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# The Influence of Autoantibodies to Myeloperoxidase on Neutrophil Function and Intracellular Signaling

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**SUMMARY**: Autoantibodies to myeloperoxidase (MPO) are associated with small vessel systemic vasculitis. Interactions of these autoantibodies with MPO target antigen, Fc$\gamma$ receptors and $\beta$2 integrins at the neutrophil surface, can set in train a sequence of intracellular signal transduction events that culminate with functional responses. These include a respiratory burst with release of superoxide ions, degranulation, cytokine release, enhanced adhesion and induction of an accelerated apoptotic program.

Myeloperoxidase (MPO) is one target within the spectrum of neutrophil and monocyte enzymes recognised by anti-neutrophil cytoplasm antibodies (ANCA) occurring in patients with small vessel systemic vasculitis. In addition to the location of MPO within neutrophil azurophil granules, MPO and other target antigens for ANCA are well described as being presented on the surface of

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'primed', i.e. cytokine-treated, neutrophils. Some evidence also suggests that soluble MPO released by activated neutrophils may bind to unstimulated neutrophils, thereby making them reactive to anti-MPO antibodies, see (1). Certainly MPO is detectable on the surface of neutrophils isolated from patients with acute vasculitis (2).

In vitro, the presence of MPO on the surface of primed neutrophils allows the autoantibody to bind. This binding event is not sufficient for functional activation of the cell. Additionally, ligation of the Fc portion of the antibody to the Fc$\gamma$ receptors on the neutrophil is required, see (1), which then gives rise to the generation of reactive oxygen species, degranulation, and the production of pro-inflammatory cytokines. It is also now becoming clear that there are additional components to the system, importantly CD18 (3). Continuous stirring of neutrophils during stimulation with ANCA inhibits the usual superoxide production (4). It may be that the involvement of CD18 stabilises the complex formed by ANCA or alternatively causes its relocalisation to areas within the plasma membrane that enable it to activate the NADPH complex. ANCA are able to induce the expression of an activation epitope on CD11b (part of the CD11b/CD18 $\beta$2 integrin complex), which may be important in ANCA-induced neutrophil adhesion to vascular endothelial cells, and possibly in the induction of signalling events also (5).

Investigation of the intracellular signal transduction pathways induced by ANCA binding to neutrophils have shown differences between the signal initiated by F(ab') 2 fragments of the antibody and those seen with whole IgG antibody, indicating that multiple pathways are involved. ANCA binding to antigen instigates activation of G protein coupled pathways (6) whereas ligation of the Fc$\gamma$ receptor targets Syk kinase (3), protein kinase C$\beta$ - see (1), and calcium release (3). Both portions of the antibody are required for protein kinase B activity (6) and both are able to activate Src kinase(s) (3) and phosphatidylinositol-3 kinase $\gamma$ - see (1). Also stimulated is the small GTPase p21ras, which is known to be a molecular switch providing the connection point for a number of pathways and this may be pivotal in uniting the signals to provide a functional response (6,7). It is noteworthy that the pathways triggered by ANCA in neutrophils are clearly different to those seen with cross-linking of Fc$\gamma$ receptors, which may provide opportunities for therapeutic interventions without concomitant down-regulation of the entire immune response (1).

As well as the above effects, ANCA is also capable of inducing neutrophils to undergo an accelerated rate of apoptosis (2). This has been shown to be dependent on the production of reactive oxygen species. Additionally the process is dysregulated with delayed externalisation of phosphatidylserine occurring and consequently reduced phagocytosis by macrophages. This leads to apoptotic neutrophils progressing more readily into secondary cell lysis, releasing their proteolytic cell contents which may cause bystander injury. In a different process but still involving apoptosis, naturally apoptotic neutrophils express increased amounts of MPO on their surface (2) and potentially this could lead to opsonisation of the cells by ANCA and their uptake by macrophages in a pro-inflammatory manner. However, cells expressing MPO during the process of apoptosis are unresponsive to ANCA-induced signalling and do not produce superoxide (2).

Less work has taken place to determine the role of the monocytes/macrophages in development and progression of small vessel vasculitis. Monocytes are capable of expressing MPO and are therefore a legitimate target for ANCA. The cells are seen within granulomas and glomerular crescents during active disease and so, by implication, play a part. Monocytes are able to release reactive oxygen species, cytokines/chemokines (MCP-1, IL-8, TNF$\alpha$, IL-1$\beta$, IL-12) and thromboxane in response to ANCA and consequently may contribute to the local pro-inflammatory environment (8).

MPO-ANCA tend to be associated with non-granulomatous forms of small vessel vasculitis, in contrast to proteinase 3 (PR3) ANCA which are associated with granulomatous phenotypes. This has prompted speculation as to whether the pathophysiology of vasculitis is related to the type of ANCA. In our hands, MPO-ANCA collectively tend to be more activating of neutrophils than PR3-ANCA (2). However, the molecular reasons for this difference are not clear. The ability of soluble MPO, but not PR3, to bind to the neutrophil surface and support ANCA-induced activation has been proposed as an explanation for the differences in the pathologic and clinical expression of MPO-ANCA versus PR3-ANCA vasculitis. Further elucidation of this intriguing problem may come from new murine models involving MPO-ANCA and PR3-ANCA.

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