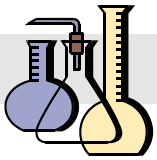

Distinct Differences in Autoantigen Specificity of Anti-Neutrophil Cytoplasm Antibodies in Systemic Vasculitides and Other Inflammatory Diseases

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Abstract

Background: Anti-neutrophil cytoplasm antibodies in necrotizing vasculitides need to be distinguished from ANCAs[1] in other inflammatory conditions to avoid clinical misinterpretation.

Objectives: To help clinicians and laboratory scientists recognize and utilize vasculitis-related ANCAs as an aid in diagnostic workup and patient follow-up, and be aware that ANCAs with different characteristics are commonly found in other chronic inflammatory conditions that persistently engage neutrophils in the inflammatory process.

Methods: Indirect immunofluorescence and enzyme immunoassay methods were used to detect ANCAs with both known and unknown neutrophil autoantigenic targets.

Results: Primary necrotizing small vessel vasculitides such as Wegener's granulomatosis, Churg-Strauss syndrome, microscopic polyangiitis, and renal-limited rapidly progressive necrotizing glomerulonephritis target either the serine protease proteinase 3 or myeloperoxidase in azurophilic granules. In ulcerative colitis and rheumatoid arthritis, we found multiple ANCA targets contained in azurophilic and specific granules, the cytosol and the nucleus, whereas PR3[2] and MPO[3] were not, or only weakly, recognized.

Conclusions: ANCAs typically found in active SVV[4] are demonstrable both by indirect immunofluorescence and antigen-specific enzyme immunoassay, and strong reactivity to either PR3 or MPO is characteristic. Strong ANCA with MPO reactivity is also found in some patients with drug-induced syndromes (lupus, vasculitis). Intermediate to strong perinuclear ANCAs are found in a substantial proportion of patients with UC[5] (40–60%) and RA[6] (30–70%), but in these conditions the ANCAs have many antigen targets that are only weakly recognized.

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Autoantibodies to neutrophils were demonstrated by the indirect immunofluorescent technique already in the early 1960s [1,2], and their common presence in patients with rheumatoid arthritis and especially Felty's syndrome was shown in the later part of that decade [3, reviewed in 4]. Until the mid-sixties the IIF[7] technique using ethanol-fixed human leucocytes as substrate and fluoresceinated polyclonal anti-human immunoglobulin G antibodies was the only way to demonstrate these antibodies. At that time researchers began to use radioactive immunoprecipitation [5] and enzyme immunoassay [6], since immunoblotting was found unsuitable for demonstrating most ANCAs.

The ANCAs first shown in relationship to glomerulonephritis and systemic small vessel vasculitides [7] gave rise to a granular cytoplasmic neutrophil and monocyte fluorescence pattern (C-ANCA) that was quite distinct from the neutrophil-directed antibodies found in rheumatoid arthritis and ulcerative colitis [4,8], which stained the nuclear/perinuclear regions of neutrophils and monocytes. The latter antibodies were initially called "granulocyte-specific antinuclear antibodies," but were later renamed "perinuclear ANCA" (P-ANCA) [9] on the assumption that all reactivity was directed to cytoplasmic antigens, which had relocated to the nuclear vicinity upon ethanol fixation as described with myeloperoxidase-ANCA [10]. In sera from non-vasculitic patients, autoantibodies to neutrophils may give rise to complex or less typical IIF ANCA patterns, generally designated "atypical ANCA." This probably reflects the fact that many different antigens are simultaneously reactive with ANCA contained in one serum [11,12]. Among the antigens recognized in such conditions are lysozyme, cathepsin G, leucocyte elastase, and bacterial permeability-increasing protein in azurophilic granules, lactoferrin in specific granules, and a number of less well-identified antigens in the cytosol and nucleus. It is important to note that some of the antigens targeted in these conditions are not neutrophil-specific, but are present also in lymphocytes and tissues. In this paper, only antibodies towards neutrophil/monocyte-specific constituents are termed ANCA. In accordance with studies on autoantibodies to neutrophils that did not include appro-

[1] ANCA = antineutrophil cytoplasm antibody
[2] PR3 = protease proteinase 3
[3] MPO = myeloperoxidase
[4] SVV = small vessel vasculitides
[5] UC = ulcerative colitis
[6] RA = rheumatoid arthritis
[7] IIF = indirect immunofluorescent

priate negative control cells, control cellular extracts must be interpreted with caution regarding neutrophil specificity. Hence the designation ANCA [Figure 1].

