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ANCA Are Pathogenic—Oh Yes They Are!

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During the past two decades, remarkable progress has been made in our understanding of anti-neutrophil cytoplasmic autoantibodies (ANCA) and the associated diseases. The early years were marked by the discovery and elucidation of the antigens that were the targets of the ANCA autoimmune response (1). Myeloperoxidase (MPO) and proteinase-3 (PR3) were rapidly determined to be the major ANCA autoantigens. Clinical and pathological correlates of ANCA were described and refined. The reclassification of small-vessel vasculitis ensued through the efforts of the International Consensus Conference on the Nomenclature of Systemic Vasculitis, which brought together investigators whose interest in vasculitis had emerged or had been renewed by the discovery of ANCA (2). Many of these events have been described in this issue by Kamesh *et al* (3).

In 1990, we first observed that ANCA were more than a serologic marker of disease and could stimulate leukocytes to undergo a respiratory burst and degranulate primary granular constituents. This supported a direct pathogenic role for ANCA. Since those early observations, investigators from across the globe have demonstrated that ANCA are capable of activating neutrophils and monocytes in a wide variety of ways resulting in the release of reactive oxygen species, granule proteins, cytokines, chemokines, and adhesion molecules. Leukocytes that have been activated by ANCA adhere to endothelium and cause endothelial cell damage (4). These *in vitro* observations show that ANCA can activate neutrophils and monocytes, which indicates that an activation signal is generated by the interaction of ANCA with leukocyte cell surfaces.

The signal transduction pathways by which this activation process occurs have been the subject of extensive investigations. The signaling mechanisms rely on both Fc gamma engagement as well as Fab'2 engagement of antigens on the surface of the cell (5). These two signaling pathways conspire to either signal neutrophils or monocytes to spill their noxious constituents or, more subtly, to transcribe and translate a number of molecules that perpetuate or modulate the immune response. For example, in this issue of the JASN, Kettritz *et al.* (6) explore a role for phosphatidylinositol 3 kinase in controlling the ANCA-induced respiratory burst in neutrophils.

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It could be argued that all of these *in vitro* studies are merely laboratory artifacts that have little or no pertinence to the pathogenesis of the human condition. Until recently, only a paucity of *in vivo* data could be marshaled to counter this contention. There are, however, emerging clinical and *in vivo* (animal model) observations that provide compelling evidence that ANCA are primarily and directly involved in the pathogenesis of small-vessel vasculitis. From the clinical human perspective, ANCA small-vessel vasculitis is an inflammatory disease that is tightly correlated with the presence of anti-neutrophil cytoplasmic autoantibodies. Less than 10% of patients with clinically and pathologically identical diseases do not have ANCA, but at least 90% of patients with Wegener's granulomatosis, microscopic polyangiitis, and the Churg-Strauss syndrome have either MPO-ANCA or PR3-ANCA. There is a loose correlation between ANCA titer and disease activity; however, these studies may be hampered by the imprecision of the ANCA assays themselves (1). The most convincing observations in humans that support a pathogenic role for ANCA are the many reported instances of induction of MPO-ANCA by drug treatment, especially with propylthiouracil, minocycline, and pimagedine, resulting in some patients in the development of necrotizing glomerulonephritis and small-vessel vasculitis. (7)

The most compelling data proving that ANCA are pathogenic stem from recent animal investigations. Early animal models of ANCA small-vessel vasculitis relied on examples of polyclonal B cell activation in animal models of MRL/lpr or SCG/KJ mice (8). In these animals, glomerulonephritis was associated with antibodies to myeloperoxidase. The next generation of experiments were performed first by Kobayashi *et al.* (9) and then Heeringa *et al.* (10). Animals were given sub-nephritogenic doses of antiglomerular basement membrane (anti-GBM) antibodies with or without immunization with MPO that produced anti-MPO antibodies. Animals with both anti-GBM and anti-MPO antibodies developed necrotizing and crescentic glomerulonephritis, whereas animals that received only anti-GBM antibodies developed only minor disease.

