The Many Effects of Complement C3- and C5-Binding Proteins in Renal Injury
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Summary: The complement system is an important component of the innate immune system and a modulator of adaptive immunity. The entire complement system is focused on C3 and C5. Thus, there are proteins that activate C3 and C5, those that regulate this activation, and those that transduce the effects of C3 and C5 activation products; each can affect the kidney in renal injury. The normal kidney has the inherent capacity to protect itself from complement activation through cellular expression of decay-accelerating factor, membrane cofactor protein (in human beings), and Crry (in rodents). In addition, plasma factor H protects vascular spaces in the kidney. Although the main function of these proteins is to limit complement activation, there is now considerable evidence that they can transduce signals on engagement in immune cells. The G-protein–coupled 7-span transmembrane receptors for C3a and C5a, and the integral membrane complement receptors (CR) for C3b, iC3b, and C3dg, are expressed outside the kidney, particularly in cells of hematopoietic and immune lineage. These are important in renal injury through their infiltration of the kidney and/or by affecting kidney-directed immune responses. There is mounting evidence that intrinsic glomerular and tubular cell C3aR and C5aR expression and activation also can affect renal injury. CR1 on podocytes and the β2 integrins CR3 and CR4 in kidney dendritic cells have functions that remain poorly defined. Cells of the kidney also have the capacity to produce and activate their own complement proteins. Thus, intrinsic renal cells express decay-accelerating factor, membrane cofactor protein, Crry, C3aR, C5aR, CR1, CR3, and CR4. These can be engaged by C3 and C5 activation products derived from systemic and local pools in renal injury. Given their capacity to provide signals that influence kidney cellular behavior, their activation can have substantial effects in renal injury. Defining these in a cell- and disease-specific fashion is an exciting challenge for future research.

Keywords: Complement, innate immunity, regulators of complement activation, β2 integrins

The complement system contains more than 30 plasma and cell-associated proteins, many of which are alike as a consequence of gene duplication events during evolution. Complement is the first line of defense against some microorganisms and an integral component of innate and adaptive immune responses to many others. Complement proteins also are important to clear immune complexes (ICs) and material derived from apoptotic cells; in so doing, they can shape the immune response to diverse antigens, including those derived from self and allogeneic tissue.1-3

The complement system has 3 main pathways of activation: the classic, alternative, and mannose-binding lectin (MBL) pathways. All proteins in these pathways come from the liver and circulate in plasma. Nonetheless, cells throughout the body, including those in the kidney, have the capacity to produce complement proteins for local or even systemic use.4,5

The circumstances behind this, including particular stimuli, cells, and produced proteins, can be relevant to kidney disease.

Each complement pathway has its own initiators. Initial activation of the classic pathway occurs through binding of C1 to immunoglob-
ulin M or immunoglobulin G antibodies (Abs) in ICs, activation of the MBL pathway occurs by the binding of MBL to terminal carbohydrate groups on certain microbes, and activation of the alternative pathway occurs through the spontaneous hydrolysis of C3. Activated C1 (as the multiprotein C1qr2s2 complex) and MBL-associated serine proteases cleave both C4 and C2 to generate the C4b2a C3 convertase. Hydrolyzed C3 binds factor B in the fluid phase, leading to a conformational change in factor B, allowing its cleavage by factor D, thereby forming an initial alternative pathway C3 convertase. This then can amplify itself to generate many copies of the alternative pathway C3 convertase, C3bBb.

Activation through each complement pathway leads to the cleavage of C3 and C5. In so doing, C3a, C3b, C5a, and C5b are generated. C5b 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. In contrast, C3a, C3b, and C5a have specific partners in the complement system (Table 1). The receptors for C3a and C5a are G-protein–coupled (GPC) 7-span transmembrane (7TM) proteins of the rhodopsin receptor family, whereas C3b-binding proteins are in the regulators of complement activation (RCA) and β2 integrin families. The events that follow binding of these various protein pairs can have considerable relevance to renal diseases.

