

growth factor production and angiogenesis. Inflammation-induced neovessels are prominent in vasculitis and are the main site of adhesion molecule expression. Therefore, newly formed vessels contribute to the development of vascular inflammatory infiltrates by recruiting leukocytes. On the other hand, angiogenesis may prevent isohemia by providing new blood supply.

Vascular response to inflammation may eventually lead to vessel occlusion generating ischemia in tissues supplied by involved vessels. Organ ischemia is the main cause of organ dysfunction and serious complications in patients with vasculitis. Vessel occlusion may develop through spasm, thrombosis and, more frequently, intimal hyperplasia and fibrosis. Several cytokines and growth factor with prothrombotic, vasoactive and fibrogenic properties have been demonstrated to be produced in vasculitis lesions.

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## Antineutrophil cytoplasmic autoantibodies in pathology: Major autoantigens and disease associations

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Antineutrophil cytoplasmic autoantibodies (ANCA) were described in 1982 by Davies and coworkers in patients with necrotizing glomerulonephritis (GN). In 1985, Van der Woude and coworkers reported ANCA as being a characteristic marker of Wegeners granulomatosis. In the 1990s, ANCA have become a commonly used diagnostic test and the term ANCA-associated vasculitides, including Wegeners granulomatosis, microscopic polyangiitis and Churg-

Strauss syndrome, as well as pauci-immune necrotizing crescentic GN, has been generally accepted. In addition to being an important diagnostic tool, it has been shown that the dynamic of ANCA titers in the majority of patients reflects the activity of the disease. In view of the clinical serologic correlation, as well as recent *in vitro* and *in vivo* experimental studies, ANCA presumably play a key role in the pathogenesis of and vascular lesions in ANCA-associated diseases. However, positive ANCA occasionally occur, most probably as an epiphenomenon, in a variety of pathological conditions, such as inflammatory bowel diseases, autoimmune liver diseases, connective tissue diseases, tumors, infectious diseases and others.

### Detection of ANCA

The standard approach for the detection of ANCA is the indirect immunofluorescence (IIF) technique followed by one of the antigen-specific quantitative assays.

IIF is performed on ethanol-fixed normal human leukocytes. Routinely, immunoglobulin (IgG) antineutrophil antibodies are detected, although in some pathologic conditions, IgM or IgA isotypes should also be tested. Two main patterns are observed by IIF: cytoplasmic (C-ANCA) and perinuclear (P-ANCA). P-ANCA, which is actually an artifact of alcohol fixation, can be confused with positive antinuclear antibodies, so a control testing on Hep-2 cells is needed. Atypical C- and atypical P-ANCA patterns have also been described.

ANCA antigen specificity is quantitatively determined by enzyme-linked immunosorbent assay (ELISA), radioimmunoassay or Western blotting technique.

### ANCA antigens

It has been established that ANCA are directed against various proteins, mostly enzymes in the cytoplasm of neutrophils and monocytes. The most common, and diagnostically the most important, are myeloperoxidase (MPO) and serin protease proteinase 3 (PR3). Both are contained in azurophilic granules and are translocated to the cell surface during the activation of neutrophils. Anti-MPO antibodies mostly exhibit P-ANCA pattern (128/171 in our study), rarely atypical F- (12/171) or atypical C- (26/171) and exceptionally C-ANCA (1/171). Anti-PR3 antibodies regularly show C- (46/69) or atypical C-ANCA (14/69) and rarely P- or atypical F-ANCA patterns in IIF.

Other known ANCA antigens, such as lactoferrin, elastase, lysozyme, cathepsin G, rs-enolase, azurocidin, bactericidal permeability increasing protein, human lysosomal-associated membrane protein 2 (h-lamp-2) and defensin occur rarely, according to the literature in less than 5%. In addition, there is still a conspicuous number of ANCA positive sera with undetermined antigen specificity.

The majority of ANCA-positive sera are specific for a single antigen, although the co-occurrence of different ANCA antigens has not been studied systematically. In our large series of 374 ANCA-positive patients, 31 exhibited simultaneous positivity for anti-MPO and anti-PR3, the association of anti-MPO or anti-PR3 antibodies with rare specificities and four the co-occurrence of rare ANCA antigen specificities. In addition, the specificity of ANCA changed in the course of the disease in five patients.

### ANCA in classification of vasculitides

It has long been recognized that pathological lesions in diseases that are now determined as ANCA-associated share many light and immunohistological similarities. Vascular lesions in various

organs, involving capillaries, arterioles, venules and small arteries and veins are characterized by focal segmental necrosis of the vessel wall and pleomorphic cellular infiltration with predominance of neutrophils in the acute phase. Thromboses can occur at the site of the lesions. Renal glomeruli are very frequently involved and show focal segmental necrotizing crescentic GN. Infiltration of mononuclear cells as well as endoproliferative and sclerotic lesions occur at later phases. The coexistence of necrotizing vasculitis and healed lesions is a very characteristic feature, which suggests acute recurrences during the course of the disease. Since only scanty immune deposits can be found in the majority of cases, ANCA-associated vasculitides have been designated as pauciimmune. However, not so rarely, quite conspicuous irregular deposits of complement C3 can be observed. In addition to these common lesions, Wegener's granulomatosis is characterized by focal granulomatous reaction with multinucleated giant cells, although this feature can rarely be confirmed in biopsies. In Churg-Strauss syndrome, eosinophils predominate in inflammatory cell infiltration.

