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Antineutrophil Cytoplasmic Antibodies (ANCA) – Associated Diseases and Their Role in Pathogenesis

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Introduction

Antineutrophil cytoplasmic antibodies (ANCA) were first described in 1982 by Davies and coworkers (1) in patients with necrotizing glomerulonephritis. In 1985, Van der Woude and coworkers (2) reported ANCA as being a characteristic marker of Wegener's granulomatosis. Myeloperoxidase (MPO) and proteinase 3 (PR3) have been discovered as the major target antigens for ANCA in patients with small vessel vasculitides (3,4). In the nineties, ANCA have become commonly used in clinical diagnostic testing and the term ANCA-associated vasculitides, including Wegener's granulomatosis, microscopic polyangiitis and Churg-Strauss syndrome, as well as pauci-immune necrotizing crescentic glomerulonephritis, has generally been accepted (5). In addition to being an important diagnostic tool, it has been shown that the dynamic of ANCA values in sera in the majority of patients reflects the effect of the therapy and occurrence of recidives, respectively.

ANCA antigens

ANCA are specific for various proteins, mostly enzymes that are localized in the cytoplasm of neutrophils and monocytes (6). The two major target antigens for ANCA in patients with pauci-immune small vessel vasculitides are MPO and PR3. Both are translocated to the cell surface during the activation of polymorphonuclear leukocytes. The MPO epitope, recognized by patient antibody, is expressed by the 130 kDa native molecule. The major C-ANCA antigen is PR3, a 28 kDa serine proteinase, which is colocalized with MPO in the azurophilic granules of polymorphonuclear leukocytes. The plasma inhibitor of PR3 is α1 antitrypsin. Interestingly, an association of deficient α1 antitrypsin with PR3-ANCA positive vasculitis has been observed. PR3-ANCA are highly specific for Wegener's granulomatosis and MPO-ANCA significantly predominate in patients with microscopic polyangiitis, pauci-immune crescentic glomerulonephritis and Churg-Strauss syndrome (5, 7, 8). Our results, which accord with the reported data, are presented in table 1.

Other known ANCA antigens are lactoferrin, elastase, lysocyme, cathepsin G, alpha-enolase, azurocidin, bactericidal permeability increasing protein, human lysosomal-associated membrane protein (h-lamp-2) and defensin. They occur rarely (less than 5%) as target antigens in patients with ANCA-associated vasculitis (9), but may be frequently the specific antigens for positive ANCA in patients with other diseases, such as inflammatory bowel diseases, autoimmune hepatitis, primary biliary cirrhosis, systemic connective tissue diseases, particularly systemic lupus erythematosus and rheumatoid arthritis, Henoch Schönlein purpura, infectious diseases and malignancies (7, 10, 11). The pathogenetic role of ANCA in these diseases in uncertain, but they are more probably an epiphenomenon than the cause of the disease.

Table 1. Clinico-pathologic diagnosis in relation to ANCA antigen specificity in 423 ANCA positive patients at the Institute of Pathology, Faculty of Medicine, Ljubljana (1989-1999)

Clinico-pathologic	Patients	ANCA antigen specificity				
diagnosis	No.	PR3	MPO	MPO+ PR3	Other	Undeter-mined
Wegener's granulomatosis	56	45	6	2	2	1
Microscopic polyangiitis	54	3	45	2	1	3
Pauci-immune necrotizing GN	28	2	24	0	0	2
Churg-Strauss syndrome	2	0	1	0	1	0
Classic polyarteritis nodosa	3	1	1	1	0	0
Skin vasculitis	8	1	3	0	1	3
Goodpasture's syndrome	5	0	5	0	0	0
Suspected vasculitis	59	12	26	6	7	8
IBD, HBD	83	11	15	2	7	48
Miscellaneous diseases	125	8	60	19	17	21

Abbreviations: GN - glomerulonephritis, IBD - inflammatory bowel disease, HBD - hepato-biliary disorders

ANCA testing

The standard approach for detection of ANCA is the indirect immunofluorescence (IIF) technique and one of the antigen-specific quantitative assays, most often enzyme-linked immunosorbent assay (ELISA). IIF and particularly ELISA need to be well standardized (8). An international

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group of ANCA researchers published a consensus statement on ANCA testing demanding that in case of positive IIF for ANCA, an ELISA test is obligatory as a minimum requirement (12, 13). The recommendation for optimal testing includes both IIF and ELISA on all samples. However, there might be significant differences in sensitivity and specificity among available commercial ELISA kits and moreover, IIF and ELISA do not always correlate.