**RECEPTORS FOR THE C3a AND C5a ANAPHYLATOXINS: C3aR AND C5aR**

**Structure/Function**

C3aR and C5aR have 21 and 30 amino acid extracellular N-termini, respectively, 7TM regions, 3 extracellular and intracellular loops, and intracellular C-termini. The extracellular regions confer the specificity of binding to their respective C3a and C5a ligands and couple subsequent activation events through other portions of the molecules. Removal of just the single arginine at the C-terminus of C3a or C5a, which occurs physiologically by carboxypeptidase N, eliminates C3a binding to C3aR and considerably decreases the binding affinity of C5a to C5aR.

C3aR and C5aR can be up-regulated by bacterial lipopolysaccharide (LPS) in a variety of cells, including those of the kidney.
From promoter analyses, this appears to require activator protein 1 (AP-1) and v-ets erythroblastosis virus E26 oncogene homologue (Ets) sites in the C3aR promoter, and CCAAT box promoter elements and transcription factor CP2 sites in the C5aR promoter, thereby indicating an underlying specificity behind their transcriptional regulation.

As is true for all G-protein coupled receptors (GPCRs), the effects of C3aR and C5aR occur from their associated specific heterotrimeric G-proteins (αβγ). Typically, C3aR/C5aR are coupled to $G_{al}$ (pertussis-sensitive) and/or $G_{a16}$ (pertussis-resistant) GPCRs. Ligand binding leads the α subunit to acquire guanosine triphosphate in exchange for guanosine diphosphate and its separation from βγ subunits. The subsequent downstream events mediated by the free α and βγ subunits involve phosphorylation of intermediates and ultimate transcriptional events, which are shared by other cellular signals (eg, receptor tyrosine kinases). For instance, activation of phospholipase C, mitogen-activated protein kinases, nuclear factor κ B, and signal transducers of activation can ensue on C3aR or C5aR engagement and activation. The intermediates and ultimate cellular effects can vary considerably from cell to cell, and in a given cell depending on its prevailing state. Even in the same cell, activation of C3aR and C5aR can lead to similar or disparate actions, thus reflecting a specificity to C3a and C5a.

Location Relevant to Renal Diseases

C3aR and C5aR are on most hematopoietic cells, including neutrophils, monocytes/macrophages, and basophils/mast cells. The former 2 are certainly relevant to inflammatory renal diseases, whereas the latter are more relevant to immediate hypersensitivity diseases (eg, asthma) that are not typical in renal disease pathophysiology. It also is appreciated that T cells, B cells, and dendritic cells (DCs) bear C3aR and C5aR. The influence of signals through these receptors can affect how these cells respond to a given antigenic stimulus.

Both C3aR and C5aR are present in intrinsic cells of the kidney. C3aR is on podocytes and C5aR is on mesangial cells, although both are present on proximal tubular epithelial cells. C5aR also is present on mouse microvascular endothelial cells, including those derived from the kidney (our unpublished data). In MRL/lpr lupus mouse kidneys, C3aR and C5aR expression was up-regulated significantly at both the mRNA and protein levels and was accompanied by a wider cellular distribution. This up-regulated expression started before the onset of kidney disease, supporting that C3aR and C5aR may be involved in the development of disease, rather than simply a consequence. In the case of renal expression, an elegant series of studies have shown that C5aR expression in mesangial cells is up-regulated by activation of receptors for urokinase-type plasminogen activator and interleukin (IL)-6 (gp130), which converge to activate signal transducers of activation.