The criteria for the classification of vasculitis were developed in 1990 by the American College of Rheumatology and definitions of vasculitides were proposed in 1994 by the Chapel Hill Consensus Conference. However, the diagnosis and classification of vasculitides, particularly if they are expressed in a limited form, are still problematic. The most difficult problem is delineating between Wegener's granulomatosis and microscopic polyangiitis with pulmonary involvement. Testing of ANCA provides significant help in the diagnosis and classification of vasculitides but for the inclusion of ANCA among the diagnostic criteria, or revision of the classification system, larger numbers of patients would need to be studied systematically.

Our experience is based on the examination of 6,570 sera of patients with vasculitides and other diseases (Table 1), among which 374 patients were found to be ANCA-positive. Anti-PR3 antibodies predominated in Wegener's granulomatosis. Many studies have confirmed the high sensitivity of positive ANCA (PR3 and MPO) for the diagnosis of Wegener's granulomatosis, being as high as 95% for an extended form and 75% for a limited form of the disease. Anti-PR3 antibodies were rarely found to be positive in other forms of ANCA-positive vasculitides.

Anti-MPO antibodies were associated mostly with microscopic polyangiitis and pauciimmune necrotizing crescentic GN. Before the ANCA era, the latter were referred to as idiopathic. The discov-

ery of ANCA showed that it is actually a microscopic angiitis limited to the kidney, which is very important in managing the therapy in these patients. The sensitivity of ANCA (MPO and PR3) in these forms of vasculitis has been reported to be 70-90% and in a very rare disease, Churg-Strauss syndrome, in which MPO also predominates as ANCA antigen, it has been reported as 60-70%.

Anti-PR3 or anti-MPO ANCA occurred in rare patients with classic polyarteritis nodosa, which is most probably mediated by heterogeneous immune mechanisms.

Positive ANCA, particularly with anti-MPO or undetermined antigen specificity, were, however, less rarely detected in patients with inflammatory bowel diseases, autoimmune liver diseases, antglomerular basement membrane disease and systemic lupus erythematosus. It was detected incidentally in other rheumatic diseases, different forms of ON, infectious diseases and tumors. It should be stressed that in these various pathologic conditions, the characteristic feature of ANCA were mostly low titers in ELISA and often atypical patterns in IF.

Other ANCA antigen specificities, which were found to be positive in 18 out of 169 tested patients (11%) in our study, were equally distributed in different forms of ANCA-associated vasculitides as well as in a large group of other diseases. Their diagnostic significance and pathogenetic role remain obscure. ANCA-positive sera with undetermined antigen specificity were mostly from the patients with inflammatory bowel diseases and other diseases and very rarely from the patients with ANCA-associated vasculitides.

#### Pathogenetic role of ANCA

The hypothesis that ANCA are not only of a great practical diagnostic significance but also play a key role in the pathogenesis of glomerular and vascular lesions in ANCA-associated diseases has been supported by clinical observations as well as by *in vitro* and *in vivo* experimental studies. A positive correlation between ANCA values and disease activity has been reported in many studies in the majority of patients. Furthermore, ANCA can be induced by certain drugs and the subsequent vasculitis resolves after withdrawal of the drug and introduction of immunosuppressive treatment.

Experimental data suggest that ANCA can activate neutrophils and induce blood vessel wall injury by multiple synergistic events, which are not yet fully understood. According to one hypothesis, in a patient with circulating ANCA, a viral infection causes the gener-

**Table 1. ANCA antigen specificity in relation to clinicopathological diagnosis in our series of 374 ANCA-positive patients (Institute of Pathology, Ljubljana, 1989-1998).**

Clinicopathological diagnosis	ANCA positive cases	ANCA antigen specificity				
		PR3	MPO	MPO + PR3	Other	Undetermined
Wegener's granulomatosis (WG)	47	37	5	2	2	1
Ocular pathology (limited WG?)	4	2	1		1	
Microscopic polyangiitis	67	4	53	6	1	3
Unclassified systemic vasculitis	26	6	11	3	3	3
Pauciimmune necrotizing GN	24	2	20			2
Churg-Strauss syndrome	2		1		1	
Classic polyarteritis nodosa	3	1	1	1		
Skin vasculitis	12	2	4	1		5
Inflammatory bowel diseases						
Autoimmune liver disease	70	8	14	1	4	43
Other diseases	119	7	61	17	6	28

ANCA = antineutrophil cytoplasmic autoantibodies; GN = glomerulonephritis; MPO = myeloperoxidase.

