

# Pathophysiology of ANCA-associated vasculitides: Are ANCA really pathogenic?

P. HEERINGA and J.W. COHEN TERVAERT<sup>1</sup>

Department of Clinical and Experimental Immunology, Cardiovascular Research Institute Maastricht, University Maastricht, Maastricht, The Netherlands

**Pathophysiology of ANCA-associated vasculitides: Are ANCA really pathogenic?** The strong association of antineutrophil cytoplasmic autoantibodies (ANCA) with certain forms of small vessel vasculitis suggests a pathogenic role of these autoantibodies in the disease process. In vitro, ANCA can activate neutrophils and monocytes to produce reactive oxygen intermediates, to release lysosomal enzymes, and to secrete proinflammatory cytokines. More recently, it was demonstrated that antimyeloperoxidase ANCA can induce systemic vasculitis and glomerulonephritis in mice. Taken together, these data provide convincing evidence that ANCA are indeed pathogenic.

Within the group of small vessel vasculitides, Wegener's granulomatosis, microscopic polyangiitis, Churg-Strauss syndrome, and the renal limited forms of these disease (i.e., idiopathic necrotizing crescentic glomerulonephritis) are strongly associated with antineutrophil cytoplasmic autoantibodies (ANCA) [1]. ANCA are a group of autoantibodies directed against enzymes contained in neutrophil granules and lysosomes of monocytes. The main antigenic targets of ANCA are proteinase 3 (PR3), a 29 kD serine protease, and myeloperoxidase (MPO), a 140 kD enzyme involved in the generation of reactive oxygen species. To date, many clinical studies have confirmed that PR3-ANCA and MPO-ANCA are highly specific for Wegener's granulomatosis and microscopic polyangiitis, respectively [1].

Since the discovery of ANCA in the early 1980s, the detection of these autoantibodies has proven to be an important diagnostic marker and, currently, is routine practice in many laboratories around the world. Also, given the strong association of ANCA with these diseases, hypotheses have been raised about the possible pathogenic role of ANCA in the disease process.

Here, we will briefly summarize the evidence obtained from in vitro and in vivo studies that ANCA are a ma-

ajor player in the pathophysiology of ANCA-associated vasculitides.

## IN VITRO EVIDENCE THAT ANCA ARE PATHOGENIC

It is generally accepted now that ANCA are capable of activating the cells that are the source of the ANCA antigens (i.e., neutrophils and monocytes [2]). In this respect, the best studied effects of ANCA on these cells are induction of the respiratory burst and the extracellular release of lysosomal enzymes, including the ANCA antigens themselves [2]. Coincubation of ANCA-activated neutrophils and endothelial cells results in endothelial cell lysis, suggesting that these effector pathways can be responsible for the vessel wall injury observed in vivo [3, 4]. Moreover, ANCA can stimulate monocytes to produce proinflammatory cytokines such as interleukin (IL)-1 $\beta$ , tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), IL-6, IL-8, and monocyte chemoattractant protein-1 (MCP-1) [5–7]. Through recruitment and activation of leukocytes, these cytokines may contribute to the amplification and propagation of the inflammatory response associated with necrotizing vasculitis.

Although the exact mechanisms involved in ANCA-induced neutrophil activation are not completely understood, most studies have shown a requirement for neutrophil priming with proinflammatory stimuli, in particular TNF- $\alpha$  [2]. Neutrophil priming refers to the process where the response of the cell to an activating stimulus is enhanced by prior exposure to low nonactivating concentrations of the same or another stimulus. In the case of TNF- $\alpha$ , priming induces up-regulation of adhesion molecule expression and facilitates the reduced form of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase complex formation through the translocation of cytochrome b558 to the cell surface [8, 9]. Importantly, it also results in expression of the ANCA antigens on the cell surface making them accessible for interaction with ANCA [10, 11]. Simultaneous engagement of the Fab portion of ANCA with ANCA antigens on the cell

<sup>1</sup>Participant and speaker.

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surface and interaction of the Fc part of the antibody with Fc receptors is generally thought to be a prerequisite for ANCA-induced neutrophil activation [1, 12, 13]. In some studies, however, F(ab)<sub>2</sub> fragments of the ANCA antibodies have been found to activate neutrophils, suggesting that Fc receptor independent pathways are involved in ANCA-induced neutrophil activation as well [14]. The observation that ANCA-induced neutrophil activation requires prior priming of the neutrophil correlates well with the clinical observation that, in patients suffering from systemic vasculitis, exacerbations are often preceded by infections.

