With Complements from ANCA Mice

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ANCA is found in the vast majority of patients with active small-vessel vasculitis. This disease group includes what is now known as granulomatosis with polyangiitis, microscopic polyangiitis, eosinophilic granulomatosis with polyangiitis, and a renal-limited disease form featuring necrotizing crescentic pauci-immune GN (NCGN).[1] ANCA is directed against neutrophils and monocytes and was initially considered a helpful clinical diagnostic and disease activity-monitoring tool.[2] Over the last 3 decades, numerous ANCA-induced inflammatory responses mediated by ANCA-antigen expressing neutrophils and monocytes were described.[3] However, a pioneering step was taken when a murine disease model for antimyeloperoxidase (MPO) antibody-induced NCGN was established, allowing the exploration of novel avenues.[4] The role of complement provides an ample example for such a novel and, for most clinicians, unexpected disease mechanism.

A hallmark of ANCA disease is the scantly deposited Igs and complement components. In fact, this finding distinguishes ANCA NCGN from other NCGN entities that manifest with similar glomerular fibrinoid necrosis and crescents but with either granular or linear Ig as well as complement deposits. Moreover, until recently, the lack of complement consumption in ANCA-patient plasma by routine tests (*e.g.*, C3 and C4) led clinicians to believe that complement is of no or, at most, trivial significance in this condition. Consequently, complement was not rigorously pursued in clinical and basic research on ANCA. However, not considering complement mechanistically may also have resulted in a missed chance for therapeutic intervention because promising complement-targeted strategies are emerging in clinical nephrology and beyond.

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Xiao *et al.* were the first to take advantage of a murine ANCA model by demonstrating that the alternative pathway, but not the classic or the lectin-binding complement pathways, was needed to induce NCGN.[5] The complement system consists of >30 plasma and membrane-bound proteins and the next steps were aimed at narrowing down the suspects. Huugen *et al.* identified C5 as a pivotal complement component that was essential in mediating NCGN in mice. They also showed that a C5-inhibiting antibody protected from NCGN.[6] Neutralizing C5 could be achieved with eculizumab, a humanized mAb that is already successfully used in patients with another C5-mediated renal disease, namely atypical hemolytic uremic syndrome. However, could the culprit be further delineated? C5 is processed by the C5 convertase (C3bBbC3b) yielding C5a and C5b. Together with C6, C7, C8, and C9, C5b forms the C5b-9 membrane attack complex (MAC).[7] The MAC is instrumental in pathogen recognition and elimination and should therefore be preserved if possible. Another aspect is that C5a not only activates the C5a receptor CD88 but also engages the inhibitory C5a-like receptor (C5L2). Thus, neutralizing C5 might have unnecessary drawbacks that could be avoided if C5a receptor (CD88) engagement by C5a was of utmost importance for ANCA-induced NCGN and could be specifically targeted. Using a murine disease model and bone marrow from gene-deficient mice, our group established that ANCA-activated neutrophils promote C5a generation and that the C5a–C5a receptor (CD88) interaction on myeloid cells was pivotal for NCGN.[8] These data implicated C5a receptor (CD88) blockade as a potential therapeutic strategy in ANCA vasculitis.

Despite these advances, we still faced several unresolved issues at this point. We did not know what the roles of the MAC and the C5L2 receptor were, nor did we have the tools to actually pharmacologically block the C5a (CD88) receptor. Xiao *et al.* rose to the challenge and now report the results of their study in this issue of *JASN*.[9] Using passive transfer of antibodies to mouse MPO, the investigators induced pauci-immune NCGN. Anti-MPO IgG transfer into a C6 deficiency background showed no protection. These data answered our first question, namely the assumption that MAC had no role in the disease and thus MAC depletion was not a therapeutic goal. In fact, undisturbed C5, C5b, and thus MAC formation, would enable patients with vasculitis to fight infections under immune-compromising treatment protocols. The next question was to determine the role of the C5L2 receptor. Mice deficient in this receptor more than doubled the percentage of glomerular crescents with anti-MPO–antibody transfer, establishing that C5L indeed had an inhibitory function in ANCA vasculitis. These data indicate that preventing C5a generation by inhibiting C5 would unnecessarily abolish an important protective C5a