Routine ANCA Serology

Although early studies showed a clear association between C-ANCAs and the diagnosis of Wegener's granulomatosis [13-15], the patient materials were derived from highly specialized tertiary referral centers; accordingly, results only pertained to the selected clientele seen there. Later studies indicate that C-ANCAs are found in about one-third of patients with microscopic polyangiitis and rapidly progressive necrotizing glomerulonephritis, and in only one-fourth of Churg-Strauss syndrome patients [16,17]. The C-ANCAs in these vasculitis patients have the same predominant specificity towards serine protease proteinase 3, although unconfirmed data point to the presence of some ANCAs directed to bacterial permeability-increasing proteins in primary SVV [18]. An optimal serologic diagnosis of C-ANCA for primary SVV must include a positive EIA reaction with PR3 to achieve a high diagnostic specificity towards inflammatory disease controls [19]. Since P-ANCA can be found in many chronic non-vasculitic inflammatory diseases [4,17], P-ANCAs relate significantly to primary SVV only if the antibodies selectively and strongly target myeloperoxidase [19]. The diagnostic specificity of a positive combined P-ANCA/MPO-ANCA test can be chosen to be satisfactorily high if the cut-off for the MPO-ANCA EIA is selected with due respect to "gray area" positivity in inflammatory disease controls.

An international panel of experts has now put together a consensus document on how to test for, report, and interpret ANCA results based on available literature data and expert experience [20]. This document recommends the continued use of published names and definitions for ANCA [9] and the Chapel Hill nomenclature for the vasculitic diseases that may be associated with ANCA [21], but also the use of both IIF ANCA and specific ANCA testing by EIA to rule out misinterpretation of ANCA in diagnosing vasculitides. It is very likely that well-defined capture EIA techniques may replace or supplement direct EIA in the future [22].

In a large routine serology setting, the majority of IIF ANCA-positive results usually relate to non-vasculitic diseases such as rheumatoid arthritis, ulcerative colitis, or chronic hepatitis. This means that it is very important to confirm IIF ANCA findings by a specific enzyme immunoassay ANCA result as an indication of vasculitis or a drug-induced syndrome. Otherwise an IIF ANCA result has uncertain utility for diagnostics and follow-up. There are data indicating that the presence of ANCAs in a patient with RA (whether RF positive or not) predicts an increased risk for erosive disease and hence a worse functional prognosis [23]. It would appear that the presence of rheumatoid factor or antinuclear antibodies does not add to this risk of erosive disease (Wiik et al., unpublished). Using immunoblot techniques, Brimnes et al. [12] showed that patients with RA commonly recognize a number of neutrophil-specific autoantigens [Figure 1], especially lactoferrin. Although some patients had low levels of ANCA directed to MPO, most sera lacked MPO- or PR3-ANCA,

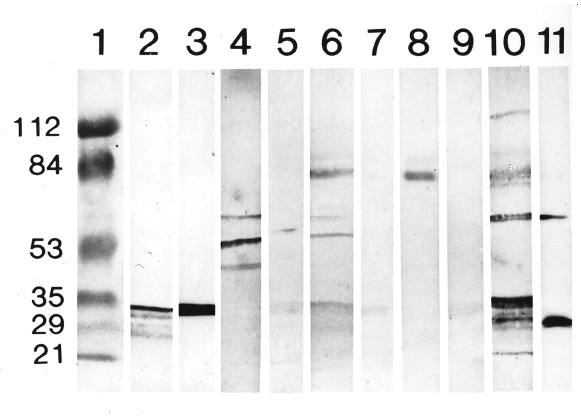

Figure 1. Immunoblot on extracts of whole human neutrophils (lanes 2,4,6,8,10) and whole human lymphocytes (lanes 3,5,7,9,11) incubated with sera of four RA patients in a pairwise fashion. Lane 1: the molecular size standard; lanes 2–9: sera from RA patients having IgG ANCA and no antinuclear antiodies on lymphocytes and HEp-2 cells; lanes 10 and 11: serum from an RA patient having both ANCA and ANAs [modified from ref. 12]. Note the strong reactivity with different neutrophil antigens in individual sera, except for one patient in whom — despite also having lymphocyte antigens — ANAs were not found by IIF (lane 3). The line around 78 kD in lanes 6,8 and 10 most likely represents lactoferrin reactivity.

