Published in final edited form as:
Semin Nephrol. 2013 November ; 33(6): 575–585. doi:10.1016/j.semnephrol.2013.08.008.

COMPLEMENT REGULATION IN RENAL DISEASE MODELS

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Abstract

Activation of the complement system is tightly regulated by plasma and cell-associated complement regulatory proteins (CRPs), such as factor H (fH), decay-accelerating factor (DAF), and membrane cofactor protein (MCP). Animal models of disease have provided considerable insights into the important roles for CRPs in the kidney. Mice deficient in fH have excessive fluid phase C3 activation *and inactivation* leading to deposition of iC3b in glomerular capillary walls (GCW), comparable to dense deposit disease. In contrast, when fH lacks C-terminal surface targeting regions, local activation on the GCW leads to a disease reminiscent of thrombotic microangiopathy. The uniquely rodent protein, CR1-related y (Crry), has features analogous to human MCP. Defective Crry leads to unrestricted alternative pathway activation in the tubulointerstitium (TI) resulting in pathological features ranging from TMA, acute kidney injury and TI nephritis. In the presence of initiators of the classical or lectin pathways, commonly in the form of immune complexes in human glomerular diseases, complement regulation on self is stressed, with the potential for recruitment of the spontaneously active alternative pathway. The threshold for this activation is set by CRPs; pathology is more likely when complement regulation is defective. Within the endocapillary region of the GCW, fH is key, while DAF and Crry are protective on mesangial cells and podocytes. Arguably, acquired alterations in these CRPs is a more common event, extending from pathological states of cellular injury or production of inhibitory antibodies, to physiological fine tuning of the adaptive immune response.

Activation through classical, alternative or lectin complement pathways leads to the cleavage of C3 and C5 and generation of C3a, C3b, C5a and C5b. The latter can combine with C6, C7, C8 and C9 to form C5b-9 in any receptive cell membrane; although this is fairly promiscuous, the effects of such C5b-9 formation appear to have some specificity in terms of cellular pathways that become activated (see chapter 9, this issue).[1–3] In contrast, C3a, C3b and C5a have specific cellular and plasma protein ligands. Anaphylatoxin

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Disclosures: AN, SS and RJQ have nothing to disclose.

receptors C3aR and C5aR (CD88) are in the rhodopsin family of seven-span transmembrane proteins. C3b-binding proteins include the regulators of complement activation (RCA) proteins (discussed below), and β_2 integrin (CD18) heterodimers with α_M (Itgam, CD11b) and α_x (Itgax, CD11c), also termed complement receptors 3 and 4 (CR3 and CR4) because they bind C3b products. C3aR and C5aR couple to G-proteins to transduce their signals, while the β_2 integrins may generate outside-in signals via immunoreceptor tyrosine-based activation motif proteins and spleen tyrosine kinase. Despite the limited ligand-receptor systems, the net effect of complement activation can vary considerably depending on the context.

The vestiges of the alternative complement pathway were present one billion years ago in sea anemones of the phylum Cnidaria. Over time, genome, chromosome and individual gene duplications have resulted in over 40 complement genes in higher vertebrates.[4;5] It is presumed that evolutionary pressure from infectious microorganisms led to an increasingly active complement system. The benefits of this protective system were limited by the negative effects of complement activating on self tissues, which led to the evolution of a multitude of regulatory checkpoints. There is considerable genetic, structural and functional variability within the complement system proteins.[6;7] This level of complexity is very relevant when considering the mechanics of complement activation; for example, antibody-mediated complement activation beginning with the classical pathway, recruiting the alternative pathway, and ending in the terminal pathway, requires 14 activating proteins, and can be affected by 11 regulatory proteins (Figure 1). This has led Harris, de Cordoba and Morgan to propose the term “complotype” as reflecting the pattern of genetic variants in complement genes inherited by an individual, which alters risk for both inflammatory disorders and infectious diseases involving complement.[8]

COMPLEMENT REGULATION

The complement system is heavily regulated, including by time and space; several complement intermediates have little time to find particular acceptor sites before inactivation. For example, in the fluid phase, the exposed thioester in C4b and C3b must find a receptive carbohydrate or amino group within 0.1 s before inactivation (i) by hydrolysis.[9] Similar constraints occur upon formation of the trimolecular C5b-7 complex, in which the exposed hydrophobic domain must find an acceptor lipid membrane in approximately 0.1 s.[10]

There are also dedicated complement regulatory proteins (CRPs), the majority of which block C3 and/or C5 activation, presumably reflecting the importance of their activation.[11] These are the RCA family members, C4 binding protein (C4bp), factor H (fH), decay-accelerating factor (DAF, CD55), membrane cofactor protein (MCP, CD46), complement receptors 1 and 2 (CR1 and CR2), and the rodent specific CR1-related y (Crry). Proteins which prevent formation of C5b-9 include plasma clusterin and S protein, and cellular CD59. The complement inhibitory properties of all these CRPs are conferred by their fairly low affinity binding for C3, C4 and/or C5 products.