effect mediated by the C5L2 receptor. How did the investigators tackle the issue of C5a receptor (CD88) blockade? They replaced the murine receptor by its human analog and were still able to induce NCGN by anti-MPO IgG injection, because both murine and human C5a stimulated human C5a (CD88) receptor–expressing leukocytes. This human C5a (CD88) receptor knock-in model was then suitable for testing a small molecule receptor antagonist *in vitro* and *in vivo*. The compound, CCX168, decreased chemotaxis of human C5a (CD88) receptor–expressing leukocytes *in vitro* and in a peritonitis model. Most importantly, daily oral CCX168 treatment dose-dependently abrogated ANCA-induced NCGN in these human C5a (CD88) receptor knock-in mice.

Where are we now? Further research is necessary because there are more questions to be answered. What activates the alternative complement pathway in ANCA disease? Reactive oxygen species, serine proteases, and properdin are still candidates to be inspected. We have not yet explored the occurrence of mutations in complement-inhibitory proteins or the presence of neutralizing antibodies to complement inhibitors in ANCA patients. Is complement a component of ANCA-induced neutrophil extracellular traps? Are C5a-mediated neutrophil priming and chemotaxis the only mechanisms promoting NCGN? Despite these open questions, we find it amazing how the complement story in ANCA vasculitis began with so little evidence for complement activation in patients and where this story stands today. Mouse experiments took a little more than 5 years to teach us that C5a and its receptor are important disease mediators in ANCA-induced NCGN and are worthy targets for human therapeutic intervention. Now, with the animal data in mind, investigators should look harder into the human condition. We had learned that we have nothing to expect from assessing plasma C4, because the classic complement pathway has no role, at least not in mice. Plasma C3 might just not be sensitive enough to reveal the alternative pathway activation. Investigators went back to their patients and looked for complement activation by more sensitive tools. Chen *et al.* detected C3c in the glomerular capillary walls by direct immunofluorescence in a third of patients with active ANCA,[10] again raising the question as to "how pauci is pauci"? Importantly, patients who exhibited positive staining on their biopsies had more proteinuria and worse renal function at presentation. Gou *et al.* found that plasma C5a was significantly higher in patients with active ANCA vasculitis compared with patients in remission.[11] Additional plasma complement components were elevated with active disease, and Bb correlated with the extend of crescent. Immunohistochemistry showed that Bb, C3d, and MAC were deposited in the glomeruli of patients with NCGN. In another clinical study, C5a was also increased in the plasma and urine of patients with active ANCA vasculitis compared with patients in remission. Renal C5a (CD88) receptor expression was downregulated and the C5L2 receptor was upregulated.[12] Thus inspired by mouse data, increasing evidence from ANCA vasculitis patients has now accumulated, establishing alternative complement activation with increased anaphylatoxin C5a.

These recent findings provided an encouraging rationale for translating knowledge obtained in murine-model systems from bench to bedside. ChemoCentryx has launched a clinical multicenter trial to explore the effect of the very CCX168 compound that was used in the mouse experiments by Xiao *et al.* published in this issue of *JASN*.[9] The study is designed as a placebo-controlled phase II clinical trial in patients with ANCA-associated renal vasculitis. The main objective is to determine whether CCX168 could at least partially substitute for glucocorticoid treatment in remission induction. We await these results with great anticipation. A truly translational research story could come to an (preliminary) end and encourage clinician scientists to stay on the complement path.

DISCLOSURES

None.

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See related article, "C5a Receptor (CD88) Blockade Protects against MPO-ANCA GN," on pages 225–231.