probably indicating that the mechanism leading to ANCA production in RA is different from that leading to ANCA production in primary SVV. Some antibodies in RA patients recognized nuclear antigens, and thus conceptually supported the earlier designation "granulocyte-specific ANA" [4]. It may be that the antibodies to neutrophils in RA reflect an immune response to apoptotic neutrophil constituents appearing in excessive amounts in the joint fluid. A strikingly similar immune response to neutrophil constituents is found in patients with UC [11].

Clinical Utility of ANCA

Our main knowledge about the utility of ANCA in daily clinical work stems from the extensive literature on patients with various forms of SVV [reviewed in 24]. There is general agreement that C-ANCAs appear especially in active Wegener's granulomatosis [14,15], and that a large proportion of patients having a disease relapse show reappearance or increased levels of ANCA [25,26]. A strongly increased risk of relapse exists in patients found to be intermittently or persistently positive for ANCA compared to persistently ANCA-negative patients [27], indicating that serologic follow-up is valuable in WG[8] [25].

The occurrence of P-ANCA with clear-cut MPO specificity is strongly associated with a diagnosis of SVV, mainly microscopic polyangiitis, rapidly progressive necrotizing glomerulonephritis, and CSS[9], but this serologic profile can also be seen in drug-induced vasculitis/lupus syndrome as well as in a small proportion of patients with WG [28]. There is also an association in MPO-ANCA-positive patients between ANCA levels and disease activity [29], but the relationship is less firm than in PR3-ANCA-positive patients.

There may be some clinical and histopathological differences between SVV patients producing PR3-ANCA and MPO-ANCA [30]. PR3-ANCAs are related to a predomi-

[8] WG = Wegener's granulomatosis
[9] CSS = Churg-Strauss syndrome

nance of vasculitis symptoms in upper airways, ears and eyes, whereas renal and lower pulmonary involvement is more common in MPO-ANCA-positive patients. Granulomatous lesions around affected vessels are common when PR3-ANCAs are present, but very rare when MPO-ANCAs are found. Disease relapses are more common in PR3-ANCA-related vasculitis than in vasculitis associated with MPO-ANCA. Chronic nasal carriage of *Staphylococcus aureus* is mainly found in WG patients with PR3-ANCAs, and these chronic bacterial carriers have more clinical relapses than do noninfected cases.

In rheumtoid arthritis the ANCAs may belong to any of the five known immunoglobulin classes, but the majority comprise the IgG class [4]. Complement-fixing properties are mostly absent — except in patients who spontaneously develop autoimmune neutropenia, commonly RA patients with Felty's syndrome [31] who form large amounts of circulating immune complexes involving ANCA [32].

In ulcerative colitis the ANCAs predominantly belong to the IgG class, and the antibodies lack complement-fixing abilities [8]. Most studies in the literature have not found any important association between positive ANCA serology and clinical variables, such as disease activity, extent or severity. The main antigens recognized are lactoferrin and BPI[10]. In patients with Crohn's disease the presence of ANCA appears to reflect large bowel involvement [33].

Both in RA and UC, the whole neutrophil leucocyte is the antigenic target of ANCA, and IIF using a standard technique and anti-IgG conjugates [34] is the only widely accepted method for their demonstration. In both diseases the levels of IgG ANCA do not relate to particular clinical variables. The production of ANCAs in RA and UC patients may actually be regarded as expanded populations of natural autoantibodies whose role is to attempt to limit tissue damage by acting as part of a scavenging system for damaged cells, which involves also the monocyte/macrophage and the complement systems [35,36].