In contrast, the plasma proteins, factor I (fI) and carboxypeptidase N (CPN) structurally inactivate complement intermediates, C3b and C4b, and C3a and C5a, respectively.[11] Here again the importance of the C3/C5 step is evident. Both are produced in their active forms; fI requires a cofactor from among C4bp, fH, MCP, CR1, or Crry, which facilitates fI binding and also allows full expression of its catalytic domain.[12]

In a physiological sense, the distinction between complement regulation and its activation is exploited by the adaptive immune system by utilizing C3/C5-binding proteins on lymphocytes and dendritic cells (DCs). For example, the B lymphocyte signaling complex contains CR2, which can be activated by immune complexes (ICs) containing natural antibody, foreign antigen, and activated C3, thereby facilitating an adaptive humoral response to that antigen.[13] More recent evidence has shown complement impacts cellular immunity (covered in detail in Chapter 7 in this issue). Here, it appears the T cell and its antigen-presenting DC partner both generate complement proteins and downregulate DAF, the net result being local complement activation. Signals through C3aR and C5aR affect T cell proliferation and differentiation in normal T cell responses, as well in autoimmunity and alloimmunity.[14–18] The relevance of these largely *in vitro* observations has held up in correlative human studies.[19;20]

All RCA proteins are composed of short consensus repeat (SCR) domains of ~ 60 amino acids, and are highly related within and between even distant species.[21–23] Human, mouse and rat fH are very similar, containing solely 20 SCRs arranged in tandem.[24–26] DAF and MCP each have only 4 SCRs along with membrane proximal O-glycosylated regions. DAF is linked to the plasma membrane through a glycosylphosphatidylinosoitol (GPI) anchor, while MCP is a type I transmembrane protein. Although mouse *Crry* was first discovered with a human *CR1* cDNA probe (giving rise to its unique name), it is a short transmembrane protein most similar to MCP.[27–29]

The functional activities of each RCA family member are attributable to their binding to C3 products, with the exception of C4bp which has limited binding to C3b in physiological conditions. Most other RCA members can also bind C4b, which expands their repertoire to classical and lectin pathway C3 convertases. Importantly for some of the conclusions reached to-date, fH is not a C4b-binding protein, and is therefore limited to inhibiting the formation and accelerating the decay of intermediates with C3b. Factor H does serve as cofactor for fI-mediated cleavage and inactivation of C3b to iC3b (to be distinguished from C3bi occurring from hydrolysis of C3b).[30] In spite of their similar SCR compositions, DAF, MCP and Crry have distinct functions as complement regulators. DAF has decay-accelerating activity towards C3 and C5 convertases of all pathways, while MCP is a fI-cofactor only. Interestingly, Crry has the features of DAF and MCP, and hence is a versatile and potent complement regulator.[31]

Factor H is produced primarily in the liver and circulates in human and rodent plasma at relatively high concentrations (~ 500 μg/ml or 3.3 mmol/L),[26;32] a fact underlying attempts at liver transplantation to treat genetic *fH* defects. [33] Although there is evidence for fH production by other cells of the mononuclear phagocyte lineage, bone marrow-derived cells do not contribute substantially to the circulating fH pool.[34;35]

DAF, MCP and Crry protect the kidney from complement activation. Each of these proteins tend to have their distinct localization patterns by immunohistochemical techniques. In humans, MCP appears to be fairly uniformly distributed in all three intrinsic glomerular cells,[36;37] while DAF is highly expressed in the juxtaglomerular apparatus.[38] Functional DAF is also on cultured human podocytes,[39] which is relevant to studies with rodent podocyte DAF (discussed below). Other sites in human kidneys served by DAF and/or MCP include endothelial and tubular cells.[37]

Complement regulatory proteins have evolved somewhat differently in rodents, yet perform nearly identical functions to those in humans. In rodent kidneys, DAF is primarily a podocyte and vascular endothelium protein.[40;41] Unlike in humans in which MCP has widespread distribution throughout the body, MCP expression in mice is limited to spermatozoa, making its absence in knockout mice of no consequence to renal disease models.[42] Overall, it is likely that the rodent uses Crry in place of MCP and/or DAF in many sites as its complement regulator, which is not surprising, as Crry combines the functions of both.[31] In the rodent kidney, this appears to be true in non-glomerular endothelial, mesangial and tubular cells.

CR1 on human erythrocytes is relevant to IC metabolism, where it binds IC C3b, inactivates it and then facilitates the transfer of IgG/iC3b to Fcγ receptor/CD11b-bearing mononuclear phagocyte cells.[43] In contrast, rodents use fH as their C3b-binding fI-cofactor RCA protein, present on platelets, another abundant blood cell (Figure 2a).[44] Human podocytes have CR1, while rodent podocytes have fH in its place (Figure 2b).[45] Studies in rodents have shown platelet and podocyte fH are responsible for systemic and transglomerular IC metabolism, respectively[35;46], which is consistent with the believed role for CR1 in humans.