Role in Renal Diseases

The anaphylatoxins C3a and C5a have long been considered to play a role in leukocytic accumulation, which occurs in various inflammatory diseases including glomerulonephritis (GN). The presence of C3aR and C5aR on inflammatory cells such as neutrophils and monocytes and the alteration of several disease models in mice in which C3aR and C5aR were deleted is further evidence for a role of these proteins in inflammatory diseases. Evidence for the role of C5aR in glomerular diseases has come from cryoglobulinemia and nephrotoxic serum nephritis models in which neutrophil infiltration was C5-dependent but C6-independent. Chronic administration of a specific C3aR antagonist to MRL/lpr lupus mice significantly reduced kidney disease, prolonging their survival. Similarly, when C5a signaling was blocked in our studies with a specific antagonist, MRL/lpr mice displayed attenuated renal disease and prolonged viability. The effects of blocking C3aR and C5aR in lupus mice had certain features in common, including a reduction in renal neutrophil and macrophage infiltration, apoptosis, and IL-1β expression. The effects on chemokine expression were distinct, with C3aR- and C5aR-inhibited MRL/lpr mice having reduced chemokine (C-C motif) ligand 5 (CCL5) regulated upon activation, nor-
mally T-expressed, and presumably secreted (RANTES) and chemokine (C-X-C motif) ligand 2 (CXCL2) [macrophage inflammatory protein 2 (MIP-2)] expression, respectively. C3aR-inhibited mice also had increased phosphorylation of protein kinase B, which may have accounted for the protection from apoptosis with receptor antagonism. Consistent with these studies, C5aR-deficient MRL/lpr mice had attenuated renal disease and prolonged survival.\(^{49}\) In these mice, there was a reduction in CD4\(^+\) T-cell renal infiltration, lower titers of anti-double-stranded DNA Abs, and inhibition of IL-12 p70 and interferon (IFN)-\(\gamma\) production, supporting that C5aR signaling links Th1 responses in lupus nephritis.\(^{49}\)

Relevant to the capacity of LPS to induce C5aR expression are Ab-mediated renal disease models in which LPS pretreatment generated a C5aR-dependent process. In a model of glomerular thrombotic microangiopathy induced by nephrotoxic serum, a specific C5aR antagonist blocked disease expression.\(^{50}\) This has similarities to the model of antiphospholipid Ab-induced fetal loss, in which complement activation led to C5aR-dependent placental infiltration with neutrophils.\(^{51}\) In addition to its chemotactic effects, activation of neutrophil C5aR results in extracellular signal-regulated kinase activation and production of Bad, which can protect these cells from apoptosis.\(^{24}\) Yet, in other circumstances, C5a can be proapoptotic.\(^{52}\) For example, in a model of IC-mediated glomerular disease, the simultaneous administration of LPS led to a C5aR-dependent tubulointerstitial nephritis, with prominent apoptosis occurring through activation of up-regulated tubular cell C5aR.\(^{53}\)

The often disparate effects of C3aR/C5aR activation extend to the adaptive immune system. In models of sepsis, T cells are stimulated to increase expression of C5aR; given the co-existent systemic complement activation, these C5aRs are activated leading to downstream events culminating in T-cell apoptosis.\(^{57}\) Although C5aR activation appears to be detrimental in sepsis, activation of C3aR can confer protection from downstream inflammatory events.\(^{43}\) C5aR activation can promote expression of activating Fc\(\gamma\) receptors by lung and liver macrophages.\(^{59,60}\) Interestingly, intravenous immunoglobulin can reverse these effects of C5aR, potentially explaining the therapeutic efficacy in certain disease states.\(^{61}\) Inflammatory cells also can produce\(^{60}\) and cleave their own C5,\(^{62,63}\) completely bypassing the need for plasma complement proteins. Although, as noted earlier, C5aR engagement can promote the development of a Th1 immune response in T cells, there are also instances in which it can suppress Th1 responses. This is true in macrophages, in which C5a potently suppresses LPS- and/or IFN-\(\gamma\)-induced IL-12 production in a dose-dependent fashion.\(^{64}\) Defining some of these nuances in a site- and disease-specific manner is one of the exciting prospects for upcoming research in complement pathobiology.

THE FLUID PHASE COMPLEMENT REGULATOR: FACTOR H

Structure/Function

All members of the RCA family are composed primarily of short consensus repeat domains (SCRs; also termed complement control protein or sushi domains). These have a high degree of relatedness within and between species. Structurally, the SCR is a globular ellipsoid approximately 3.5 nm in length created by 5 short antiparallel \(\beta\)-strands dictated by 2 invariant intra-SCR disulfide bonds.\(^{55,66}\) Complement factor H (CFH) is composed of 20 SCRs arranged in tandem, with a high degree of similarity among human, mouse, and rat CFH.\(^{67,69}\) By best-fit models, CFH is approximately 38 nm in length, which is nearly half that of a fully extended molecule of 20 SCRs, reflecting that the native molecule is folded back on itself (like the Greek letter \(\sigma\)).\(^{70,71}\)

