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REVIEW

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

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## ABSTRACT

**The strong relation between antineutrophil cytoplasmic autoantibodies (ANCA) and primary vasculitic syndromes suggests a pathophysiological role for ANCA. Experimental evidence for the pathogenic potential of ANCA has been derived from *in vitro* studies that demonstrate that ANCA can activate tumour necrosis factor α primed neutrophils, monocytes and/or endothelial cells. The binding of ANCA to primed neutrophils results in activation of these cells by a process that is largely dependent on engagement of β-2 integrins and on the interaction of the Fc portion of ANCA. An Fc-independent mechanism is, however, also operative. In experimental animal models, it has been demonstrated that immunisation with myeloperoxidase (MPO) induces MPO-ANCA. The induction of ANCA, however, is not sufficient to induce vasculitis *in rats* since immune complexes first have to be deposited along the vessel wall before lesions develop. When MPO-deficient *mice* are, however, immunised with murine MPO, anti-MPO immunoglobulins are purified and subsequently injected into mice that are not deficient for MPO, systemic vasculitis and glomerulonephritis is induced. These experiments suggest that ANCA indeed induces vasculitis. Risk factors for breaking self-tolerance to ANCA antigens are genetic factors, drugs, chemical substances and/or infectious agents.**

## INTRODUCTION

Within the group of small-vessel vasculitides, Wegener's granulomatosis (WG), microscopic polyangiitis (MPA), Churg Strauss Syndrome (CSS) and the form of these diseases that is limited to the kidneys, namely idiopathic necrotising crescentic glomerulonephritis (NCGN), are closely associated with antineutrophil cytoplasmic autoantibodies (ANCA). The lesions in those diseases, particularly demonstrated in the kidneys, are 'pauci-immune', meaning that no immune deposits are found in most cases. ANCA in these diseases are directed against proteinase 3 (PR3) or myeloperoxidase (MPO) (*table 1*). Besides being a helpful diagnostic tool, determination of ANCA levels can also be useful for monitoring disease activity, since relapses of disease are often preceded by rises in ANCA levels.[1,2] Furthermore, persisting high levels of ANCA are associated with a poor renal outcome.[3] The strong relation between ANCA and primary vasculitic syndromes suggests an important role for ANCA in the pathophysiology. Experimental evidence for the pathogenic potential of ANCA has been derived from both *in vitro* and *in vivo* studies and will be reviewed.

## PATHOPHYSIOLOGY OF ANCA-ASSOCIATED VASCULITIS: *IN VITRO* DATA

*In vitro*, ANCA can activate neutrophils primed with tumour necrosis alpha (TNF-α) for the production of reactive oxygen intermediates (ROI), the release of lysosomal enzymes, and the secretion of interleukin-1β.[4,5] Furthermore, it has been demonstrated that ANCA are able to stimulate neutrophils to adhere to cultured human endothelial cells, a process that can be inhibited by anti-CD18 antibodies.[6] Johnson *et al*. have clarified the mechanism by which ANCA might

**Table 1**
*Characteristics of ANCA-associated vasculitides*

| DISEASE | CLINICAL | MPO ANCA | PR3 ANCA |
|---|---|---|---|
| Churg-Strauss syndrome | Asthma, eosinophilia, neuropathy | >70% of patients | <10% of patients |
| Wegener's granulomatosis | Nose bleeds, nephritis, lung lesions | 10-30% of patients | >70% of patients |
| Microscopic polyangiitis | Nephritis, purpura, haemoptysis | 30-70% of patients | 30-70% of patients |
| Idiopathic NCGN | Nephritis | >70% of patients | 10-30% of patients |

*NCGN = necrotising crescentic glomerulonephritis.*

stimulate neutrophil adherence by showing that ANCA stimulate the upregulation of CD11b on neutrophils *in vitro*.[7] Finally, it has been demonstrated that ANCA-stimulated primed neutrophils can lyse cytokine-pretreated cultured endothelial cells.[8,9] Apart from inducing the activation of neutrophils, ANCA also activate monocytes[10] and/or endothelial cells.[11,12]