HUMAN DISEASES ATTRIBUTABLE TO ABNORMAL COMPLEMENT REGULATION

The importance of normal complement regulation can be appreciated from the disease paroxysmal nocturnal hemoglobinuria, in which there is impaired GPI linkage of DAF and CD59 to the erythrocyte membrane.[47] Spontaneous activation of complement through the alternative pathway leads to hemolysis; this now can largely or completely be prevented with eculizumab, a humanized antibody to C5.[48]

An early success with genome-wide association was the linkage of fH polymorphisms with age-related macular degeneration (ARMD).[49–51] The importance of fH to the glomerular capillary wall (GCW) is illustrated by the association of abnormal or absent fH with atypical hemolytic uremic syndrome (aHUS) and membranoproliferative GN type 2 (also termed dense deposit disease, DDD). The latter is now included in a spectrum of so-called C3 glomerulopathies. This is a rapidly evolving area covered in detail in this issue (Chapters 2 and 3); overall, it is clear mutations conferring gain-of-function to activators and/or loss-of-function to regulators within the complement system can underlie a number of diseases, including those affecting the kidney.[7;52]

Complement activation in the renal tubulointerstitium (TI) from excessive activation and/or abnormal regulation appears relevant in a number of disease states. That ammonia-induced C3 activation could underlie the progression of renal diseases originates from the studies by Nath and Hostetter;[53] this provides theoretical support for the considerable recent interest in correcting acidemia. [54] Alloantibody-mediated complement activation appears etiologic in humoral renal allograft rejection. Acquired abnormalities of MCP, a complement regulator present in the renal tubulointerstitium[37;55] may underlie acute kidney injury.[56] Inherited defects in MCP can clearly lead to aHUS; unlike those due to fH, in these instances, disease does not recur with renal transplantation.[57]

In spite of DAF being present on most endothelia and an effective regulator of the alternative pathway, there is no evidence yet that DAF deficiency or defects are relevant to aHUS, DDD or ARMD.[58] DAF only displaces Bb from C3b; once it moves on, the surface-bound C3b remains capable of binding factor B to again form a C3 convertase. In contrast, fH and MCP function to permanently inactivate C3b.[58;59]

ANIMAL DISEASE MODELS

Our understanding of complement regulation in the normal and diseased human kidney has been aided by work in experimental animals. Characterization (“cloning”) of human CRPs occurred in the 1970s; the next decade saw identification of CRPs from other species. With this came a number of studies in traditional rat renal disease models using function-neutralizing antibodies. These types of experimental approaches have largely been supplanted by informative studies in mouse strains with targeted gene deletions, which have dominated the past 15 years.

As mentioned earlier, there is compartmentalization of complement regulation in the kidney;[37;55] in rodents, DAF is on glomerular endothelial cells and podocytes, Crry is on mesangial, non-glomerular endothelial and tubular cells, and fH is recruited from plasma to the GCW. Perhaps because it is the only cellular regulator of C5b-9, CD59 has a widespread distribution. Since the particular anatomic site within the kidney where complement is activated determines disease phenotype,[60] a range of diseases can occur in the setting of altered CRPs. Experimental animals develop spontaneous disease when either fH or Crry are abnormal or absent; as true in humans, the absence of DAF does not appear to lead to spontaneous disease. Yet, each of the CRPs in the kidney can be relevant in circumstances of heightened complement activation, such as occurs through the classical pathway in IC-mediated disease.

MEMBRANOPROLIFERATIVE GLOMERULONEPHRITIS

The Norwegian group of Jansen, Hogåsen, Mollnes described a heritable porcine model of MPGN type II that led to early mortality.[61] In a series of biopsies from birth to death they identified C3 and C5b-9 localized within GCW at birth in fetal kidneys without intramembranous deposits or mesangial hypercellularity. Subsequently subendothelial then intramembranous deposits developed, followed by mesangial hypercellularity and expansion associated with development of renal failure. The plasma of these pigs showed very low circulating C3 and highly elevated C5b-9 levels, suggesting unrestricted complement

activation, potentially due to altered complement regulation. Consistent with this premise, transfusion of normal porcine plasma to affected piglets inhibited complement activation and increased their survival. This group went on to prove disease was attributable to an inherited type 1 defect in fH, such that fH protein was absent.[62] These series of studies convincingly showed the absence of fH was associated with unrestricted complement activation and renal disease akin to human MPGN type II (now known as dense deposit disease, DDD).

Over the eleven years since their initial description of $fH^{-/-}$ mice,[63] the Imperial College group led by Pickering, Botto, and Cook have provided remarkable insights into the pathophysiology of MPGN occurring in these animals.[64–68] These animals develop a spontaneous inflammatory glomerular disease, histologically containing a mixture of MPGN I and II features, including early complement deposition in GCWs followed later by GCW double contours with mesangial cell interposition, glomerular hypercellularity, and neutrophil influx.[63;68] Consistent with an alternative pathway mediation, $fH^{-/-}$ mice with a coexistent fB deficiency are protected from disease.[63] Spontaneous disease in $fH^{-/-}$ mice requires C5, but not C6 activation, supporting a role for C5a and C5a receptor activation.[68]

More recent findings in this model include the unexpected improvement of disease with fI absence and worsening with fP absence.[64;67] Similar findings to the latter were also noted by Wen-Chao Song's group.[69] Taking these animal data together, development of MPGN in $fH^{-/-}$ mice requires complement activation and inactivation in the fluid phase to generate iC3b, the latter attributable to fI with other cofactors besides fH. As expected, the absence of fP does impair spontaneous complement activation, but on net, this simply provides more fluid-phase C3 to feed into nephritic glomeruli.

