Chronic Progression of Tubulointerstitial Damage in Proteinuric Renal Disease Is Mediated by Complement Activation: A Therapeutic Role for Complement Inhibitors?

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Abstract. The mechanisms by which increased urinary protein concentrations lead to nephrotoxic injury are certain to be multifactorial and involve complex interactions between numerous pathways of cellular damage mediated by both cellular and humoral pathways. These may include a major role for the podocyte in glomerular diseases leading to chronic renal failure, the loss of microvascular endothelium, the albumin-induced upregulation of renal cytokines and growth factors that promote tubulointerstitial injury by inflammation and fibrogenesis, and the role of complement-mediated tubulointerstitial injury due to proteinuria. This review will focus on the last mechanism, and emphasize recent studies implicating a primary role for activation of complement in proteinuric urine as the principal mediator of tubulointerstitial damage and progressive renal disease in various experimental animal models of nephrosis. It will be our contention that intraluminal activation of the terminal complement cascade leading to the formation of the C5b-9 membrane attack complex is the principal mediator of chronic progressive interstitial damage and progressive renal failure irrespective of the type of primary glomerular injury. This paradigm has important implications for the potential therapeutic role of complement inhibitors that are currently being developed.

The Role of Proteinuria in Progressive Renal Disease: Evolving Paradigms

Although historically proteinuria has been considered as simply a surrogate marker of the severity of underlying glomerular damage, clinical and experimental data reported during more than a decade of intensive investigation indicate that proteinuria is an independent risk factor and plays an important role in the pathogenesis of the progression of renal disease (1–4). The relationship between proteinuria and poor prognosis in renal disease, as well as the benefits of dietary and pharmacotherapeutic strategies aimed at lowering urinary protein excretion, have been firmly established by several large randomized prospective studies (5–10).

The mechanisms by which increased urinary protein concentrations lead to nephrotoxic injury are certain to be multifactorial and involve complex interactions between numerous pathways of cellular damage mediated by both cellular and humoral pathways. These may include a major role for the podocyte in glomerular diseases leading to chronic renal failure (11), the loss of microvascular endothelium due to alterations in the balance between local expression of angiogenic (vascular endothelial growth factor) and antiangiogenic (thrombospondin 1) factors mediated by cytokines and vasoactive mediators (12), the albumin-induced upregulation of renal cytokines and growth factors that promote tubulointerstitial injury by inflammation and fibrogenesis (13), and the role of complement-mediated tubulointerstitial injury due to proteinuria (14–16). This review will focus on the last mechanism, and emphasize studies implicating a primary role for activation of complement in proteinuric urine as the principal mediator of tubulointerstitial damage and progressive renal disease in various experimental animal models of nephrosis. It will be our contention that intraluminal activation of the terminal complement cascade leading to the formation of the C5b-9 membrane attack complex is the principal mediator of chronic progressive interstitial damage and progressive renal failure irrespective of the type of primary glomerular injury.

Since the original observation by Risdon et al. (17), which was rapidly developed by others (18–22), it has become a widely accepted paradigm that the extent of tubulointerstitial damage is better correlated with impaired renal function than the degree of glomerular damage. In addition, accumulating evidence suggests a strong link between heavy proteinuria, subsequent tubulointerstitial injury, and progressive kidney failure (23,24). Although several mechanisms are likely to contribute to this common pathway of progressive renal failure (25–29), mounting evidence indicates that intratubular complement activation, leading to tubular cell activation or injury and the release of proinflammatory cytokines, is the principal mediator of progressive tubulointerstitial damage (30–33).
Complement Activation Is the Principal Mediator of Chronic Progressive Tubulointerstitial Damage

Hostetter and colleagues first demonstrated in a remnant kidney model that protein overload in the absence of antibody deposition is associated with the activation of complement components on the apical membrane of proximal tubules (34–36). The proposed mechanism involved augmented intrarenal levels of ammonia, a nitrogen nucleophile that has the capacity to activate C3 and thereby generate the terminal complement cascade. However, it is likely that tubular cells injured via any mechanism may become alternative pathway activators (37), whereas multiple mechanisms by which C5b-9 results in tubular and interstitial damage also likely exist (1,38,39).