CFH is unique among RCA members in that it contains solely SCRs, which it puts to good use to provide its complement inhibitory functions as well as specificity of binding to host surfaces. This distinguishes CFH from other RCA members with C-terminal transmembrane and intracytoplasmic regions (membrane cofactor protein, CR1, CR2), or points of attachment to the membrane (decay-accelerating factor) or neigh-
boring molecules in multimers (C4-binding protein). CFH has 3 C3b-binding sites, with the first in SCRs 1–4 also responsible for factor I cofactor activity, and 3 heparin/sialic acid-binding sites, which allows CFH to attach to the receptive surfaces. In particular, the high density of positively charged residues in SCRs 19 and 20 confers its binding to corresponding negatively charged residues in molecules such as heparin. It appears that when native CFH binds to a surface bearing C3b and/or heparin (or related anions) it can straighten out, thereby revealing its multiple binding sites and permitting full functionality.

Because CFH has affinity for C3b and not C4b, CFH inhibits the formation and accelerates the decay of the alternative pathway C3 convertase, C3bBb, and also serves as cofactor for factor I–mediated cleavage and inactivation of C3b to iC3b. Because it is the predominant fluid-phase alternative pathway complement regulator, abnormalities in CFH quantity or function result in uncontrolled alternative pathway activation. The C3 convertases of each of the complement activating pathways are converted to C5 convertases by the addition of C3b molecules, which localize C5 for cleavage by C2a or Bb. By virtue of its actions on C3b, CFH is an effective inhibitor of C5 convertases.

Location Relevant to Renal Diseases

CFH 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). This fact underlies attempts at liver transplantation to treat genetic CFH defects (discussed further later). Although there is evidence for CFH production by other cells of the mononuclear phagocyte lineage, bone marrow–derived cells do not contribute substantially to the circulating CFH pool. Similar to many complement proteins that are synthesized in the liver and circulate in plasma, there is evidence that CFH also is produced by intrinsic cells of the human kidney. Nonetheless, this is not sufficient to impact local or systemic complement regulation; thus transplantation of a kidney with normal CFH genes does not reverse local or systemic alterations in individuals with defective CFH. CFH also appears to be uniquely expressed on the rodent platelet and podocyte, where it functions in place of CR1 (discussed later).

Besides the key role of hepatic-derived plasma CFH to limit systemic alternative pathway complement activation, it is very clear that CFH is necessary to limit local complement activation within organs such as the kidney and eye. This is attributable to the specific binding of CFH to anionic sites in cellular and acellular regions of blood vessels, particularly those of the glomerular and choroidal capillaries.

Role in Renal Diseases

Abnormalities in CFH can underlie membranoproliferative (MP) GN type II (dense deposit disease) and atypical hemolytic uremic syndrome (aHUS). Both typical HUS and aHUS are characterized by acute kidney injury together with consumptive anemia and thrombocytopenia, but are different because aHUS is not associated with diarrheal infections (caused by Shiga toxin–producing Escherichia coli) and its propensity to recur. A substantial proportion of aHUS cases are attributable to defects in complement regulation. Thus, genetic defects in genes for CFH, factor I, and membrane cofactor protein (MCP, CD46), as well as inhibitory autoAbs to CFH are associated with MPGN. As a general rule, MPGN type II is attributable to type I mutations in CFH, leading to its altered appearance in plasma and the ensuing unrestricted systemic alternative pathway activation, whereas aHUS is attributable to type II mutations clustering in the terminal SCRs. These lead to impaired ability of CFH to bind anionic sites such as on endothelium and to provide local protection against complement activation. Overall, aHUS occurs under the right genetic and environmental conditions, with excessive complement activation occurring on endothelia. Exactly what initiates this complement activation has been elusive.