These studies of the $fH^{-/-}$ mouse model have yielded a wealth of information, mainly relevant to MPGN in a general sense. Because of the added role of fH on mouse platelets and podocytes to process ICs, immunoglobulin accumulates over time in $fH^{-/-}$ mouse glomeruli; thus, this is not a true C3 glomerulopathy, such as DDD. As developed in greater detail in Chapter 2, this distinction is important in humans, as immunoglobulin-positive MPGN will typically have an underlying trigger driving chronic antigenemia or circulating ICs, while (immunoglobulin-negative) C3 glomerulopathy has been attributed to dysregulated complement activation. Whether all cases of C3 glomerulopathy are due to abnormal CRPs remains to be established.

IMMUNE COMPLEX-MEDIATED GLOMERULONEPHRITIS

Chronic serum sickness

Given its distinctive physiological features, the glomerulus can become involved in IC-mediated diseases such as post-infectious GN and lupus nephritis (LN). As a model of circulating IC excess, a number of variations on chronic serum sickness (CSS) have been employed, in which IC deposits accumulate in the mesangium and GCW.[70–72] Yet, many strains of mice do not develop GN, despite the presence of substantial glomerular IC deposits.[73;74] Therefore, this is useful to examine whether CRPs are protective in this disease.

In the rodent glomerulus, Crry is primarily in the mesangium.[75] Thus, in a mesangial proliferative GN model in rats, blocking Crry worsened disease.[76] Because maternal C3 deficiency is needed for Crry-deficient fetal survival,[77] $Crry^{-/-}$ $C3^{+/-}$ mice are commonly studied (i.e., providing a wildtype *C3* allele of paternal origin). $Crry^{-/-}$ $C3^{+/-}$ mice actively immunized with horse spleen apoferritin had increased development of GN relative to controls, which could be attributed to neutrophil infiltration; absent staining for alpha-smooth muscle actin and proliferating cell nuclear antigen suggested that mesangial cell proliferation did not play a significant role in this model.[78] In comparable studies to examine potential roles for DAF and CD59, both $DAF^{-/-}$ and $DAF^{-/-}$ $CD59^{-/-}$ mice had significantly increased glomerular C3 deposition which correlated with development of GN relative to wildtype controls.[79] There were no histological or functional disease features in $CD59^{-/-}$ or wildtype mice with CSS, which is indirect evidence that C5b-9 has a limited role in this model.

While cellular CRPs appear to limit endocapillary and mesangial IC models of GN, their absence only results in a mild glomerular inflammatory disease. In contrast, fH recruited from the plasma is a critical CRP for the GCW. Thus, all $fH^{-/-}$ mice with CSS developed diffuse proliferative GN.[80] This is well prior to their development of spontaneous glomerular disease, as shown specifically by the inclusion of control $fH^{-/-}$ mice "immunized" with saline, which consistently have normal glomerular histology.

As with spontaneous disease models, studies with $fH^{-/-}$ mice are complicated by the role for fH in IC processing. Factor $H^{-/-}$ mice with CSS had more glomerular ICs, which could also lead to excessive glomerular complement activation. Because platelet-associated fH arises intrinsically (i.e., from megakarocytes, Figure 2a) while plasma fH arises from the liver, they could be separated in mice by generating bone marrow (BM) chimeras between $fH^{-/-}$ and wildtype C57BL/6 mice (Figure 3).[35] Wildtype mice with $fH^{-/-}$ BM lacked platelet fH; thus, systemic IC metabolism was impaired in the CSS model, leading to extensive glomerular deposition of IgG together with comparable C3. By immunoblotting this C3 was as iC3b. Despite considerable glomerular IgG and iC3b, there was not histopathological (nor functional) GN. In contrast, IC processing in $fH^{-/-}$ mice with wildtype BM (and fH on platelets) was similar to wildtype mice, with moderate amounts of glomerular ICs containing IgG and C3. Although these mice had less C3b and the majority was as iC3b, there was also active C3b 110-kDa α' chain. Despite the lesser quantities of glomerular IgG and total C3, all animals developed GN and thus, IC quality rather than quantity dictate whether inflammation ensues or not. In follow-up studies, the GN occurring in $fH^{-/-}$ mice was C5aR dependent, as it was prevented in $C5aR^{-/-}$ $fH^{-/-}$ mice with CSS (Figure 3f);[81] of note in this figure is the small, but significant increase in BUN in wildtype mice with CSS compared to controls, indicating there is measurable disease expression in CSS in wildtype mice.

