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Complement Inhibitors And Glomerulonephritis: Are We There Yet?

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I recently gave a talk at a European nephrologic gathering on future therapies for glomerulonephritis (GN). It relied heavily on data from established animal models of renal disease. At the end, the first comment came from a very stern and unhappy-looking clinical nephrologist at the back of the room who said in a booming voice, "You research guys have been standing up there for decades and telling us we are on the verge of new and better therapies, but in the 30 years I have been taking care of patients I have yet to see one of these predictions come true." He went on to chastise me for wasting the audience's time listening to tales of success in reducing the severity of GN under very controlled conditions in a laboratory, a situation that bears little resemblance to real human life.

While this obviously heartfelt response to my carefully crafted talk was not heartwarming to the speaker, the clinician indeed had a point. In fact, the history of therapeutic changes in the clinical management of GN over the past several decades has been woefully depressing for those of us who work in the area. Consider that therapy with cytotoxic drugs has been with us since the 1950s (1), pulse steroids since the late 1970s (2), and plasmapheresis, the crudest of all procedures, for about the same amount of time (3). Yet our understanding of the mechanisms that underlie GN, both acute and chronic, has mushroomed over that same time period.

Is it all a fantasy that new approaches to therapy will emerge from clearer understanding of the mechanisms involved? Was my cynical tormenter correct that nothing of therapeutic relevance in humans can ever emerge from the cellular, molecular, and animal model studies we continue to pursue, speak about, and publish?

In this issue of *JASN*, Bao *et al.* (4) from Richard Quigg's laboratory at the University of Chicago provide compelling data showing that administration of a soluble form of a recombinant complement regulatory protein (Crry) to MRL/*lpr* mice that normally develop lupus nephritis caused a marked reduction in both structural and functional manifestations of inflammatory disease in the kidney. Why is that so important? Didn't Dixon and colleagues show that complement depletion could protect from acute, antibody-induced glomerular injury decades ago? (5)

Indeed they did, but by using a model of uncertain relevance to humans and the standard reagent to inhibit complement activation, cobra venom factor, which is lethal to man. In the rapidly expanding world of new therapies for glomerulonephritis, complement inhibitors have very special promise and appeal. First of all, most forms of glomerular injury involve deposition of immunoglobulins, presumably antibodies, in the glomerulus. With the possible exception of a few very specific monoclonal antibodies, there is little evidence that antibody deposition alone is nephritogenic. The injury that results from antibody deposition is mediated largely through complement activation. Second, inhibition of complement activation, unlike inhibition of most other participants in inflammatory reactions, does not simply improve the resulting lesions but often essentially abolishes them. Third, we now recognize an important role for the complement C5b-9 membrane attack complex in non-inflammatory diseases such as membranous nephropathy as well as in acute inflammation. Fourth, it has also become appreciated very recently that complement mediates not only inflammatory reactions in glomeruli, but also the interstitial inflammatory response that occurs in chronic proteinuric disorders and leads to progressive renal failure (6). Finally, in the recent past a series of both circulating and cell-bound complement regulatory proteins have been discovered that represent naturally occurring molecules that inhibit complement activation with no known toxic side effects or immunogenicity, thus making them ideal reagents for treating complement-mediated human diseases.

What exactly is complement doing in GN that makes it such an attractive target for therapy? In the 1950s and 1960s, it was believed that complement activation injured glomeruli only indirectly when the cleaving of C3 and C5 produced C3a and C5a, potent neutrophil chemotactic molecules that attracted leukocytes to the site of immune deposits. There they became activated and released oxidants and proteases that were the actual agents of tissue injury. Although it was considered central to understanding inflammation in the kidney through the 1980s, the C5a hypothesis had never been tested directly or verified *in vivo*. In 1980, a new role for complement in GN was discovered when it was shown that proteinuria in experimental membranous nephropathy, a totally non-inflammatory lesion without neutrophil participation, was totally complement-dependent (7). This process was hypothesized to involve the

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C5b-9 membrane attack complex interaction with podocytes consequent to deposition of anti-podocyte antibodies, an hypothesis later verified in the intact animal, the isolated perfused kidney, and even the isolated glomerulus (8). This process of sublytic injury was found to characterize any lesion induced by antibody to cell membrane proteins, or deposits near cell membranes, that provided lipid bilayers for C5b-9 insertion, including mesangial and endothelial cells as well as podocytes (9,10). Sublytic quantities of C5b-9 act through a non–receptor-dependent mechanism to produce cell activation and local overproduction of oxidants, proteases, and other inflammatory molecules that induce substantial functional injury, such as loss of the integrity of the glomerular filtration barrier, to cause proteinuria (8). In fact, it now seems likely that C5b-9–induced upregulation of endothelial expression of leukocyte adherence molecules is probably more important in the generation of a neutrophilic inflammatory response than is C5a (11). This C5b-9 mechanism has been convincingly shown to mediate tissue injury in the passive Heymann nephritis (PHN) model of membranous nephropathy (12), the anti-thymocyte serum (ATS) model of immune mesangial injury as seen in IgA nephropathy, lupus nephritis, and Henoch-Schönlein purpura (9), the traditional "nephrotoxic nephritis" model of anti-GBM disease (13), and a model of thrombotic microangiopathy induced with anti-endothelial antibodies (10).