It is important to note that IIF ANCAs can be found in a number of inflammatory and infectious diseases, but a clear antigen specificity has not been established in most of these conditions. An exception to this is the ANCA produced in patients with cystic fibrosis, where a large proportion of the patients harbor IgG and IgA ANCAs directed to BPI, probably as part of an immune response to *Pseudomonas aeruginosa* infection in the small airways [37].

Pathophysiologic Potential of ANCA

Much of the data in the literature point to a potential role of PR3-ANCA and MPO-ANCA in vasculitis [extensively reviewed in 17,24,28]. It is also likely that immune complex-forming, complement-fixing IgG ANCAs in Felty's syndrome contribute to the neutropenia and splenomegaly in these patients [reviewed in 4].

Conclusions

From the early phase of excitement that was generated a decade ago by the description of a new serologic tool to be used as an aid in diagnosing SVV, we have now reached a more critical state of knowledge about ANCAs and their targets in diagnostics. Among the important issues to resolve are better standardization of specific ANCA tests [19], and multicenter evaluation of more sensitive techniques that have shown promise without losing diagnostic specificity for SVV. The much less focused immune response to the whole neutrophil in other chronic inflammatory conditions can be interpreted as an immune epiphenomenon of the inflammatory process, the clinical implications being much less certain.

[10] BPI = bacterial permeabilty-increasing protein

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Capsule

Treating influenza with zanamivir

The MIST Study Group reports that in young adults who inhaled zanamivir, 10 mg twice daily within 36 hours of onset of symptoms compatible with influenza, the syptoms lasted for 1–5 days less than in those inhaling placebo. In a high-risk subgroup the symptoms were alleviated at a median of 2–5 days earlier, and the use of complication-associated antibiotics was lower in the zanamivir group than in the placebo group. As in the study reported by Hayden and colleagues, the use of zanamivir seemed to be safe. In particular, there was no excess of cough among patients inhaling the active drug. Taken together, these studies suggest that in the working population of young adults, zanamivir — provided it is inhaled shortly after the onset of symptoms — can reduce time lost from school or work by 30–50%. The effect is largest in those patients who are febrile when they present for treatment.

Zanamivir, an inhibitor of the influenza-virus enzyme neuraminidase, is a result of rational, computer-assisted drug design. Neuraminidase is essential for the release of newly synthesized virions from infected cells, and inhibition of this enzyme by zanamivir interrupts propagation of influenza virus within the human respiratory tract. This property distinguishes zanamivir from amantadine and its analogue rimantadine, which act by blocking the ion-channel function of the virus protein M2. This protein is not present in influenza B subtype, which is therefore insensitive to these drugs.

The MIST Study Group found a favorable effect on time to recovery and antibiotic usage in a high-risk subgroup, but mild asthma was the reason for the high-risk classification in most of this group, and only 18% were aged 65 years or older. Patients at highest risk are the very elderly and those with moderate to severe chronic cardiac or pulmonary disease. However, until the drug is investigated in such patients, caution should be exercised in extrapolating these data to the general population.

Will this drug be used to treat influenza? A major disadvantage is that it has to be given within 30 hours of onset of symptoms, and patients rarely seek medical attention at this stage. The MIST Study Group employed a media campaign to recruit patients early. Furthermore, doctors would have to prescribe empirically because of the limitations of confirmatory tests. Even when nasopharyngeal aspirates are tested with modern rapid antigen techniques and polymerase chain reaction, microbiological diagnosis within 24 hours is unlikely. Sensitive and specific rapid diagnostic techniques will not be available to many family doctors within the foreseeable future.

A safe and effective oral drug would be most valuable for outbreak control in nursing and residential homes. The disadvantage of zanamivir is that its bioavailability is poor when taken orally, and it is rapidly eliminated by the kidneys. Hence it can be applied only topically (by inhalation or intranasally), which may limit its usefulness for very young or very old people.

The studies by the MIST group and by Hayden and colleagues have shown that the neuraminidase inhibitors are effective in the treatment of influenza. For these drugs to be widely used, the medical community will want evidence that they can reduce the rates of complications, hospital admissions, and mortality in truly at-risk populations, and that their use in young people makes a worthwhile economic impact. Development of oral agents, and evidence that they are better than amantadine and rimantadine, would argue for their deployment chemoprophylactically.

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