Overall, these data support that: 1) Glomerular-bound ICs activate C3 to C3b, irrespective of complement regulation; 2) While there is considerable C3b inactivation in the absence of fH, presumably by fI and other cofactors, fH facilitates the inactivation of all C3b; 3) In the latter case, despite the presence of abundant IgG and iC3b, inflammatory cells bearing Fcγ receptors and β2 integrins remain quiescent; 4) Intact C3b in ICs is necessary to form an

active C5 convertase; and, 5) Generation of C5a and signaling through C5aR are necessary events for inflammatory cell activation around glomerular-bound ICs.

Lupus nephritis

Autoimmune-prone MRL/Mp mice with the *lpr* gene (due to a retroviral insertion within the *Fas*/*Tnfrsf6* gene) develop many features of human systemic lupus erythematosus, including the presence of circulating autoantibodies and consumptive hypocomplementemia.[82;83] They also develop lupus nephritis (LN) characterized by progressive accumulation of ICs in the GCW and endocapillary proliferative GN.[84] Thus, this represents an alternative model to CSS to examine complement regulation around glomerular IC deposits.

Factor H-deficient MRL-lpr lupus mice died at a young age, with ⅔ dead by 14 wks of age, compared to none of $fH^{+/+}$ and $fH^{+/-}$ MRL-lpr control animals[85] Prior to death animals were azotemic (average BUN values of 82.9 mg/dl within 2 wks of death), consistent with their dying of renal failure. By 12 wks of age, $fH^{-/-}$ MRL-lpr mice developed severe diffuse LN characterized by mesangial, endocapillary, and extracapillary (i.e., crescentic) cellular proliferation, and hyalinosis lesions in glomeruli representing large subendothelial IC deposits (so called “wire loops” in human LN). Thus, fH deficiency in MRL-lpr mice markedly accelerated renal disease in the 4 wk period between 8 and 12 wks of age. This provides another example of the critical role played by fH to limit complement activation initiated by ICs within the GCW.

MEMBRANOUS NEPHROPATHY

Heymann nephritis (HN) is a widely utilized rat model of membranous nephropathy (MN). The fundamental paradigm in HN that antibodies directed towards podocyte antigen(s) progressively accumulate within the subepithelial space appears to have been upheld in human MN; this is then followed by complement activation, C5b-9-mediated podocyte injury and proteinuria (see Chapters 4 and 9, this issue). Active HN depends on the presence of rat IgG2b antibodies, which are efficient at binding C1q and leading to subsequent complement activation.[86] A similar requirement exists in the passive HN model for complement-activating sheep γ1 anti-Fx1A antibodies.[87]

Podocytes actively regulated complement activation; in the rat, this is attributable to DAF, Crry and CD59.[88] As true anywhere, for productive complement activation to occur, this regulation must be overcome.[89] In cultured podocytes, sheep anti-Fx1A antibodies potently activated the alternative pathway, independent of the classical pathway, attributable to impairing complement regulation.[90] Because the Fx1A antigen preparation used to induce HN is a crude extract of rat kidney, it was not very surprising to find it contained CRPs. Whether inhibitory anti-CRP antibodies were relevant in HN was investigated. Rats actively immunized with Fx1A depleted of Crry developed glomerular IgG deposits, but these lacked C3 and animals failed to develop abnormal proteinuria.[91] Complement activation and proteinuria were restored either by reconstituting the depleted Fx1A preparation with recombinant Crry or by passive administration of anti-Crry IgG or $F(ab')_2$ antibodies.[91] In a passive model, anti-megalin monoclonal antibodies were “planted” on the rat podocyte *in vivo*, which activated complement as evidenced by the presence of C3d on podocytes, yet

did not result in abnormal proteinuria. In this setting, the simultaneous neutralization of Crry and CD59 led to productive complement activation and proteinuria.[92] However, anti-Fx1A immunodepleted of reactivities towards these CRPs was fully capable of inducing the passive form of HN.

DAF is present on both human and rodent podocytes.[39;41] In careful histological studies in the rat, DAF is present on the podocyte apical surface, but not the basal surface abutting the GCW. In the puromycin aminonucleoside nephrosis model, DAF-inhibited rats had delayed recovery, consistent with the premise podocyte DAF limits complement activation in glomerular proteinuria.[41]

The conclusions reached from these studies were that Crry, DAF and CD59 are functional on the rat podocyte, where they regulate spontaneous complement activation in proteinuria, and by subepithelial ICs. However, in passive HN, their regulatory capacity is overwhelmed by the tempo of complement activation by podocyte-bound anti-Fx1A antibodies.

Cultured rat podocytes contain both fH and fH-related protein 5.[45;93] The role for fH in mouse podocytes in vivo was investigated by transplanting kidneys between wildtype and $fH^{-/-}$ mice.[46] Normal wildtype kidneys in a $fH^{-/-}$ host accumulated C3 in the mesangium and GCW over several weeks. By immunofluorescence microscopy, these kidneys had prominent GCW staining for fH, which colocalized with the podocyte marker, α-actinin 4 (Figure 2b). Yet, fH was not present in other renal or extrarenal sites, including sera. In every $fH^{-/-}$ recipient of wildtype or $fH^{-/-}$ kidneys in which CSS was induced, diffuse proliferative GN occurred with features identical to those seen in previous studies, affirming the importance of plasma fH to dictate outcome in this model. Interestingly, $fH^{-/-}$ kidneys in wildtype hosts with CSS had substantial GCW deposits of IgG and C3, which ultrastructurally were present in both subendothelial and subepithelial electron dense deposits. These also had a modest impairment in function (BUN and albuminuria in recipients with bilateral native nephrectomies). The persistence of ICs in subendothelial and subepithelial locations, as well as absent (potential) intrinsic complement regulation by fH are explanations for these mild phenotypic features. These data supported that fH was an intrinsic podocyte protein and the kidney does not contribute to systemic fH pools. By analogy to mouse platelet CfH/human erythrocyte CR1,[35;44] mouse podocytes use fH in place of CR1.