Others have focused on the characterization of complement components in patient samples of proteinuric urine as mediators of tubulointerstitial injury (32,40,41). Schulze et al. were the first to document experimentally that urinary C5b-9 excretion was increased in proteinuric urine, particularly in experimental membranous nephropathy when antibody deposition and C5b-9 attack on the podocyte were occurring, an observation that allowed ongoing immunologic injury in the glomerulus to be accurately monitored by measuring urinary C5b-9 excretion, whereas protein excretion persisted long after glomerular immune deposit formation ceased (42). Similar findings in human membranous nephropathy were reported by the same group (43) and confirmed by others with suggestions that urinary C5b-9 excretion may serve as a prognostic marker in human membranous (44,45). Morita et al. have also observed differential expression of complement activation products (CAP) at both the C3 level (iC3b and Bb) and C9 level (C5b-9) in patients with different glomerular disease processes (32). Patients with focal glomerular sclerosis and diabetic nephropathy showed the highest urinary CAP excretion rates, whereas those with minimal change nephrotic syndrome exhibited no increase. Thus, increased urinary excretion of CAP is specific to urine with nonselective proteinuria. Notably, patients with membranous nephropathy showed an isolated increase in the C5b-9 excretion rate. In addition, the urinary excretion rate of CAP significantly increased in patients with nephrotic range proteinuria and correlated with the serum creatinine level, whereas the rate significantly decreased 2 wk after bicarbonate administration despite no change in the level of proteinuria or plasma CAP. The authors concluded that the degree of intra-tubular complement activation may be determined by the type of glomerular disease, level of proteinuria, degree of renal impairment, and metabolic acidosis. Montinaro et al. have also reported that in idiopathic membranous nephropathy, urinary excretion of C5b-9 may induce intrarenal synthesis of C3, mainly at the tubular level (41). The demonstration that the kidney has the capacity to synthesize most of the activation pathway components of the complement cascade has led to the suggestion that intrarenal synthesis of complement may play an important role in the pathogenesis of renal injury (46).

The strongest evidence implicating C5b-9 as the principal mediator of chronic progressive tubulointerstitial injury due to proteinuria has come from the study of various rodent models of induced glomerulonephritis or reduced renal mass, using three distinct models of complement depletion or inhibition: (1) injection of either cobra venom (to deplete complement) or the C3/C5 convertase inhibitor scCr1 (soluble complement receptor type 1) (30,47), and use of antisense oligodeoxynucleotides to knock down mRNA transcript levels of Crry (48), a rodent gene encoding a complement regulatory protein that controls the activation of the human complement inhibitors decay accelerating factor (DAF) and membrane cofactor protein (MCP); (2) use of C3 and C4 double knockout mice characterized by absolute deficiency in C3 and C4 (49); and (3) use of Piebald Viral Glaxo (PVG) rats which exhibit an absence of C6 inherited in an autosomal recessive pattern (31,33). We will confine our discussion to the third strategy, that which was used by Couser and colleagues.

