Leading Article

Anti-neutrophil cytoplasmic antibodies and vasculitis

B. Leaker and G. Cambridge

Medical Unit Rayne Institute, University College and Middlesex School of Medicine, University Street, London WC1E 6JJ, UK.

Anti-neutrophil cytoplasmic antibodies (ANCA) have been described in a variety of vasculitic syndromes. Although first described in patients with renal disease^{1,2} ANCA are also found in vasculitis in the absence of renal disease. Their clinical relevance is now under active investigation.

The most widely used diagnostic test for ANCA at present, is an indirect immunofluorescence technique using alcohol-fixed normal human neutrophils as substrates for binding of sera.³ This test is easy to perform and provides a useful technique for screening sera rapidly. Several staining patterns of neutrophils have been observed using this method leading to confusing nomenclature in the literature. At a recent workshop the following classification was proposed:⁴ C-ANCA: cytoplasmic pattern, consisting of fine granular staining, often brighter around the nucleus; P-ANCA: perinuclear pattern, a rim of staining outlining the nucleus. This pattern of staining is an artefact which can be abolished by using formalin instead of alcohol as a fixative. Alcohol solubilizes the constituents of the primary granule which then diffuse and bind to the nuclear membrane giving rise to the perinuclear staining pattern.

C-ANCA is the commonest pattern of staining and is associated with Wegener's granulomatosis and microscopic polyarteritis. P-ANCA staining is associated with antibodies against myeloperoxidase as detected by enzyme-linked immunosorbent assay (ELISA) and is seen with systemic vasculitis and idiopathic crescentic nephritis^{5,6} although it has been described rarely in rheumatoid arthritis and systemic lupus erythematosus (SLE).⁷ Anti-nuclear antibodies detected on a conventional substrate of rat liver are negative in the above patients with C-ANCA and P-ANCA staining patterns on human neutrophils. A combination of a positive anti-nuclear and weak P-ANCA staining pattern may be seen in a variety of autoimmune disorders, and is not specific for vasculitis.

The immunofluorescent assay gives a diagnostic sensitivity and specificity of 80% to 100% for

Wegener's granulomatosis microscopic polyarteritis, and idiopathic crescentic nephritis.^{8–13} Positive indirect immunofluorescence tests have also been reported in other vasculitides such as Churg-Strauss syndrome and Kawasaki syndrome although in small numbers only. False positive tests have not been reported in the C-ANCA group; in three combined studies^{8,9,13} no false positives were reported in 1712 control patients of whom 141 had various types of renal disease and including patients with rheumatoid arthritis or SLE. In the P-ANCA group (anti-nuclear antibody negative) a few false positive tests have been reported in rheumatoid arthritis, SLE, and other causes of renal disease but none in normals.^{5,7}

False negative results are seen frequently in patients with limited forms of vasculitis and inactive or treated disease. Titres of ANCA are variable in Wegener's granulomatosis; using serial dilution of sera, Specks *et al.*¹¹ reported that a titre of >1:16 ANCA was associated with limited Wegener's granulomatosis whilst a titre >1:32 ANCA was associated with generalized disease. In the same study the authors described a reduction in titre of ANCA when the disease was adjudged to be in remission.

More sophisticated assays using ELISA¹⁴ and radioimmunoassay (RIA)¹⁵ have been developed using neutrophil cellular extracts as antigens and are currently being evaluated. Preliminary data suggest increased sensitivity and specificity.^{14,15} The clinical correlation between organ involvement, clinical syndromes and corresponding staining pattern on immunofluorescence⁶ and in ELISA is poor.

The nature of the antigen with which ANCA reacts remains controversial.^{14–19} Earlier studies using several different techniques have reported alkaline phosphatase,¹⁵ primary granule components,^{5,17,19} and secondary granule components.¹⁴ Such diverse results may be explained by poor methodology and inherent difficulties of neutrophil research. The early report of Lockwood *et al.* was correctly criticized^{16–18} because of their failure to use proteolytic enzyme inhibitors in their cell preparation. This is important as neutrophils are rich in neutral proteases. Additionally, a variety of

methods has been employed to detect the antigen-antibody reaction (for example Western blotting or ELISA systems) and studies have used a range of sera from different clinical sub-sets of vasculitis.