FOCAL AND SEGMENTAL GLOMERULOSCLEROSIS

Using passive HN as a guide, sheep antibodies to cultured mouse podocytes were passively administered to mice.[94] Given the relevance of DAF on podocytes (discussed above), DAF-deficient mice were also studied. As anticipated, there was deposition of the heterologous sheep IgG on podocytes, followed by mouse (anti-sheep) IgG. Surprisingly, Balb/c $DAF^{-/-}$ mice developed focal and segmental glomerulosclerosis (FSGS), but not MN, within 30 days (Figure 4). In addition to histopathologic FSGS, there were periglomerular collections of $Thy1.2^{+}$ T and $F4/80^{+}$ mononuclear cells, and mice developed clinical nephrotic syndrome.

Mice generated an active cellular immune response to heterologous IgG which was heightened in the global absence of DAF. Consistent with a T cell-mediation, $CD4^+$ T cell depletion 10 and 17 days post-anti-podocyte IgG injection prevented disease. Wildtype Balb/c kidneys transplanted into $DAF^{-/-}$ mice developed FSGS (as did native DAF-deficient kidneys), while $DAF^{-/-}$ kidneys in wildtype hosts were protected from FSGS. As such, systemic and not local (e.g., podocyte) DAF deficiency was relevant. In contrast, $Balb^{nu/nu}$ mice reconstituted 4 weeks prior with T cells were susceptible to FSGS only if the T cell donor was DAF-deficient. These data provide evidence that podocyte-associated antigens can generate an exuberant immune response by DAF-deficient T cells, leading to podocyte injury and FSGS.[94]

THROMBOTIC MICROANGIOPATHIES

Nangaku, Johnson, Couser et al generated an experimental model in which direct injection of heterologous anti-glomerular endothelial cells antibodies into the renal artery of rats led to acute renal disease with features of thrombotic thrombocytopenic purpura (TTP);[95] this could largely be attributable to C5b-9-induced endothelial cells apoptosis, as it was reduced in the C6-deficient PVG/c^- rat[96] and worsened with CD59 inhibition.[97] In other studies, rats injected with a mouse anti-rat renal endothelial mAb (K6/1, IgG2aκ)[98] developed dose-dependent thrombocytopenia, hemolytic anemia, functional acute kidney injury, and histological renal disease including glomerular thrombi and tubular injury.[99] The renal disease and thrombocytopenia were eliminated if animals were complement depleted with cobra venom factor prior to K6/1 injection, and worsened when the activity of Crry was inhibited with function-neutralizing antibodies. Thus, these are examples of models of thrombotic microangiopathies attributable to anti-endothelial cell antibody-induced complement activation.

The genetic and pathological differences between ARMD, DDD and aHUS, supported the premise the latter alone is attributable to effective plasma C3 regulation but defective control of complement activation on renal endothelium. To model this, Pickering et al generated transgenic mice exclusively expressing fH protein lacking the terminal five SCR domains ($fH^{-/-}$.fHΔ16–20).[100] These mice had effective complement regulation in the plasma, yet considerably reduced GCW deposition of C3 products. Mice developed clinical and histopathological features of thrombotic microangiopathy, including renal functional insufficiency and thrombocytopenia. In follow-up studies, a requirement for C5 was shown by comparing C5-sufficient and -deficient strains.[101] Taken together, these provided evidence that effective regulation of C3 activation in the fluid-phase but not on surfaces within the GCW, with resultant C5 activation, underlie the pathogenesis of aHUS associated with *fH* variants.

TUBULOINTERSTITIAL NEPHRITIS

In normal kidneys, C3 (and C5b-9) staining is evident along the basement membranes of Bowman's capsule and renal tubules[74] which can be attributed to alternative pathway activation, which is enhanced in warm ischemic-reperfusion injury.[53;102] Despite the inability to regulate the alternative pathway systemically, unmanipulated $fH^{-/-}$ mice lack

this typical C3 staining.[46] Yet, within 30 minutes after being transplanted into a wildtype host, kidneys from fH-deficient mice exhbited small amounts of discontinuous staining for C3 around tubules and glomeruli. Thus, unlike the GCW, these sites do not appear to recruit systemic fH, with activation of C3 from the circulation occurring fairly rapidly. From a histological or functional standpoint, this did not appear to be of significance even up to 5 weeks after transplantation.