Most recently, two different groups of investigators have demonstrated that anti-MPO antibodies alone can cause necrotizing and crescentic glomerulonephritis. In our own studies, murine MPO knockout mice were immunized with murine MPO and developed anti-MPO antibodies. Splenocytes from these animals were transferred into Rag2 knockout mice that lack functioning T and B lymphocytes. In a dose-dependent fashion, these mice developed circulating anti-MPO and necrotizing and crescentic glomerulonephritis and a small-vessel

vasculitis in the lung, lymph node, and spleen (11,12). Control Rag2 knockout mice populated with splenocytes stimulated by bovine serum albumin developed no glomerular necrosis or crescents and no systemic vasculitis. All Rag2 knockout mice that received immune-competent splenocytes developed glomerular immune complex accumulation, but only mice with circulating anti-MPO developed crescentic glomerulonephritis and vasculitis. In a separate series of experiments, anti-MPO IgG derived from immunization of MPO knockout mice were transferred alone without splenocytes; a focal pauci-immune necrotizing and crescentic glomerulonephritis was found. Although the number of glomeruli injured was fewer than in the experiments in which splenocytes were transferred, these lesions were pauci-immune in nature, resembling human MPO-ANCA necrotizing and crescentic glomerulonephritis.

In another recent study, Smyth *et al.* (13) induced glomerulonephritis and pulmonary hemorrhage by immunization with human MPO that resulted in the production of circulating anti-MPO that cross-reacted with rat MPO. Injection of low doses of anti-GBM antibodies into these rats with anti-MPO resulted in the development of severe necrotizing and crescentic glomerulonephritis.

ANCA do not act alone. It is reasonable to conjecture from the existing animal studies that low-level immune complex deposition or the presence of sub-nephritogenic does of anti-GBM antibodies provide an inflammatory environment that is amplified by the effects of MPO-ANCA–primed leukocytes. This results in necrotizing vascular damage.

In humans, there may be multiple factors that contribute to the initiation of the ANCA autoimmune response and the induction of injury by ANCA. For example, there is evidence that environmental pressures play a role in the development of ANCA disease, including silica exposure (14). There may be genetic factors as well that provide additional "hits" for the full expression of small-vessel vasculitis. This may explain the many patients who have persistently high levels of circulating MPO or PR3-ANCA yet have no overt evidence of clinical small-vessel vasculitis. Infections may be able to provide synergistic inflammatory stimuli that interact with ANCA to cause disease. For example, the onset of a flu-like illness with high local and systemic levels of proinflammatory cytokines or the presence of *Staphylococcus aureus* infection in the upper airways (15) may be sufficient stimuli to provide a synergistic inflammatory stimulus resulting in the development of widespread vasculitis in a patient with ANCA.

An interesting and exciting area of future research is the elucidation of the factors responsible for the amplification and perpetuation of the ANCA small-vessel vasculitis and glomerulonephritis. In addition, we must understand why this disease process is so focal. For example, why one segment of a glomerulus may be completely destroyed by a necrotizing lesion while an adjacent segment or an adjacent glomerulus is apparently unscathed. Although this pattern of focal disease may be stochastic, it is more appealing to postulate that the endothelium has been preferentially damaged in that area, making it susceptible to damage by leukocytes that have been stimulated by ANCA to cause lytic destruction.

Another fruitful area of future investigation will focus on understanding the cause of the ANCA autoimmune response, *i.e.*, what triggers ANCA production in one group of individuals and not another? Such studies have gained renewed importance now that there is convincing evidence that, yes, ANCA *are* pathogenic.

Editor-in-Chief Note

Dr. Falk, a pioneer in the area of ANCA-positive vasculitis and glomerulonephritis, makes a compelling case for a pathogenic role for ANCA in these diseases. It should also be remembered, however, that there is now equally compelling evidence that cell-mediated immune mechanisms, absent ANCA or other antibodies, can also mediate severe, pauci-immune, crescentic glomerular lesions (1,2). The relative role of these two processes in human glomerular disease has not been resolved, and it is likely that both are probably involved (3,4).

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See related article, "Phosphatidylinositol 3-Kinase Controls Antineutrophil Cytoplasmic Antibodies—Induced Respiratory Burst in human Neutrophils," on pages 1740–1749.