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 antimeyloperoxidase (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 scanty 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.

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 deficient 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, 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. 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. 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. 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.

**REFERENCES**