The mechanisms involved in ANCA-mediated neutrophil activation are not completely understood. Upon priming with TNF-α, neutrophils express PR3 and MPO on the cell surface which then become accessible for interaction with ANCA.[4] It is thought that PR3 and MPO binding to the cell membrane is both through charge interactions and receptor mediated. Binding of ANCA to primed neutrophils may result in activation of neutrophils, a process that is largely dependent on engagement of $\beta_2$ integrins and the interaction of the Fc portion of ANCA.[13] A Fc-independent mechanism, however, has also been described to be operative *in vitro*.[4,14] Recently, Ben Smith *et al.* demonstrated that ligation of FcγRIIa and FcγRIIIb is necessary for ANCA-induced neutrophil activation. Since the signalling cascades that are used by ANCA are different from the signal pathways used by FcγR engagement only, it is suggested that apart from Fcγ engagement also other membrane co-factors are used by ANCA for neutrophil activation.[15] These other membrane co-factors have not been identified yet. The signals involved in neutrophil activation have been recently dissected and include p38 mitogen-activated protein kinase (MAPK) and extracellular signal-regulated kinase (ERK) as well as phosphatidylinositol 3 kinase control systems.[16]

## PATHOPHYSIOLOGY OF ANCA-ASSOCIATED VASCULITIS: ANIMAL STUDIES

Several types of pathophysiological events that may lead to vessel wall damage in vasculitis are currently recognised. These include pathogenic immune complex deposition or *in situ* formation, a 'Shwartzman-like' phenomenon in which intravascular activation and aggregation of neutrophils may be operative, antibody-dependent cell-mediated cytotoxicity, and cell-mediated immune responses.[17]

Pathogenic immune complexes (ICX) deposition-mediated vasculitis is best depicted in the serum sickness animal model. In this model, rabbits are injected with bovine serum albumin and about seven to ten days later immune complexes are found that may induce vasculitis and/or glomerulonephritis. In ANCA-associated vasculitis, however, immune complexes are generally not found in the lesions. Therefore, the classic renal lesion in ANCA glomerulonephritis is labelled 'pauci-immune'. In kidney biopsies of patients with ANCA-associated glomerulonephritis, we found no IgA or IgG deposits and only nonspecific IgM deposits in a minority of the patients.[18] Complement deposition, however, is often present (in about 50% of the cases).[18] This may point to prior ICX deposition, but there is no proof for this hypothesis.[19]

To test the hypothesis that ANCA themselves may induce vasculitis,[20] we immunised BN rats with human MPO which induced antibodies to human MPO which also cross-reacted with rat MPO.[21] Furthermore, in these rats a cellular response to MPO could be detected.[21] To our surprise, these rats appeared completely normal and no vasculitic lesions were found at autopsy. So, the induction of ANCA is not sufficient to induce vasculitis in rats. We hypothesised that there must first be ICX deposition at vessel walls. These ICX then attract neutrophils and these neutrophils then express MPO on their cell surface that may bind anti-MPO, which results in an overstimulation of the neutrophils resulting in vasculitis and also the rapid disappearance of ICX. To test this hypothesis, we injected MPO-immunised rats with an extract of neutrophils containing MPO, and hydrogen peroxide ($H_2O_2$). In this context we predicted that ICX deposition and subsequently vasculitis would occur. We observed vasculitis of the lungs and the gut in the rats that were immunised with MPO but not in the rats that were not MPO immunised and received the neutrophil extract only.[22] Unfortunately, no glomerulonephritis was found. However, after unilateral perfusion of the left kidney with the neutrophil extract and $H_2O_2$ we saw a severe form of necrotising crescentic glomerulonephritis in rats that had been immunised with

MPO and no lesions in nonimmunised rats.[21] More importantly, immediately after perfusion, ICX deposits were seen in the kidneys, but these disappeared very quickly and when the glomerulonephritis was at its maximum no further immune deposits were detected.[21] So, in the presence of ANCA, severe vasculitis and 'pauci-immune' glomerulonephritis can be induced in rats when immune complexes are first deposited along the vessel wall. The next question was what would happen if ICX other than ANCA/MPO ICX are deposited along the vessel wall. To study this, Heeringa *et al*. injected rats with an antibody to the rat glomerular basement membrane (GBM) and compared MPO-immunised rats with non-immunised rats. For these studies, a low dose of anti-GBM antibody was used that binds to the GBM but is not enough to induce a glomerulonephritis.[23] In rats with anti-MPO a severe glomerulonephritis developed whereas no lesions were found in the non-immunised rats.