Of perhaps even greater importance than appreciating the role of C5b-9 in acute forms of antibody-mediated glomerular injury has been the more recent recognition that C5b-9 is also central to mediating the interstitial inflammation and fibrosis that inevitably accompany any chronic proteinuric disorder and seem to correlate with function and progression better than the primary glomerular lesions do (14). A role for complement in this process has been recognized since the studies of Nath *et al.* (15) in the 1980s. However, the fact that this is due to C5b-9 has only been appreciated recently with the demonstration by Nangaku *et al.* (16) that, in rats with severe nephrotic syndrome, the (congenital) absence of C6 markedly reduces the degree of interstitial inflammation and improves renal function. In fact, in the standard remnant kidney model of progressive hemodynamically mediated renal fibrosis, there appears to be an early stage of glomerular hypertrophy that is C6-independent followed by development of proteinuria and then chronic interstitial fibrosis that requires the presence of C6 (17). The only recognized function of C6 relevant to kidney disease is in the formation of C5b-9 complexes; therefore, this data implies that chronic, progressive renal insufficiency due to a variety of non-immunologic processes is mediated through sublytic attack on tubular epithelial cells by C5b-9 formed from individual complement components excreted in nonselective proteinurias of any type (18).

With that understanding of why complement regulation may be central to both acute immune and chronic renal injury of any type, it is easy to appreciate why the potential availability of nontoxic inhibitors of complement activation is so exciting. Several have been developed, including synthetic, low–molecular weight molecules such as compastatin, a C3-binding peptide that inhibits C3 activation and generation of C5a and C5b-9 (19), and polysaccharide pentosan sulfate (PPS), which inhibits the alternative complement pathway and generation of C5b-9 (20). Because of their size, both of these may be effective orally, and PPS is now in clinical trial in cancer patients (21). But neither has been tested in kidney disease. One humanized anti-C5 antibody that blocks generation of C5a and C5b-9 has been shown to benefit murine lupus nephritis as soluble Crry did in this article (22) and is currently in clinical trial in patients with membranous nephropathy and lupus nephritis (23).

The most attractive of current approaches to complement blockade is the use of molecularly engineered complement regulatory proteins, because of their low toxicity and the potential for molecular regulation of their natural expression. These come in both cell-bound and fluid-phase varieties. Of the circulating complement regulators (C1 inhibitor, C4 binding protein, Factor H, factor I, S protein, and clusterin), only clusterin has been studied in renal disease, where it has been shown that clusterin depletion increases proteinuria induced by anti-podocyte antibody (24). Of more interest are the membrane-bound complement regulators, decay-accelerating factor (DAF), membrane cofactor protein (MCP), complement receptor 1 (CR1), and CD 59. The details of how each of these inhibit complement activation, or the effects thereof, are well described elsewhere (24,25). Crry (Complement receptor 1-related gene/protein y) is a rodent analog of CR1 that also serves the functions of human DAF and MCP to block C3 convertases (26).

Of these membrane regulators, CR1 and Crry have been most studied in renal disease models. Soluble CR1 (sCR1) is the extracellular portion of a cell-bound inhibitor of C3/C5 convertase that blocks generation of both C5a and C5b, the predecessor of C5b-9. It has dramatically beneficial effects on experimental glomerular diseases induced with antibodies to podocytes, mesangial cells, and an endothelial-bound ligand (27). Crry, which has more potency as a complement inhibitor than CR1, has been studied more extensively. By inactivating Crry, Matsuo and colleagues have demonstrated that it can participate in renal injury by showing that neutralization leads to increased tubulointerstitial disease (28), increased severity of mesangioproliferative glomerulonephritis (29), and anti-GBM disease (30). The group of Quigg has pioneered the study of Crry as a potential therapeutic agent by first developing a recombinant Crry molecule, linking it to an immunoglobulin, and using it to demonstrate that it decreases severity of experimental anti-GBM nephritis (31), as does overexpression of Crry *in vivo* using transgenic mice (32).

The article in this month's *JASN* is the first to demonstrate a clear benefit of chronic administration of a soluble complement regulatory protein on a naturally occurring immune complex nephritis closely resembling lupus nephritis in humans and thus the study with the closest analogy to human disease yet published. Like most good studies, it raises additional questions regarding effects of complement inhibition on immune complex handling and correlations between glomerular structure and function. However, the bottom line is that a soluble, naturally occurring complement inhibitor administered

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chronically had a very beneficial effect in markedly reducing the severity of an autoimmune nephritis like human lupus with none of the toxic effects of steroids or cyclophosphamide. That it is still an animal model study means that it cannot yet assuage the criticism of my European colleague mentioned above who will have to wait a little while longer before the first descriptions of beneficial effects of complement inhibition in human nephritis appear.

Are we there yet? No, not quite. But, spurred by the basic and applied science illustrated by the Bao *et al.* study, very similar reagents are now in human trial, and it is certainly not premature to anticipate their availability for use in patients in the next decade.

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See related article, "Administration of a Soluble Recombinant Complement C3 Inhibitor Protects against Renal Disease in MRL/*lpr* Mice," on pages 670–679.

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