To elucidate the role of C5b-9 in complement-mediated effects on renal tubular cells exposed to nonselective proteinuric urine, in vivo, equivalent levels of proteinuria were induced using the aminonucleoside (puromycin)-induced nephrosis method in normocomplementemic and genetically C6-deficient PVG rats (31). Complement-sufficient animals developed more severe tubulointerstitial damage than did C6-deficient rats, as confirmed by immunohistologic studies with three independent markers of tubular damage (vimentin, osteopontin, and proliferating cell nuclear antigen). Immunofluorescence studies showed that C3 and C5b-9 were present on the brush border of the proximal tubules, consistent with previous studies showing that the brush border of proximal tubules directly activated the alternative complement cascade (50,51). The tubular brush border localization of C5b-9 deposition also supports the hypothesis of Hostetter and colleagues that ammonia may activate C3 (34–36), because proximal tubules are the principal site of ammonium production (52). Notably, strongly positive staining for C5b-9 was observed only on the proximal tubule brush borders of complement-sufficient PVG rats, whereas the C6-deficient animals were negative for C5b-9 staining. C3 staining was also seen on tubular brush borders, but there was no significant difference in staining between complement-sufficient and C6-deficient rats. These results emphasize a specific role for C5b-9 in the pathogenesis of tubulointerstitial damage induced by proteinuric urine in a nonimmunologically mediated proteinuria model, and are supported by similar observations by Khan and Sinniah (40).

A similar strategy was used to investigate the role of C5b-9 in the progression of chronic proteinuric renal disease in a nonimmunologic model of reduced renal mass (5/6 nephrectomy in PVG rats) (33), rather than primary glomerular injury. Although this rat remnant kidney model has been widely and successfully used in the past to study mechanisms of progressive renal disease due to hemodynamic factors, glomerular sclerosis, and interstitial fibrosis, two new observations emerged from this study of the remnant kidney model in C6-deficient PVG rats. First, there appear to be two distinct phases of interstitial disease, rather than one continuous progressive one. The early phase of interstitial disease, which likely reflects effects of acute increases in glomerular, and presumably interstitial, pressures and flows after 5/6 nephrectomy, is not associated with proteinuria and exhibits revers-
Inhibitors of Complement Activation

Because complement-induced interstitial damage associated with heavy proteinuria has now been shown to be mediated principally by C5b-9, then inhibitors or regulators of complement activation may serve as potential therapeutic targets for pharmacologic intervention. Three major classes of inhibitors have been developed: synthetic molecules, recombinant natural inhibitors of complement, and antibodies to complement components or their receptors. Several synthetic small molecular weight molecules that inhibit components of the activation cascade of the complement system have been under active investigation using various models of acute inflammation in animal models. Comstatin is a synthetic peptide that binds to complement component C3 and inhibits complement activation, as demonstrated in two models of extracorporeal circulation (53). Comstatin effectively inhibited the generation of C3a, C5b-9, and the activation and binding of polymorphonuclear lymphocytes to the polymer surface, properties that make it a promising drug to avoid bioincompatibility reactions. Its simple peptide structure may allow for the development of a complement inhibitor for oral administration. The semisynthetic polysaccharide pentosan polysulfate, a polyionic molecule that resembles other glycosaminoglycans such as heparin, blocks the factor B binding site of C3b, thus preferentially inhibiting the alternative pathway. Polysaccharide pentosan polysulfate has been shown to prevent complement-mediated myocardial injury in the rabbit isolated perfused heart model (54). Unlike other glycosaminoglycans, it is orally bioavailable and has undergone phase I testing as an orally administered drug in patients with advanced cancer (55). Compound 7 (based on the structure of FUT-175 or nafamostat mesilate), a newly synthesized inhibitor of active center-directed trypsin-like serine proteases such as C1r and C1s, has been reported to exhibit greater potency and stability in vivo than FUT-175 as an inhibitor of both the classical and alternative complement activation pathways (56). To date, none of these molecules has been tested directly in progressive kidney disease.