In mice, ANCA have been identified in MRL-*lpr-/-lpr* and in SCG-/-Kj mice. In these models the role of ANCA is, however, difficult to tease out from the complex backgrounds of polyclonal B cell activation. Recently, however, convincing evidence was obtained that ANCA are sufficient to cause systemic 'pauci-immune' vasculitis and glomerulonephritis *in vivo*.[24] Two major strategies were used to demonstrate this. In the first, MPO-deficient mice were immunised with murine MPO and developed anti-MPO. Adoptive transfer of splenocytes from these mice into immune deficient *RAG2-/-* mice (lacking functioning B lymphocytes and T lymphocytes) resulted in anti-MPO and the development of glomerulonephritis and capillaritis. In contrast, transfer of splenocytes from mice that were immunised with BSA into *RAG2-/-* mice resulted in a mild form of immune complex glomerulonephritis without crescents. The nature of the background immune complex disease found in *RAG2-/-* mice that received either splenocytes from mice immunised with MPO or BSA is unclear. It was hypothesised that this relatively nonspecific response may represent a form of graft versus host disease. In the second strategy, purified anti-MPO was intravenously injected into *RAG2-/-* mice or wild type. 'Pauci-immune' necrotising and crescentic glomerulonephritis and systemic vasculitis, closely resembling the human disease, were observed.[24] These experiments indicate that ANCA can produce vasculitis without the further participation of T lymphocytes and/or B lymphocytes. This suggests that ANCA indeed induce vasculitis.

From these experiments, we come to our current working hypothesis. ANCA induce activation of neutrophils and monocytes resulting in ICX deposition in vessel walls. Other antigens, however, may also be involved in ICX formation. In the presence of ANCA this ICX deposition results in persistent activation of neutrophils and monocytes and subsequently severe glomerulonephritis or vasculitis.

## INDUCTION OF AUTOIMMUNITY TO PR3 AND/OR MPO

The central mechanism in autoimmunity is the breaking of self-tolerance. It is now well established that autoreactive T and B cells exist in the blood of healthy individuals and that these cells can potentially induce autoimmunity if activated beyond a certain threshold.[25] A combination of risk factors may be present in patients who develop ANCA. Inherited determinants have been sought, i.e. associations with certain HLA class I or class II molecules and/or with the C3F component of complement, but have not been convincingly found in patients with ANCA-associated vasculitis and/or glomerulonephritis. Other genetic factors, however, were found to be involved in ANCA-associated vasculitis. These genes include the genes for PR3, MPO, FcγR, $\alpha_1$ antitrypsin, CD18 and/or CTLA-4.[26] In addition, environmental factors are probably important modulators. Among these, drugs such as propylthiouracil and/or hydralazine[27] and/or exposure to chemical substances such as silicon[28] have been incriminated. Infectious agents are, however, the most likely candidates to cause autoimmunity. Several mechanisms by which infectious agents might induce autoimmunity have been postulated.[29] These include molecular mimicry, abnormal presentation of self-proteins, and/or abnormal stimulation of autoreactive T or B cells by agents such as superantigens. This latter mechanism has our special attention, since superantigens produced by staphylococci that may activate autoreactive B cells, in a T-cell dependent way, to produce ANCA depositions are often present in patients with ANCA-associated vasculitis.[30]

## CONCLUSION

Vascular damage in ANCA-associated vasculitis and/or glomerulonephritis results predominantly from activation of neutrophils when these cells adhere to endothelial cells. These neutrophils may be initially attracted by immune complexes formed *in situ*. Once these adherent activated neutrophils are 'over-stimulated' by ANCA they may cause necrotising vasculitis and glomerulonephritis and, in addition, stimulate the rapid disappearance of immune complexes, thus explaining the absence of immune complexes in tissue biopsies.

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