ation of cytokines which prime the neutrophils to express ANCA antigens on their surface. The binding of ANCA induces neutrophil activation with the release of hydrolytic enzymes and toxic oxygen metabolites. Subsequent endothelial cell death, which probably involves both apoptosis and lytic necrosis, occurs if the neutrophils were previously bound to the endothelial cells. This is enabled via cytokine-induced cell adhesion molecules expressed on endothelial cells and occurs particularly at sites where leukocytes are in close contact with vessel walls, as in kidney glomerulus and in pulmonary alveolar capillaries. Furthermore, it has been shown that highly cationic ANCA antigens that are released from activated neutrophils may bind to the negatively charged endothelial cells and extracellular matrix structures, such as the glomerular basement membrane. There they can act as planted antigens for *in situ* immune complex formation and local complement activation may augment the tissue injury. Moreover, some studies suggest that endothelial cells, if influenced by cytokines, produce and express PR3 on their surface and in this case ANCA could act as anti-endothelial cell antibodies and kill endothelial cells by an antibody-dependent cytotoxicity. It has also been hypothesized that bound PR3 can be recognized by specific T lymphocytes.

There are an increasing number of experimental animal models that simulate ANCA-positive human disease at least in some features, although none of them is an ideal model for pauciimmune ANCA-positive vasculitis in humans.

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## Vascular pathology in systemic lupus erythematosus: Crossroads of immune complex vasculitis and vasculopathy, thrombotic microangiopathy and arteriosclerosis

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Systemic lupus erythematosus (SLE) is a prototype of a multisystem autoimmune connective tissue disease, marked by immune complex-mediated lesions of blood vessels in diverse organs. It is

a disease with a myriad of diverse clinical manifestations and a fairly unpredictable exacerbating course, which is influenced by the current therapeutic approaches with corticosteroids and immunosuppressive drugs. Despite the growing understanding of the cellular and molecular events, the obviously very complex etiopathogenesis of SLE still remains unsolved. It appears that the interplay of etiologic factors, genetic, hormonal and environmental, results in a failure of the immune regulatory mechanisms characterized by a T-cell dysfunction, a B-cell activation and an imbalance in the production of cytokines. A dominant consequent feature is an overproduction of a bewildering array of autoantibodies, of which over 50 are currently well characterized, particularly those against diverse nuclear and cytoplasmic components of the cell. Three basic pathogenetic mechanisms have been suggested; i) injury related to deposition of circulating and *in situ* formed immune complexes, double-stranded DNA (dsDNA) – anti dsDNA being of particular significance; ii) the direct cytotoxic effect of autoantibodies; and iii) functional effect of autoantibodies, such as autoantibodies to phospholipids involved in thrombogenesis, anti-ribonucleoprotein (RNP) and anti-DNA penetrating into live cells may cause apoptosis and thus stimulate antigen-driven production of anti-DNA nucleosome autoantibodies.

### ~~Lupus vasculopathy and vasculitis in diverse organs~~

Vascular involvement characterizes the pathology of diverse organs in SLE and seems to be crucial for the majority of clinical manifestations. Widespread vascular lesions were described in the most frequently cited classical study of SLE pathology by Klemperer *et al.* in 1941. They reported microvascular injury with "fibrinoid" degeneration and necrosis of predominantly small arteries, arterioles and capillaries. Significantly less frequent inflammatory or thrombotic effects of diverse vessels, including even large arteries and veins, have not inspired systematic investigations until recently.

Our experience is based on a systematic study of 342 kidney biopsy specimens in 266 SLE patients, 131 skin biopsy samples in 114 patients, 125 biopsy samples of skeletal muscle in 112 patients and on the tissue samples of diverse organs obtained from autopsies of 37 patients. The most consistent and characteristic were immunofluorescence microscopic findings of granular deposits of immunoglobulins, predominantly IgG, and complement components, especially C1q, in kidney glomeruli (99%), choroid plexus (82%), skin (75%), spleen (73%), heart (71%), salivary glands (57%), brain (56%), lung (49%), liver (42%) and skeletal muscle (36%). The deposition of immune reactants in all organs, with the choroid plexus as the only clear-cut exception, correlated with non-inflammatory vasculopathy and inflammatory vascular and extravascular changes. Since the pathology of the kidney in SLE has been studied by far the most extensively and systematically, the following text will be devoted to renal vascular lesions.

### ~~Lupus glomerulonephritis~~

The glomeruli are by far the most commonly affected by deposits of wide arrays of circulating and *in situ* formed immune complexes in SLE. Glomerular capillaritis results in different types of lupus glomerulonephritis. In our large series of 379 kidney biopsies and autopsy samples, the following glomerular distribution patterns were found with the following incidence; nil (1.4%), mesangial (15.5%), mesangial-subendothelial (10.6%), subepithelial (4.4%), mesangial-subepithelial (12.0%) and, the most frequent and characteristic, mesangial-transmembranous (56.1%). A clear-cut posi-