Naturally occurring complement regulatory proteins have also been intensively investigated for their potential therapeutic efficacy as complement inhibitors. Soluble complement receptor 1 (sCr1) has been reported to attenuate three different forms of acute complement-induced glomerular injury in rodent models of glomerulonephritis, including injury targeting the endothelial cell, the mesangial cell, and the podocyte (57). Similar results have been reported with acute complement-induced lung injury in rabbits (58). The sCr1 is a soluble “decoy” receptor for complement receptor 1, a single-chain membrane-bound glycoprotein that is a potent inhibitor of C3/C5 convertases of both the classical and alternate pathways of complement activation (59). In this study, treatment with sCr1 resulted in an amelioration of pulmonary edema, a decrease in thromboxane release, and a reduction in the tissue deposition of C3c and C5b-9. A novel bifunctional chimeric complement inhibitor (DC), which contains the functional domains of the C3/C5 convertase inhibitor DAF (CD55) and the membrane attack complex inhibitor CD59, has been shown in vitro to reduce cell surface activated C3 deposition on cells expressing the chimeric molecule (60). Similarly, phospholipid anchored transmembrane versions of either DAF or the structurally related C3/C5 convertase inhibitor MCP (CD46), show equal efficiency in protection from complement mediated cell damage (61). Welch has argued that using one of the several naturally occurring complement inhibitors (sCR1, DAF, MCP, or CD59) is likely to be therapeutically successful in the treatment of human proteinuric glomerulonephritis (62). Thus far, AVANT Immunotherapeutics has developed TP-10, a recombinant soluble sCR1, which has been shown to be well tolerated in phase I clinical trials in adults at risk for adult respiratory distress syndrome and in adults with first-time myocardial infarction and in myocardial infarction (63). However, after completion of a phase IIa trial in nine patients with adult respiratory distress syndrome, AVANT ceased development of TP-10 for this indication. Interest has shifted to its use in xeno- and allo-transplantation and the treatment of human autoimmune inflammatory disorders such as multiple sclerosis, rheumatoid arthritis and lupus.

Along with sustained interest in the use of naturally occurring complement inhibitors as a clinically effective therapeutic approach to control inflammatory disorders and prevent solid organ transplant rejection (64–66), an alternative immunotherapeutic strategy based on the use of complement-specific blocking antibodies (67) is currently being evaluated in early phase clinical trials for a host of inflammatory diseases including idiopathic membranous glomerulonephritis and systemic lupus erythematosus (www.alexionpharmaceuticals.com). Membranous nephropathy was selected for study because in a rodent model closely resembling the human disease (passive Heymann nephritis) glomerular injury and proteinuria can be totally abolished by treatment to block C5b-9 activation in the intact animal, isolated perfused kidney, and isolated glomerulus (68–70). Based on the above, however, success in ameliorating a chronic proteinuric disease like membranous nephropathy by complement blockade might result as well from inhibition of C5b-9 induced interstitial disease as from reduction in glomerular injury. In lupus, the strategy is based on earlier studies demonstrating that systemic administration of a
monoclonal antibody (mAb) specific for murine C5 that effectively blocks the generation of the major chemotactic and proinflammatory factors C5a and C5b-9, prevents the onset of collagen-induced arthritis, and ameliorates established disease in a mouse model of rheumatoid arthritis (71). Similarly, continuous therapy for 6 mo with anti-C5 mAb resulted in significant amelioration of the course of glomerulonephritis (delay in the onset of severe proteinuria, normal glomerular and tubulointerstitial architecture with only minimal mesangial expansion in mice without detectable proteinuria) and in markedly increased survival (80% versus <5% at 40 wk) compared with the animals treated with control mAb, in the NZB/W F(N19
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designer recombinant murine single chain antibody fragment
(N19–8 scFv) was created that recognizes the human comple-
ment protein C5 and effectively blocks the cleavage of C5 into
C5a and C5b in vitro and in vivo (73), thereby inhibiting
terminal complement activation including formation of C5b-9.
Using established molecular cloning techniques, the antigen-
recognizing hypervariable loops (complementarity determin-
ing region) of the murine anti-C5 mAb was grafted on to
human framework regions to produce both humanized anti-C5
Fab and scFv molecules (74). These humanized anti-C5 mol-
ecules were shown to effectively block complement-mediated
lysis of chicken erythrocytes and porcine aortic endothelial
cells in a dose-dependent fashion, indicating that they retain
both the affinity and blocking functions of the original murine
mAb, and may serve as potent inhibitors of complement-
mediated human inflammatory diseases.

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