Clearly Crry is relevant in the TI. This was first shown by Nomura, Matsuo et al with neutralizing antibodies in the rat.[103] A fascinating series of studies from Josh Thurman have shown that the normal polarization of Crry to the basolateral aspect of mouse tubules is lost in ischemia, which leads to unrestricted alternative pathway activation and acute kidney injury upon reperfusion.[102;104;105] This appears to be relevant to acute kidney injury (tubular necrosis) in humans.[56]

In direct studies to examine complement regulation by Crry in kidneys, kidneys from $Crry^{-/-}$ $C3^{-/-}$ mice were transplanted into complement-sufficient wildtype mice; these developed marked inflammatory cell infiltration, tubular damage and interstitial fibrosis, features absent in control transplanted kidneys.[106] Strong C3 deposition in the vessels and tubules which correlated significantly with measures of disease supported that complement activation was pathogenic in this model. The functional significance of these pathophysiological findings was evaluated by removing both native kidneys so the transplanted kidney alone provided renal function. Within 21 days of transplantation, over 80 percent of Crry-deficient kidneys in complement-sufficient wildtype hosts failed (leading to fatal azotemia). The inflammatory infiltrate surrounding injured tubuli contained $F4/80^{+}CD11b^{+}Ly6C(Gr1)^{high}$ and Thy-1.2^{+} T cells, the former characteristic of a classically activated macrophage. To determine the potential roles for extrarenal C3aR and C5aR, $Crry^{-/-}$ $C3^{-/-}$ kidneys were transplanted into wildtype, $C3aR^{-/-}$ and/or $C5aR^{-/-}$ hosts. All kidneys had marked peritubular C3 deposition. Only $C3aR^{-/-}$ hosts were protected from TI nephritis, as determined by pathological criteria and functionally in recipients with bilateral native nephrectomies. Consistent with these disease criteria, TI inflammation with $F4/80^{+}$ cells and $Thy1.2^{+}$ T cells assessed by histopathology and flow cytometry was markedly reduced in $C3aR^{-/-}$ hosts alone.[107] Thus, acute C3 activation in Crry-deficient kidneys leads to C3aR-dependent TI inflammation with mononuclear phagocytic and T cells, ultimately culminating in organ scarring and functional renal failure.

CONCLUSIONS

As a necessary component of immunity towards infectious organisms, activation within the complement cascades leads to generation of injurious and pro-inflammatory proteins. Experimental animals and humans rely on CRPs to protect from unwanted complement activation. Perhaps because of exposure to relatively large quantities of plasma proteins, the endothelial surfaces of the GCW and TI are particularly at risk. Spontaneous alternative pathway activation is regulated by fH. When fH is ineffective in the fluid phase, there is generation of iC3b (by fI) which deposits in the GCW in DDD. Factor H also is retained by the GCW surface; when this is inefficient, endothelial cell injury and aHUS can result. Similarly, defective cell surface regulation by MCP in humans and Crry in rodents in

endothelial and epithelial cells of the TI, can result in pathological pictures of aHUS, acute kidney injury and TI nephritis.

If there are initiators of the classical or lectin pathways, such as ICs, the ability of complement regulators to protect “self” tissues is stressed, with the potential for recruitment of the spontaneously active alternative pathway. The threshold for this activation is set by CRPs. Hence, when complement regulation is defective, complement activation and pathological states are more likely to result. Within the endocapillary region of the GCW, fH is key, while DAF and Crry are protective on mesangial cells and podocytes. Acquired alterations in CRPs occur in cellular injury, such as ischemia, or when function blocking auto-antibodies are present, as in the HN models of membranous nephropathy. Besides disease states, altering the level of complement regulation is exploited physiologically to fine tune adaptive immune responses. Overall, the considerable body of experimental work performed by a number of laboratories world-wide has illustrated the important roles for complement activation and its regulation in the normal immune system and in pathological states involving the kidney.

Acknowledgments

Financial Support: National Institutes of Health grants R01DK41873 and R01DK55357, and a grant-in-aid from the Lupus Foundation of America.

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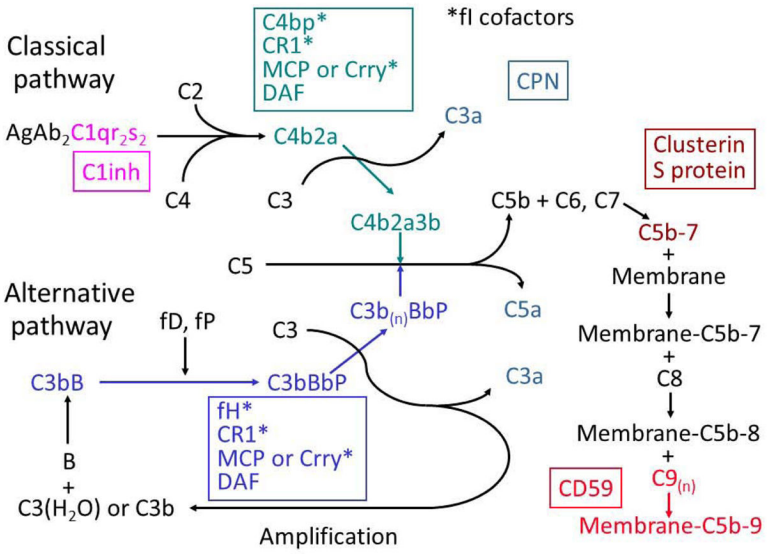

Figure 1. Complement activation and regulation

The proteins relevant to complement activation beginning with ICs in the classical pathway are shown. Regulators of complement activation are in colored boxes adjacent to their site(s) of action. Classical pathway activation can recruit the alternative pathway; if intrinsic regulation is overwhelmed, activation and generation of C3a, C3b, C5a and C5b-9 ensues, each of which has pathophysiological relevance in kidney diseases.

