

## Newer Insights into the Aetiology and Pathogenesis of Myeloperoxidase Associated Autoimmunity

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**SUMMARY:** In recent years there have been substantial developments in the understanding of the aetiology and pathogenesis of ANCA-associated vasculitides, including myeloperoxidase (MPO) associated autoimmunity. This review will describe genetic and environmental factors that may increase the risk for the disease and will summarise findings demonstrating that T-cells, B-cells and ANCA themselves are of pathogenetic significance. Leukocyte gene expression profiles indicate that the reactivation of granule protein genes contributes to the pathogenesis of AASV. Finally, data derived from closely related autoantibodies against proteinase 3 (PR3) suggest anti-idiotypic antibodies induced by antisense transcripts as potential pathological agents.

Wegener's granulomatosis (WG), microscopic polyangiitis (MPA) including its renal limited variant and Churg Strauss syndrome are strongly associated with the presence of anti neutrophil cytoplasmic antibodies (ANCA). These ANCA-associated systemic vasculitides (AASV) can serologically be separated into MPO-ANCA and PR3-ANCA positive patients. Whereas PR3-ANCA are more common in WG, those directed against MPO are predominantly seen in cases of MPA. Although PR3- and MPO-ANCA associated autoimmunity have many similarities, there is increasing awareness that both disorders represent different diseases within the spectrum of AASV. Large progress has been made during recent years in our knowledge on the aetiology and pathogenesis of these disorders. The interaction of ANCA with polymorphonuclear neutrophils (PMN) plays a central part in their pathophysiology and is summarised elsewhere in this journal. This review will focus on the role of genetic and environmental factors in the aetiology of MPO-ANCA associated vasculitis and will discuss recent findings of leukocyte gene expression profiles. The pathogenetic significance of T-cells, B-cells and ANCA will briefly be summarised. Finally, data from anti-PR3 directed autoimmunity suggests that anti-idiotypic antibodies induced by antisense transcripts may be of pathogenetic importance.

**Genetic factors** that increase the risk for MPO-ANCA associated vasculitis include the MPO molecule itself as well as molecules important for the adhesion of PMN to endothelial cells. Higher levels of circulating MPO have been found in the serum of patients with MPO-ANCA associated vasculitis as compared with control subjects (summarized by [1]). The -463 G/A promotor polymorphism of the MPO gene was shown to affect MPO protein level expression, with higher levels identified in the GG genotype that was found to be overrepresented among female, but not male, patients with MPO-ANCA associated vasculitis. Relapse free survival may be longer in cases with the -463GG genotype as compared with GA and AA genotypes. Furthermore, the -463 G/A promotor polymorphism seems to affect MPO activity in a gender dependent manner. The pathological consequences of an increased release of active MPO from PMN have recently been reviewed and include lower NO bioavailability at inflammatory sites and the prevention of protease inactivation (1).

Several polymorphisms of the CD18 adhesion molecule have been found to be associated with MPO-ANCA in MPA patients, including one that was localised in an alternate transcription initiation site which may influence CD18 gene expression (2) and may thus affect adhesion of PMN to the endothelial layer.

**Environmental induction of MPO-ANCA:** Environmental factors, that have been found to be associated with MPO-ANCA associated autoimmunity include drugs as well as silica:

The link between MPO-ANCA and drugs such as hydralazine and anti-thyroid medications including propylthiouracil, but also

carbimazole and methimazole is well reported and has been recently reviewed (3). T-cell sensitisation to self-peptides has been shown to occur in conjunction with the accumulation of reactive intermediates of propylthiouracil in PMN. It is not firmly established to which extent the underlying thyroid disease may contribute to the occurrence of MPO-ANCA. The clinical implications of the antibodies are still unclear, and in their majority, these ANCA-positive patients do not suffer of vasculitic lesions. Authors recommend close observations of the patients and the withdrawal of suspected drugs according to the clinical situation (3).

Exposure to silica, including mining and farming, is clearly associated with the occurrence of MPO-ANCA associated vasculitis, with odds ratios between 4.4 and 12.0. The underlying pathogenetic mechanisms are unclear. However, silica exposure may be related to abnormalities in apoptosis, as soluble Fas has been shown to be elevated in these patients (recently summarized by [3]). In a rat model, inhaled silica was shown to induce apoptosis of alveolar neutrophils, granuloma formation and foreign body reactions following the phagocytosis by macrophages. An association with solvent exposure has been described for PR3-ANCA positive WG patients, but not for MPO-ANCA in MPA.

**Reactivation of granule protein genes:** Under normal conditions, MPO and PR3 mRNA transcripts are found almost exclusively at the early promyelocyte stage. It has now been demonstrated that peripheral mature PMN and monocytes from patients with both MPO-ANCA and PR3-ANCA positive disease had increased transcript levels of PR3 and MPO mRNA, which correlated with disease activity and absolute PMN counts, but not with "left shift", drug regimen, ANCA titers, or cytokine levels including TNF- $\alpha$ . The changes were specifically associated with ANCA disease and were not observed in patients with SLE or other causes of renal failure (4). Further genes that were found to be upregulated in PMN from these patients included other leukocyte granule proteins such as cathepsin G, elastase and bacterial permeability increasing protein (BPI) (4). However, the increased expression of MPO and PR3 may be of special importance, as it may increase antigen availability for circulating ANCA. The pathogenetic mechanisms underlying these observations are still unclear.