In our own studies,²⁰ we have used two methods to identify the antigen; firstly, using immunoblotting (a non-denaturing system), we have shown that the antigens are located in the primary granule of the neutrophil; secondly, using polyacrilamide gel electrophoresis (SDS-PAGE) and Western blotting (a denaturing system), we have demonstrated that sera from different patients react with different neutrophil proteins although some proteins appear to be common between patients. Recently, Goldschmeding *et al.*,²¹ using Western blotting to purify the antigen from six patients with Wegener's granulomatosis, have identified serine proteinase 3 (molecular weight 29 kD) a protein found in the primary granule. However, these studies are preliminary and need to be confirmed. The cytoplasmic pattern (C-ANCA) may be caused by an antibody directed against a 29 kD protein (and possibly other antigens) whilst the perinuclear pattern may result from antibodies directed against myeloperoxidase or other primary granule components.

The clinical importance of ANCA is twofold. Firstly, ANCA remains the only diagnostic test for vasculitis; secondly the antibody is present in active, untreated disease and disappears rapidly with immunosuppressive therapy.^{8,11,15,22} Patients who experience a relapse of their disease have rises in ANCA titres whilst those who develop intercurrent illnesses such as infection show no change in ANCA titre.¹¹ This suggests that ANCA may be useful in monitoring disease activity although there are cases in which ANCA titres can rise in the absence of a clinical relapse. More serial data are needed to investigate the possibility that ANCA

levels may be a reliable guide to disease activity and control of immunosuppressive therapy.

The role of ANCA in the pathogenesis of the disease is unknown and there is at present no evidence of direct pathogenicity of ANCA. Efforts to reproduce vasculitis in a primate model have failed. Preliminary reports have suggested that ANCA stimulates neutrophil superoxide production and also release of myeloperoxidase from primary granules.^{6,23} However, we have found that ANCA produces little or no respiratory burst in comparison with other stimuli. Neutrophil superoxide production after pre-stimulation with cytokines may be enhanced by ANCA but the effect appears weak. ANCA can bind to renal endothelial cells in culture²⁴ but are not directly cytotoxic to these cells. ANCA may simply be a secondary phenomena produced in response to neutrophil destruction. However, ANCA are not seen in other conditions of high neutrophil turnover such as septicæmia.

In conclusion, ANCA are of value in the diagnosis of vasculitis and a possibly useful marker of disease activity. A cytoplasmic pattern (C-ANCA) has been predominantly associated with Wegener's granulomatosis and microscopic polyarteritis whilst the peri-nuclear pattern (P-ANCA) has been seen with microscopic polyarteritis, and idiopathic crescentic nephritis although either pattern may be seen in any of these conditions. Both types of staining are produced by autoantibodies which react with one or more constituents of the primary granule and are associated with necrotizing vascular diseases. Identification of the antigens recognized by ANCA will enable more precise assays to be developed and may allow more specific treatment such as immunoabsorption therapy²⁵ to be developed. The mechanisms of disordered immunity leading to vasculitis and the development of ANCA remain unknown.