CFH also is necessary in mice to limit spontaneous and Ab/IC-dependent complement activation. Thus, CFH-deficient mice have unrestricted activation of the alternative pathway of complement, with systemic complement con-
These mice developed factor B- and C5-dependent glomerular disease with features of MPGN type II later in life, which was fatal in some animals. Complement deposition in the glomerular capillary wall (a feature of MPGN type II) preceded IC deposition (which is a feature of MPGN type I but not type II). In an accelerated model of nephrotic syndrome nephritis using relatively low (subnephritogenic) doses of Ab, CFH-deficient mice had a greater extent of disease compared with controls; similar to the spontaneous disease, this was factor B- and C5-dependent. In a model of chronic serum sickness, C57BL/6 mice did not develop GN unless deficient in CFH.

**INTRINSIC CELLULAR COMPLEMENT REGULATORS: DECAY-ACCELERATING FACTOR, MEMBRANE COFACTOR PROTEIN, AND COMPLEMENT RECEPTOR–RELATED PROTEIN Y**

**Structure/Function**

The proteins of the RCA family that provide intrinsic cellular complement regulation in human beings are decay-accelerating factor (DAF, CD55) and MCP. DAF is a glycosylphosphatidylinositol–anchored membrane protein, whereas MCP is a type 1 transmembrane protein. Despite these differences in cell attachment, they are alike in their extracellular 4 SCRs and membrane proximal O-glycosylated sites.

As is apparent throughout this issue, the use of rodents has been invaluable to developing human disease models and obtaining an in-depth understanding of disease pathophysiology. Those of us interested in complement are aided by the high degree of similarities among human and rodent complement activation pathways and constituent proteins. There are, however, some important differences in the regulatory proteins, particularly those in the RCA family. Rodents, but not human beings, have the type 1 transmembrane protein Crry (CR1-related y), named for its similarity to human CR1. Structurally, Crry is relatively small, similar to DAF and MCP, with 5 to 7 SCRs. There are also 2 Daf genes in mice; the product of Daf1 has the most relevance to human DAF and disease states of the kidney.

Although DAF, MCP, and Crry have similar structures, they have distinct functions as complement regulators. DAF has decay-accelerating activity toward C3 and C5 convertases of all pathways, whereas MCP is a cofactor for the factor I cleavage and inactivation of C3b. Interestingly, Crry has the features of DAF and MCP, and hence is a versatile and potent complement regulator.

There has been considerable interest in defining the roles of these proteins outside of their regulating complement activation, such as their capacity to generate cellular signals and to serve as receptors for the binding and uptake of microorganisms. Related to the latter, in the 12 years between the findings that CD46 and DAF are receptors for measles virus and *Helicobacter pylori*, respectively, the list has expanded to include a number of viral and bacterial pathogens. When Abs are used to cross-link DAF on the surface of T cells, there is subsequent src family tyrosine kinase-mediated cellular activation. The potential that DAF can modulate T-cell function in vivo has been supported by studies in DAF knockout mice. Although C3b is a natural ligand for DAF, the binding affinity is several logs lower than that of Abs. This has led to a search for other natural ligands of DAF, one of which is CD97, a 7TM protein with 3 to 5 epidermal growth factor repeats in its N terminus. The question remains whether the functions of DAF in T-cell immunity are related to signals through engagement of DAF by ligand and/or are a consequence of its complement inhibition. The study by Heeger et al showed augmented mouse primary T-cell responses in the absence of DAF, a phenomenon that appears to require intrinsic production of complement alternative pathway constituents (eg, factor D) and C5. This has similarities to previously discussed studies in liver macrophages, which have an apparent autocrine loop of C5a production-C5aR stimulation. However, recent studies with a human T-cell system have shown that DAF engagement with Abs or recombinant (CD97)2-Fc provides a costimulatory signal to those delivered through CD3; this appeared to be independent of complement activation products. Thus, the current understanding is that DAF limits generation of C5a and its
potential stimulatory effects on T cells, and it also can provide a productive second signal to CD3, at least when cross-linked by fluid phase CD97 or anti-DAF Abs.