**Direct pathogenetic role for MPO-ANCA:** The evidence for a pathogenetic role of MPO- and PR3-ANCA is now broadly accepted. ANCA are present in about 90% of AASV patients, and active disease is rarely observed in individuals with a negative ANCA test result who were shown to be ANCA positive at diagnosis. In vitro data show that both MPO- and PR3-ANCA can bind to their target antigens, when they are expressed on the surface of PMN following priming with TNF- $\alpha$ . Infections, which are often observed prior to the first manifestations of the disease or prior to relapse, might be clinically important in the context of priming. ANCA bind to PMN not only via their Fab portions, but also via Fc $\gamma$  receptors IIa and IIIb and thus cause neutrophil activation and respiratory burst, as recently summarized (3). Much progress has been made in understanding the interaction of ANCA, PMN and endothelial cells, which

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is beyond the scope of this review. It should only be mentioned that binding of ANCA-IgG and ANCA-F(ab)2 to leukocytes from healthy blood donors was recently shown by the Chapel Hill group to stimulate the transcription of distinct inflammatory subsets of genes including those of IL-8 and cyclooxygenase 2 (COX-2), as well as differentiation dependent gene-2 (DIF-2), as assessed by microarray technique. IL-8 mRNA expression was increased in leukocytes from patients in remission, but not in leukocytes from patients with active disease. In contrast, DIF-2 levels were increased in active AASV, but not in remission. These observations indicate a previously unrecognised role of DIF-2 in AASV, which may be an important one, as DIF-2 is thought to be involved in monocyte differentiation and thus in the regulation of inflammation.

**Animal models further substantiate a direct pathogenetic role of MPO-ANCA:** Xiao et al. have convincingly shown that anti-mouse MPO-ANCA derived from MPO deficient mice immunised with mouse MPO can directly induce pauci-immune crescentic glomerulonephritis and pulmonary vasculitis in both Rag2<sup>-/-</sup> and wild type mice (5). Rag2<sup>-/-</sup> animals who received anti-MPO splenocytes instead of serum from the MPO immunised mice developed more severe renal lesions that were, however, not pauci-immune but associated with granular immune deposits. Although the model can be criticised because it does not represent autoimmunity, it is the most convincing model of anti-MPO disease currently available.

**Pathogenetic role of T-cells and B-cells:** In both MPO- and PR3-ANCA associated immunity, T-cells clearly play an important role summarized by (3,6), although there is more data available from PR3-ANCA positive cases. Soluble markers of T-cell activation such as the soluble IL-2 receptor are elevated in active disease, and T-cell infiltration is seen in active vasculitic lesions in patients suffering of MPA and WG. Peripheral blood mononuclear cells were shown to proliferate after exposure to ANCA antigens. The cytokine profile of these antigen specific T-cells is a subject of ongoing investigation, with a Th1 profile found by most investigators. However, the latter studies have all been performed in WG, and data from MPA patients are not available. CD28<sup>+</sup> T-cells were found to be very prevalent in the expanded, activated T-cell subset in WG, implying an effector role for these cells (3). Finally, the good clinical response of anti-T-cell directed therapy in refractory patients favours a pathogenetic role of these cells (6).

B-cells: In some AASV patients, circulating B-cells seem to spontaneously produce ANCA, without the need of T-cell help and presentation of ANCA antigen. It can be speculated whether these B-cells might have escaped regulatory control and perpetuate inflammation (7). In this context it is interesting that depletion of B-cells by a monoclonal anti-CD 20 antibody (rituximab) was shown to be an effective treatment in refractory WG.

**Antisense transcripts of ANCA antigens:** Finally, recent data derived from anti-PR3 directed autoimmunity implicate antisense transcripts as potential pathological agents in AASV. It could be

shown that patients with PR3-antibodies did also harbour antibodies to a peptide translated from the antisense DNA strand of PR3 (complementary PR3, cPR3). Furthermore, immunisation of mice with cPR3 derived peptides led to the production of antibodies directed against both cPR3 and PR3, indicating the presence of anti-idiotypic antibodies. The authors speculate whether autoimmunity in human AASV might be initiated by an immune response directed against peptides that are complementary or antisense to the autoantigen (8).

## CONCLUSION

Substantial developments in the understanding of the aetiology and pathogenesis of MPO-ANCA-associated vasculitides have been made during recent years including the identification of genetic and environmental factors that increase the risk for the disease, and factors that mediate the pathogenetic process such as B-cells, T-cells and ANCA themselves. Data from expression profiling studies indicate that reactivation of MPO and PR3 genes contributes to the pathogenesis of AASV. These concepts begin to open new therapeutic strategies for AASV patients.

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