Investigations into the signal-transduction pathways activated in ANCA-induced neutrophil stimulation have demonstrated the involvement of p38 mitogen-activated protein kinases (MAPK), extracellular signal-regulated kinases (ERK), as well as phosphatidylinositol 3 (PI<sub>3</sub>) kinase signaling systems [15–17]. However, this area of ANCA research is still in its infancy and much remains to be discovered. In this respect, Yang et al [18] recently made use of the microarray technology to study differential gene expression in neutrophils activated by whole ANCA IgG or ANCA F(ab)<sub>2</sub> fragments. They observed that expression of some genes was unique to activation by whole ANCA IgG, whereas other genes were preferentially induced by F(ab)<sub>2</sub> fragments of the antibodies. In the future, this approach, together with detailed biochemical and molecular studies, may help in elucidating the mechanisms of ANCA-induced neutrophil activation and provide more insight into the underlying signaling pathways.

### IN VIVO EVIDENCE THAT ANCA ARE PATHOGENIC

Although *in vitro* experiments as described above support the contention that ANCA are pathogenic, animal models are required to prove causality. The development of an animal model for ANCA-associated vasculitis has, however, proven to be a difficult task. Using various experimental approaches, several animal models have been proposed but all of these have important limitations. For example, MPO-ANCA can be detected in mercury chloride-treated rats and in autoimmune prone mouse strains such as MRL-lpr/lpr mice and SCG/Kj mice [19–21]. However, in these animals, various other autoantibodies are induced due to polyclonal B-cell activation, which complicates evaluation of the pathogenicity of the MPO-ANCAs.

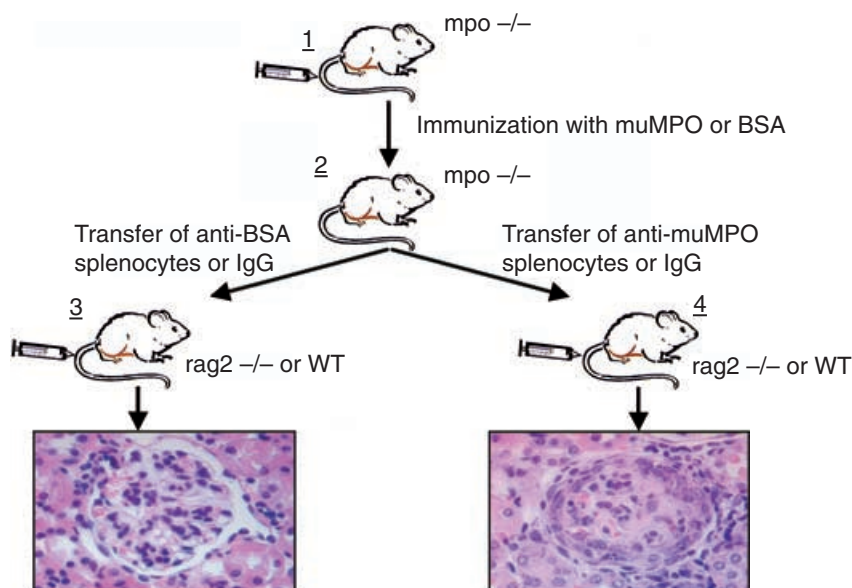
Active immunization of rats with human MPO induces antibodies to human MPO that crossreact with rat MPO. In these rats, subsequent renal perfusion or systemic administration of human neutrophil lysosomal extracts induces crescentic glomerulonephritis or pulmonary and

intestinal vasculitis, respectively [22, 23]. Also, crescentic glomerulonephritis develops in human MPO-immunized rats treated with doses of anti-glomerular basement membrane (GBM) antibodies that in control immunized rats lead to mild anti-GBM disease only [24]. These studies indicate that MPO-ANCA can render mild glomerular injury into clinically severe disease but do not prove that ANCAs are an independent cause of disease. Moreover, these models are hampered by the use of heterologous MPO and neutrophil extracts and in that sense are not autoimmune models.