Similar fascinating yet confusing issues present themselves for MCP. Cross-linking MCP with Abs can provide a costimulatory signal to anti-CD3-treated T cells. These are specific to those with a T-regulatory 1 (Tr1) phenotype. Under more physiologic conditions, costimulation of CD3 and MCP generated poorly proliferative Tr1 cells, which could be attributed to defective protein kinase B/survivin signals. Overall, this phenotype of Tr1 cells is more reflective of their state in vivo. Further evidence for the relevance of CD46 and Tr1 cells has come from recent studies in patients with multiple sclerosis, in whom there was altered Tr1 cell–associated IL-10 secretion in response to CD46 (but not CD28) costimulation. CD46 is associated with Discs Large 4 in epithelial cells and the microtubule organizing center in T cells. In the latter, ligation of CD46 can be dominant in polarization of the immunologic synapse, which can be productive or subvertive, depending on the circumstances.

Consistent with its limited distribution in rodents, MCP is not present on rodent immune cells. Instead, Crry is present with DAF on rodent T cells. Strategies to examine MCP functions in rodent models have included the use of transgenic expression of human MCP, which revealed comparable results with those of human T cells ex vivo. In addition, the role for Crry on T cells, as a surrogate for MCP, has also been examined through the use of cross-linking Abs. These studies have shown results comparable with those with MCP, in that cross-linking of Crry can activate mitogen-activated protein kinases and Vav, thereby facilitating events through CD3 engagement, as well as providing independent costimulatory signals.

Location Relevant to Renal Diseases

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 human beings, MCP appears to be distributed fairly uniformly in all 3 intrinsic glomerular cells, whereas DAF is highly expressed in the juxtaglomerular apparatus. Functional DAF is also on cultured human podocytes, which is relevant to studies with rodent podocyte DAF (discussed later). Other sites in human kidneys served by DAF and/or MCP include endothelial and tubular cells.

In rodent kidneys, DAF is primarily a podocyte and vascular endothelium protein. Unlike in human beings 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. 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 because Crry combines the functions of both. In the rodent kidney, this appears to be true in endothelial, mesangial, and tubular cells.

Role in Renal Diseases

Clearly podocyte DAF is important to restrict complement activation locally, such as can occur in nephrotoxic serum nephritis, and recovery from puromycin aminonucleoside nephrosis, in which complement proteins are accessible to the apical portion of podocytes. Consistent with this limited role, studies from Wenchao Song in MRL/lpr mice showed that there was no effect of DAF deficiency on the inflammatory lupus nephritis.

In disease states in which there is complement deposition, DAF and MCP are present in increased intensity, perhaps owing to an appropriate protective response of cells to complement activation. The regulatory influences on DAF and MCP expression have been defined further. For instance, DAF has constitutive, cell-specific, and stimulated expression, which largely can be attributed to 2 cytosine-guanine dinucleotide (CpG)-rich regions within its proximal promoter to which Sp1 binds. As with C3aR and C5aR, the expression of DAF can be up-regulated by LPS and cytokines, once again illustrating the interrelationships among different arms of the immune system.

Given its widespread distribution in glomer-
uli and tubules, Crry is likely to be very important in renal diseases. This has proven to be difficult to study directly because Crry leads to embryonic death owing to unrestricted activation of maternal complement. Nonetheless, it is quite clear that Crry is a very important complement regulator, as shown by a series of studies by Seeichi Matsuo in which Crry function-neutralizing Abs exacerbated diseases of the mesangium, podocyte, and tubulointerstitium. A fascinating series of studies from Josh Thurman showed 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 on reperfusion. This appears to be relevant to acute kidney injury (tubular necrosis) in human beings. Consistent with this work are our studies in which transplantation of Crry-deficient kidneys into wild-type recipients led to marked complement activation in the tubulointerstitium, and the rapid development of scarring and failure of the kidney.

