and 65)).
This hyperacute rejection can be attributed to the reactivity of natural Abs with galactosyl α-(1-3)-galactosyl β-1,4-N-acetyl glucosaminyl residues (or simply, Gal) which are absent on higher primates, but widely present on cells in lower species such as the pig. Strategies to prevent or reduce this C activation include C depletion/inhibition in the recipient, and the expression of natural C regulators such as decay-accelerating factor and membrane cofactor protein on donor cells, that can restrict activation of recipient C.

Interestingly, there has been a resurgence of interest in the role of C activation in leading to humoral rejection in renal allografts (reviewed in Ref. 66). Currently, this is identified by the presence of C4d in peritubular capillaries of the allograft. The exact reactivities of C-activating Abs remain unclear, but may include HLA class I and II Ags, ABO Ags, as well as other non-HLA endothelial Ags. Whether binding to endothelial Gal epitopes in xenografts, or to other endothelial cell Ags in allografts, the resulting C activation has the potential to lead to a variety of effects, which must depend, at least in part, on the tempo of its activation. In hyperacute xenograft rejection, there is endothelial cell activation and generation of a procoagulant milieu, which results in thrombosis, platelet consumption, hemorrhage, and infarction (65), while in acute humoral rejection of the allograft there is neutrophil infiltration, arterial fibrinoid necrosis, and acute tubular injury (66). There are experimental rodent models to substantiate that Ab reactivity with endothelium can lead to C-dependent endothelial cell alterations similar to those described in transplant rejection (67–69). In addition, C inhibition with soluble rCR1 has been shown to be beneficial in allograft rejection in the rat (70).

An interesting and somewhat unexpected finding has recently come from Pratt et al. (71), in which C3 deficiency in a renal allograft was protective against rejection. Proximal tubular cells are capable of expressing MHC class II molecules and presenting alloAg. The data from both in vivo and ex vivo experimentation supported that local C3 production facilitated this alloAg presentation by tubular cells. Absent this, the immune response directed against the allograft was considerably attenuated. Although there is good evidence showing that the interaction of C3 fragments with B lymphocyte and/or follicular dendritic cell CR1/CR2 is an important second signal in an acquired humoral immune response (72–74) these data suggest that such a signaling mechanism may also exist on T lymphocytes (71).

Genetic associations

As noted by Walport et al. (75), the strength of association of inherited C deficiency and development of autoimmunity is inversely correlated with the position of the deficient protein in the classical pathway. Homozygous deficiencies of C1 and C4 genes are strongly associated with the development of SLE and in some cases, lupus nephritis. Recently, animal models have

![FIGURE 1](http://www.jimmunol.org/)

**FIGURE 1.** Proposed role of C proteins in a renal disease such as murine lupus nephritis. C is activated on immune complexes, which are either processed by platelet-associated factor H (or E-associated CR1 in primates) and hepatic macrophage CR3 and FcγR, or deposited in mesangial, subendothelial, and superepithelial sites in the glomerulus, where they generate C3a, C3b, and C5a, which interact with intrinsic and inflammatory cell C3aR, C5aR, CR3, and FcγR. C5b-9 is formed on all glomerular cells, as well as proximal tubular cells and fibroblasts, leading to cellular injury and activation. Progressive glomerular/tubular inflammation and injury leads to accumulation of inflammatory/immune cells and matrix components in the tubulointerstitial compartment. Complement regulators (such as Crry in rodents, not drawn) are present in all cells depicted and can limit complement activation. Portions adapted from Segerer et al. (94).
provided insight into these clinical observations. A proportion of mice with targeted deletion of C1q developed spontaneous GN (76) and in MRL/Mp+/+ mice, deficiency of C1q accelerated glomerular disease (77). C4-deficient mice on a 129/C57BL/6 background can develop autoantibodies (32, 78). Of these mixed background C4-deficient mice, some females developed features of lupus nephritis, as did those in which a coexisting deficiency of Fas was present (32, 79). The role of C4 signaling through CR1/CR2 is not fully defined, as CR1/CR2-deficient mixed background mice did not develop increased levels of autoantibodies (32), but, with a coexisting deficiency of Fas, features of lupus nephritis developed (79). Altogether, these data have been interpreted to indicate that classical pathway activation on apoptotic debris, rich in nuclear components, is necessary for appropriate clearance of these autoantigen-containing immune complexes as well as maintenance of tolerance, in part as signaled through the B lymphocyte CR1/CR2 (32, 78–82). Immune complexes containing autoAbs and autoantigens may end up in glomeruli, but, the resulting pathology is dependent upon C activation, Fc interactions, as well as other genetic components that are slowly being defined (83).

Genetic abnormalities in factor H have been associated with the renal diseases hemolytic uremic syndrome (HUS) and membranoproliferative GN (MPGN) (84–86). It is interesting that the vast majority of factor H-associated HUS can be attributed to polymorphic variation in the terminal (20th) C control protein (CCP) of factor H (87). This particular CCP is conserved among the factor H family members, and has a sialic acid/heparin binding domain that allows it to become associated with cells, a means by which factor H discriminates non-C activators from activators (86). An explanation for why heterozygous inheritance of such an abnormal factor H molecule predisposes to HUS may be that under normal circumstances half maximal levels of functional factor H can restrict alternative pathway activation. However, under conditions of endothelial insult, this is insufficient to prevent alternative pathway activation on the endothelium (87).

From a clinical perspective, the association of factor H deficiency with MPGN is not as clear, particularly in terms of the molecular defect and the resulting clinical disease (84, 85). However, there are illuminating studies in animals. Norwegian Yorkshire pigs with homozygous factor H deficiency developed a disease similar to MPGN type II, which can be rescued by treatment with factor H (88). The Zipfel laboratory (89) has shown this to be the result of a mutation in the 20th CCP of factor H, leading to an impairment of the secretion of factor H into the circulation. Mice with targeted deletion of factor H generated by the Botto laboratory spontaneously developed GN with some features of human MPGN (90). In these studies, C deposition in glomeruli preceded immune complex deposition, and the morphological expression of disease was dependent upon alternative pathway C activation, as coexistant factor B deficiency abrogated disease.

Conclusions

The current use of sophisticated genetic techniques in humans as well as elegant studies in animals has permitted unparalleled insight into how the C system plays an important role in the kidney. Particularly through the use of mice with targeted deficiencies in one or more C genes, a paradigm for the involvement of the C system in kidney disease is slowly coming. In Fig. 1, a schema is provided that incorporates some of these findings, plus some speculation. Over time, this will be refined. One hopes that with our growing knowledge of how and where the C system is involved in renal pathophysiology, we will be able to manipulate it to come up with rationale and beneficial therapeutics, although the translation from bench to bedside can be slow (16, 17, 60, 91–93).

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References