In preliminary studies [abstract; Smyth et al, *J Am Soc Nephrol* 13s:170S, 2002], it was recently found that Wistar Kyoto (WKY) rats also develop circulating anti-rat MPO antibodies upon immunization with human MPO. Interestingly, these rats developed proteinuria and hematuria and, 8 weeks after immunization, showed segmental inflammation of glomeruli, deposits of fibrin with and without crescents, tubular red cell casts, and tubulointerstitial inflammation. In the lungs, signs of alveolar hemorrhage, perivascular cuffing by leukocytes and fibrin deposits in vessel walls were found. The paucity of immune complexes observed on immunohistochemical evaluation closely resembles the human disease. This promising study emphasizes the importance of the background rat strain and timing in the development of models of ANCA-associated vasculitis.

In a recent study, Xiao et al [25] provided direct and convincing evidence that MPO-ANCA can cause pauci-immune glomerulonephritis and vasculitis in mice. The strategy used in this study is depicted in Figure 1 and is based on immunization of MPO-deficient mice to circumvent tolerance to MPO that would normally occur in MPO-expressing mice. Indeed, it was shown that when MPO  $-/-$  mice were immunized with murine MPO, circulating antimurine MPO antibodies developed. Adoptive transfer of anti-MPO splenocytes into immune-deficient recombinate activating gene-deficient mice (Rag2  $-/-$ ) that lack functioning T and B cells resulted in circulating anti-MPO-ANCA and the development of crescentic glomerulonephritis and systemic vasculitis. In contrast, Rag2  $-/-$  mice receiving splenocytes from bovine serum albumin (BSA)-immunized MPO  $-/-$  mice developed only mild glomerulonephritis without necrosis or crescents.

To test the pathogenic potential of the antibodies alone, a second series of experiments was performed in which purified IgG from murine MPO or BSA immunized MPO  $-/-$  mice was intravenously injected into Rag2  $-/-$  mice or wild-type mice. These studies showed that passive transfer of anti-MPO IgG induces a pauci-immune, focal necrotizing crescentic glomerulonephritis and systemic vasculitis mimicking the human disease and provide direct evidence that MPO-ANCA are pathogenic.



**Fig. 1. Strategy for the development of a mouse model of anti-myeloperoxidase (MPO)-associated glomerulonephritis/vasculitis.** **1,** MPO-deficient mice (*MPO*<sup>-/-</sup>) are immunized with murine MPO (muMPO) or bovine serum albumin (BSA). **2,** Splenocytes and IgG are obtained from immunized *MPO*<sup>-/-</sup> mice. **3,** Adoptive transfer of BSA+ splenocytes into *Rag2*<sup>-/-</sup> or passive transfer of BSA+ IgG into *Rag2*<sup>-/-</sup> or wild-type mice induces no disease. **4,** Adoptive transfer of muMPO+ splenocytes into *Rag2*<sup>-/-</sup> or passive transfer of muMPO+ IgG into *Rag2*<sup>-/-</sup> or wild-type mice induces vasculitis and necrotizing crescentic glomerulonephritis. Images represent glomerular lesions as observed 6 days after passive transfer into wild-type mice of anti-BSA IgG and anti-MPO IgG, respectively [periodic acid-Schiff (PAS) staining, ×400].

## CONCLUSION

Evidence derived from *in vitro* studies in conjunction with the *in vivo* observation that MPO-ANCA induce systemic vasculitis in mice implies that the association in patients between MPO-ANCA and pauci-immune glomerulonephritis and vasculitis is due to causation. Importantly, the mouse model will be a powerful tool for the study of pathogenic processes involved in ANCA-induced vasculitis, to identify new targets for treatment, and to test new experimental therapies. It will be interesting to see whether, using a similar approach, an animal model for PR3-ANCA-induced vasculitis can be developed. This would not only increase the evidence for a pathogenic role of ANCA but could also give more insight into the possible pathophysiologic differences between MPO- and PR3-ANCA associated disease as has been proposed by some investigators [26].

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Reprint requests to Prof. Dr. J.W. Cohen Tervaert, Department of Clinical and Experimental Immunology, University Maastricht, Universiteitssingel 50 6229 ER, Maastricht, The Netherlands.  
E-mail: jw.cohentervaert@immuno.unimaas.nl

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