RECEPTORS FOR C3: COMPLEMENT RECEPTORS 1 THROUGH 4

Structure/Function

CR1 (CD35) and CR2 (CD21) are type 1 transmembrane proteins with extended extracellular domains made up of tandemly arranged SCRs. Human and mouse CR2 contain 15 SCRs. Similar to CFH, the SCR arrangement in CR2 is flexible, facilitating interactions with its ligands. Human CR1 is the product of a separate gene, whereas mouse CR1 is generated by alternative splicing from the Cr2 gene, leading to the addition of 6 SCRs to the N-terminus of CR2. There are 4 human CR1 allotypes, distinguished by the number of 7 SCR-containing long homologous repeats, ranging from 3 to 6. CR3 and CR4 are members of the β2 integrin family of heterodimeric transmembrane proteins, containing the β2-subunit (CD18) and αM (CD11b) or αV subunit (CD11c). As opposed to the relatively simple make-up of RCA proteins consisting of SCRs, integrins contain a variety of distinct functional domains contributed by both subunits. The integrin ectodomain consists of head, upper leg, and lower leg segments formed by the noncovalent association of the αβ subunits. Under basal circumstances, integrins are expressed as inactive forms characterized by a bent structure in which the head segment contacts the lower leg. Activation induced by inside-out signals rapidly and reversibly switch the integrin to a ligand-binding state, most typically characterized by a linear form, although this is not essential for high-affinity binding.

The ligands for CR1-4 include C3b and its 2 factor I cleavage products: iC3b and C3d. The highest-affinity binding interactions are C3b with CR1, iC3b with CR3 and CR4, and C3d with CR2. These receptors are somewhat promiscuous in their binding because CR2 also binds Epstein-Barr virus gp350/220, the immunoregulatory CD23 protein, and IFN-α, whereas CR3 and CR4 also bind intercellular adhesion molecule (ICAM)-1 and fibrinogen. Unlike DAF, MCP, and Crry, for which the evidence is circumstantial only, engagement of CR1-4 with their C3 ligands can lead directly to cellular signaling events. However, similar to the former, activation appears to involve accessory plasma membrane molecules rather than direct effects of these proteins. This is not surprising because CR1, CR2, αM, αV, and β2 proteins each have short intracytoplasmic domains ranging from 24 to 46 amino acids. Among the best studied are the costimulatory signals provided by CR2 to B cells. In this setting, antigen-C3d complexes are bound by the B-cell receptor and CR2 in proximity, the latter recruiting CD19 and CD81 into lipid rafts, leading to the phosphorylation of CD19 tyrosine residues, which provides a costimulatory signal to B-cell receptor activation.

Location Relevant to Renal Diseases

As can be appreciated from Table 1, CR1-4 are expressed primarily on cells of hematopoietic and immune lineage. One clear exception to this is the expression of CR1 on human podocytes, on which it is capable of binding C3b. The quantities of CR1 on podocytes can vary considerably in glomerular diseases, with loss
of the entire CR1 molecule occurring in a variety of disparate glomerular diseases.\textsuperscript{187,188} Although these circumstantial findings have been established for decades, the exact role of podocyte CR1 remains undefined.

A major obstacle to defining the functions of CR1 in human beings is that rodent CR1 is not its equal. This is true for its gene and protein structure, as discussed earlier, as well as its cellular distribution, which is much more restricted in rodents.\textsuperscript{189-192} The vast majority of CR1 in human beings is present on erythrocytes, which simply reflects the numeric predominance of these cells.\textsuperscript{172} On erythrocytes, CR1 binds C3b in ICs, which is necessary for proper delivery of ICs to the mononuclear phagocyte system.\textsuperscript{193} In rodents, platelet CFH serves this purpose,\textsuperscript{88,94} which is not totally surprising given that erythrocytes and platelets are each present in blood in large numbers, and CR1 and CFH are C3b-binding proteins with factor I–cofactor activities. A similar switch appears to occur in rodent podocytes, in which CFH takes the place of CR1.\textsuperscript{93,95}

The normal kidney contains a sizable pool of cells expressing CR3 (CD11b\textsuperscript{+}), CR4 (CD11c\textsuperscript{+}), major histocompatibility class II molecules, and FC\gamma receptors.\textsuperscript{194,195} Although some of these are tissue resident macrophages (eg, F4/80\textsuperscript{+}), many express CX\textsubscript{3}CR1 typical of resident DCs.\textsuperscript{195}