IIF is performed on ethanol fixed normal human leukocytes. Routinely, IgG antineutrophil antibodies are detected, although in some pathologic conditions, IgM or IgA isotypes should be tested, too. IgM ANCA, either isolated or associated with IgG, have been described in rare patients with pulmonary-renal syndrome, and IgA ANCA have been reported in patients with IgA glomerulonephritis and purpura Henoch-Schönlein.

By IIF, two main patterns are observed: cytoplasmic granular with central interlobular accentuation (*C-ANCA*) mostly specific for PR3 and perinuclear, often with nuclear extension (*P-ANCA*), that occurs predominantly with MPO specificity. It is important to stress that P-ANCA, which is actually an artifact of alcohol fixation, may be confused with antinuclear antibodies. The problem could be overcome by introducing formalin-fixed smears of neutrophils, but the technique is difficult and not widely used in the laboratories. P-ANCA may lack the nuclear fluorescence and show nuclei rimmed by a positive reaction. These P-ANCA usually occur with unknown antigen specificity in patients with inflammatory bowel diseases, autoimmune hepatitis and rheumatoid arthritis (12). In addition to the two main IIF patterns, *atypical C-ANCA* is determined, if cytoplasmic reaction is not diffuse and lacks interlobular accentuation. The corresponding antigen specificities include PR3 in low values, MPO and bactericidal permeability-increasing protein but are usually unknown. *Atypical ANCA* is used for immunofluorescence patterns other than those described as C-ANCA, atypical C-ANCA, or P-ANCA and most often is a combination of cytoplasmic and perinuclear staining.

Sensitivity and specificity of ANCA testing (indirect imunofluorescence+ELISA) from different studies in the literature

Disease	**Sensitivity of ANCA**
Limited Wegener's granulomatosis	50-66 %
Generalized Wegener's granulomatosis	80-98 %
Microscopic polyangiitis	82-90 %
Pauci-immune necrotizing glomerulonephritis	90-95 %
Churg-Strauss syndrome	60-70 %

Control group	**Specificity of ANCA**
Patients with various other diseases	76-91 %
Healthy subjects	94-99 %

Pathogenetic role of ANCA

The etiology of ANCA-associated diseases is unknown, while the hypothesis that ANCA have a direct role in the pathogenesis of tissue damage is supported by many in vitro studies as well as recent experimental *in vivo* model (14, 15).

At present, it seems that pathologic mechanism may be triggered by infections with the release of proinflammatory cytokines in genetically susceptible subjects. Clinical studies have provided evidence that patients with ANCA-associated vasculitis frequently suffer from virus or bacterial upper respiratory infections before the development of vasculitis. Priming of neutrophils by cytokines, particularly TNF-α, enables the surface expression of PR3 and MPO and consequent binding of ANCA via Fab and Fc portion with neutrophil activation. The activated neutrophils, adhered to the vessel wall, generate respiratory burst and induce endothelial cell damage by release of granule enzymes, superoxide products and nitric oxide. Damage and activation of endothelial cells produce proinflammatory chemokines and cytokines recruiting other inflammatory cells, such as monocytes and T cells, intensifying endothelial cell and vessel wall damage as well as crescent formation in glomeruli. Neutrophil apoptosis is dysregulated by ANCA activation of neutrophils, preventing apoptotic cell removal, which allows progression to secondary necrosis. Antigen-specific memory T cells persist following disease remission with the potential reactivation and recidive of the disease.

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