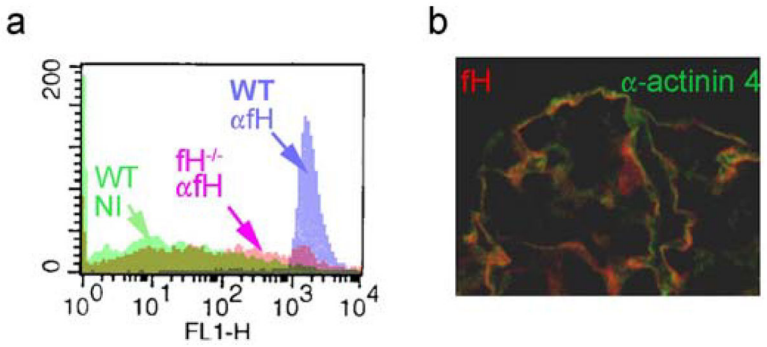

Figure 2. Characteristicization of mouse platelet and podocyte fH

a) Flow cytometric analyses of platelets released from cultured megakaryocytes derived from wildtype (WT) and $fH^{-/-}$ mice. Staining was with specific anti-fH antibodies (αfH) or nonimmune (NI) IgG. b) Staining for glomerular fH in a wildtype kidney transplanted into a $fH^{-/-}$ mouse. Staining was also performed for actinin-4 as a podocyte protein, with the merged image shown.

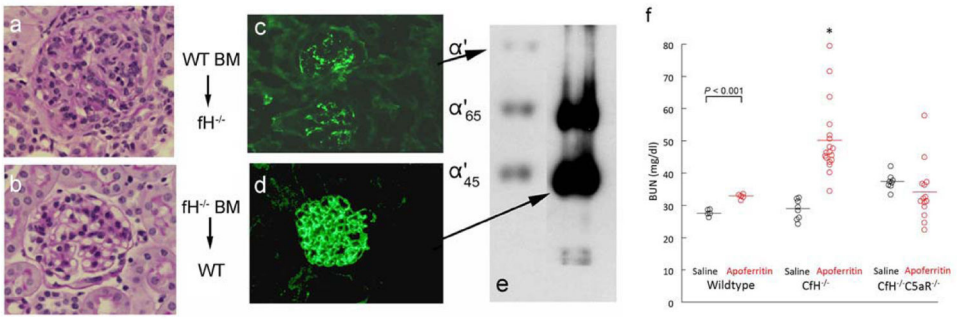

Figure 3. Dependence of experimental GN on plasma fH and C5a receptor, and association with C3b in glomerular ICs

a–e) CSS induced in bone marrow (BM) chimeras - $fH^{-/-}$ mice with wildtype BM (a, c) and wildtype mice with $fH^{-/-}$ BM (b, d). Representative glomerular histopathology and immunofluorescence staining are shown. e) Anti-C3 immunoblotting of isolated glomerular proteins. The C3 α chain generates C3a, and C3b with its 110 kDa α′ chain;; this is cleaved by fI forming α'_{65} and α'_{45} chains of inactive (i) C3b. f, BUN values in individual wildtype, $fH^{-/-}$, and $fH^{-/-}$ $C5aR^{-/-}$ mice after 5 wks of daily apoferritin or saline as controls. ANOVA/Tukey's; *P < 0.001 vs others.

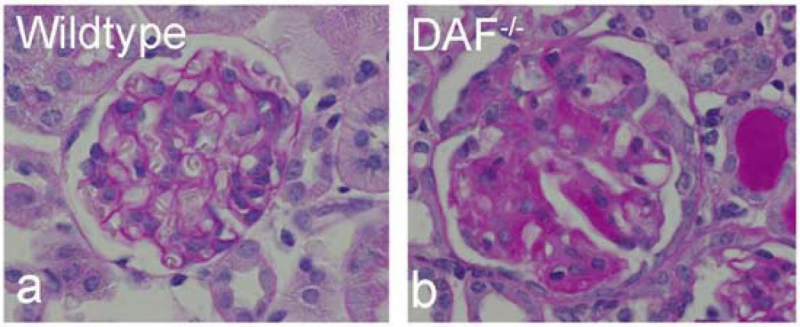

Figure 4. Experimental FSGS

Podocyte injury and segmental glomerulosclerosis occur following injection of anti-podocyte antibodies in DAF$^{-/-}$ but not wildtype mice.