Macrophage Dynamics in AKI to CKD Progression

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Stemming from the recent and robust clinical data confirming that AKI does indeed increase the likelihood of developing CKD (reviewed by Chawla and Kimmel[1]), numerous preclinical studies have offered insight into how the transition from AKI to CKD occurs. However, our understanding of this multifactorial process is incomplete. This knowledge is critical if we are to advance therapeutic strategies to combat the detrimental progression of renal injury. The development of CKD is thought to be promoted by an aberrant wound healing response involving tubular epithelial cells (TECs), fibroblasts, pericytes, myofibroblasts, fibrocytes, immune cells, and others, which leads to progressive fibrosis in the kidney and loss of viable nephrons.[2] Previous studies have demonstrated that cell cycle arrest of TECs,[3] as well as epigenetic modifications in renal fibroblasts,[4] are key components in fibrogenesis and the decline in renal function. In an integrated model,[5] cell cycle arrest of TECs leads to unchecked production of profibrotic mediators such as TGF-β and connective tissue growth factor, which induce sustained fibroblast activation and proliferation causing excessive extracellular matrix production, establishment of fibrotic lesions, and CKD.

The inflammatory nature of AKI, involving resident cells of the renal mononuclear phagocytic system[6,7] and infiltrating immune cells,[8] suggests that leukocytes and their products would also influence the fate of the injured kidney. For example, sustained leukocyte accumulation and activation inside the kidney would promote extended periods of ischemia due to vascular congestion and may induce direct tubular and endothelial cell damage by the release of inflammatory mediators. Macrophages are among the innate leukocytes that rapidly accumulate in the kidney and promote inflammation in the acute phase of AKI,[9,10] yet macrophages also have a critical role in wound healing as scavengers of proinflammatory cell debris and as promoters of regeneration.[7,10–13] It should be mentioned here that the study of renal resident and/or infiltrating monocytes, macrophages, and dendritic cells is complicated by the overlapping phenotypes and surface marker expression by these different cell types in the kidney during health and injury,[7] and use of the term *macrophage* to describe the cell types discussed herein is for simplicity.

The dual role of macrophages in AKI and subsequent repair and regeneration can be explained by the multiple phenotypes that a macrophage can adopt. There are several different major classifications of macrophage phenotype, including M1 (classically activated) and M2 (alternatively activated) macrophages. Most macrophages fall somewhere in the spectrum between M1 and M2 and may change their characteristics depending on their environment.[13] M1 macrophages are considered proinflammatory and make cytokines such as IL-1, IL-6, and TNF-α, whereas M2 are mainly anti-inflammatory and express arginase, mannose receptor, IL-10, and IL-4 receptor-α.[13] Thus, macrophages display considerable diversity and plasticity, such that the same cell can promote and inhibit inflammatory (or other) processes in different contexts.

Early studies suggested that macrophages promote initial ischemia reperfusion injury (IRI) in mice[9,14] and promote fibrosis after IRI in rats.[15] These observations were made when monocyte/macrophage migration to the kidney, through CCR2- and CX3CR1-dependent mechanisms, was attenuated,[14] or when macrophages were depleted using liposomal clodronate before injury or within 3 days after ischemic insult, respectively. Recent mouse studies have added support to the hypothesis that M1-type, clodronate-sensitive macrophages do participate in initial injury,[11,16] but have also revealed that macrophages are critical for the normal repair processes that inhibit the progression of fibrosis and CKD.[10–12] Using several sophisticated mouse models, Lin *et al.* demonstrated that macrophages responding to renal injury produce and release the Wnt ligand Wnt7b that acts on injured and regenerating TECs to promote their continuation through the cell cycle and regeneration of the tubule basement membrane, thus re-establishing renal function and reducing fibrosis.[12] Importantly, it was demonstrated that the M1 macrophages that traffic to the postischemic kidney change their phenotype *in situ* to the anti-inflammatory M2 phenotype.[11] Collectively, these studies have greatly enhanced our understanding of the role of macrophages in the normal and abnormal injury and reparative response of the kidney after AKI. However, the studies investigating the influence of macrophages on recovery/transition to CKD have relied on liposomal clodronate and/or the

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