**Role in Renal Diseases**

Much of the work on CRs in renal disease has focused on their roles in inflammatory cells. For example, nephrotoxic serum nephritis has a dependence on CR3.\textsuperscript{196,197} Although CR3 is present and functional on both neutrophils and monocyte/macrophages, the predominant cell affected in this model was the neutrophil, given that early glomerular accumulation of these cells was reduced.\textsuperscript{198} The conclusions regarding CR3-iC3b interactions were limited in these studies because CR3 has an affinity for ICAM-1, up-regulated in this model,\textsuperscript{199} and CR3 also can function to facilitate FC\gamma receptor functions,\textsuperscript{197,200} which clearly can be important in this model.\textsuperscript{201} Thus, an effect of CR3 deficiency to limit glomerular injury could be ascribed to preventing leukocyte binding to iC3b or ICAM-1, or to impaired signaling through the FC\gamma receptor, or any combination thereof. As noted, CR1 in mice is not expressed to the same extent as it is in human neutrophils and monocytes,\textsuperscript{191,202} making it difficult to fully study the role for leukocyte CR1 in experimental renal diseases.

Because platelet and podocyte CFH in mice are surrogates for human erythrocyte and podocyte CR1, respectively, studies of CFH in mice have provided circumstantial evidence for the roles of human E and podocyte CR1. To dissect the roles of plasma and platelet-associated CFH, we created bone marrow (BM) chimeras between $\text{CFH}^{-/-}$ and wild-type C57BL/6 mice.\textsuperscript{88} Plasma and platelet CFH protein largely tracked with the CFH status of recipient and BM donors. We also examined the role of CFH intrinsic to the kidney through the use of renal transplants between wild-type and $\text{CFH}^{-/-}$ mice.\textsuperscript{93} These have shown that wild-type kidneys in a CFH-deficient host bear CFH in the glomerular capillary wall, which by several lines of evidence is likely to originate from the podocyte. These results with BM and kidney transplants were consistent with those from cultured megakaryocytes and platelets in showing the intrinsic origin of their CFH.\textsuperscript{88,94,95}

Further studies were performed in these transplanted mice by actively inducing chronic serum sickness characterized by the presence of circulating ICs. Wild-type mice with $\text{CFH}^{-/-}$ BM had extensive glomerular deposition of complement-activating ICs. In contrast, $\text{CFH}^{-/-}$ mice reconstituted with wild-type BM had substantially less glomerular deposition of ICs and complement activation than mice with global or platelet CFH deficiency.\textsuperscript{88} Impaired glomerular IC processing occurred when extrarenal or podocyte-associated CFH were absent.\textsuperscript{93} These were taken as evidence that podocyte-associated CFH is necessary for the directed movement of ICs across the glomerular capillary wall,\textsuperscript{203} such that in its absence, subepithelial and subendothelial ICs persisted.

These studies with mouse platelet and podocyte CFH were likely to be reflective of human erythrocyte and podocyte CR1. By virtue of their capacity to bind C3b in ICs, they facilitate IC processing in the circulation and subepithelial space. It is notable, however, that in all
experimental conditions, absence of plasma CFH had the strongest bearing on the development of GN.\(^{88,93,111}\) The reason behind this was shown by Western blotting studies examining isolated glomeruli for C3b activation products.\(^{88}\) Even with excessive IC deposition as occurred in mice lacking platelet CFH, the large quantities of C3b were inactivated; in contrast, active C3b remained when plasma CFH was absent. Interestingly, mild disease also resulted in CFH-deficient kidneys, which could be attributable to CFH on podocytes serving as an intrinsic complement regulator.\(^{93}\) Most of the details behind this and whether the function of CFH on rodent podocytes is truly analogous to CR1 in human beings remains to be determined.

That the kidney can actively participate in immune reactions has been supported over the years by work from many accomplished investigators.\(^{204-209}\) Relevant to the complement system is the body of work by the Sacks group showing that intrinsic renal C3 production can markedly affect immunity to renal allografts, both in rodent models\(^{208,210,211}\) and apparently also in human beings.\(^{212}\) As noted, the normal kidney contains resident macrophages and DCs.\(^{194,195}\) How intrinsic renal immune cells and activation from locally and systemically derived complement can influence local immune responses remain areas of active investigation by a number of laboratories.

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