References

1. Davies, D.J., Moran, J.E., Niall, J.F. & Ryan, G.B. Segmental necrotizing glomerulonephritis with anti-neutrophil antibody: possible arbovirus aetiology? *Br Med J* 1982, **285**: 277-278.
2. Hall, J.B. McN., Wadham, B., Wood, C.T., Ashton, S. & Adam, W.R. Vasculitis and glomerulonephritis: a sub-group with an anti-neutrophil cytoplasmic antibody. *Aust NZ J Med* 1984, **14**: 277-278.
3. Venning, M.C., Arfeen, S. & Bird, A.G. Antibodies to neutrophil cytoplasmic antigen in systemic vasculitis. *Lancet* 1987, **ii**: 850.
4. Falk, R.J. & Jennette, C.J. Proposed nomenclature for autoantibodies specific for neutrophil primary granule constituents. *Proceedings of the Second ANCA Workshop, Leiden*, 1989. In press.
5. Falk, R.J. & Jennette, C.J. ANCA with specificity for myeloperoxidase in patients with systemic vasculitis and idiopathic necrotizing and crescentic glomerulonephritis. *N Engl J Med* 1988, **318**: 1651-1657.
6. Jennette, C.J. & Falk, R.J. Anti-neutrophil cytoplasm antibodies. *N Engl J Med* 1988, **319**: 1417.
7. Wiik, A. Granulocyte specific anti-nuclear antibodies. Possible significance for the pathogenesis, clinical features and diagnosis of rheumatoid arthritis. *Allergy* 1980, **35**: 263-289.
8. Van der Woude, F.J., Rasmussen, N., Lombatto, S. *et al.* Autoantibodies against neutrophils and monocytes: tool for diagnosis and marker of disease activity in Wegener's granulomatosis. *Lancet* 1985, **i**: 425-429.
9. Savage, C.O.S., Winnears, C.G., Jones, S., Marshall, P.D. & Lockwood, C.M. Prospective study of radioimmunoassay for antibodies against neutrophil cytoplasm in the diagnosis of systemic vasculitis. *Lancet* 1987, **i**: 716-720.
10. Harrison, D.J., Simpson, R., Kharbada, R., Arbenethy, V.E. & Nimmo, G. Antibodies to neutrophil cytoplasmic antigens in Wegener's granulomatosis and other conditions. *Thorax* 1989, **44**: 373-377.

11. Specks, U., Wheatley, C.L., McDonald, T.J., Rohrbach, M.S. & DeRemee, R.A. Anticytoplasmic autoantibodies in the diagnosis and follow up of Wegener's granulomatosis. *Mayo Clin Proc* 1989, **64**: 28–26.
12. Venning, M., Quinn, A., Broomhead, V. & Bird, A.G. Antibodies directed against neutrophils (ANCA and GS-ANCA) are of distinct diagnostic value in systemic vasculitis. *Proceedings of the Second ANCA Workshop, Leiden* 1989. In press.
13. Ludermann, G., Utecht, B. & Gross, W.L. Autoantibodies against cytoplasmic structures of neutrophil granulocytes in Wegener's granulomatosis. *Clin Exp Immunol* 1987, **69**: 350–357.
14. Ludermann, G., Utecht, B. & Gross, W.L. Detection and quantitation anti-neutrophil cytoplasm antibodies in Wegener granulomatosis by ELISA using affinity purified antigen. *J Immunol Methods* 1988, **114**: 167–174.
15. Lockwood, C.M., Jones, S., Moss, D.W. *et al.* Association of alkaline phosphatase with an autoantigen recognized by circulating anti-neutrophil antibodies in systemic vasculitis. *Lancet* 1987, **i**: 716–720.
16. Rasmussen, N., Borregard, N. & Wiik, A. Anti-neutrophil cytoplasm antibodies in Wegener's granulomatosis are not directed against alkaline phosphatase. *Lancet* 1987, **i**: 1488.
17. Goldschmeding, R., Tetteroo, P.A.T., The, T.H., von dem Borne, A.E.G.K.R. & Kallenberg, C.G.M. Anti-neutrophil cytoplasm antibodies in Wegener's granulomatosis are not directed against alkaline phosphatase. *Lancet* 1987, **i**: 1489.
18. Gross, W.L., Ludermann, J. & Schroder, J.M. Anti-neutrophil cytoplasm antibodies in Wegener's granulomatosis are not directed against alkaline phosphatase. *Lancet* 1987, **i**: 1489.
19. Goldschmeding, R., ten Bokkel Huinink, D., von dem Borne, A.E.G.K. & The, T.H. Wegener's granulomatosis autoantibodies: serological and immunochemical characterization of the antigen. *Br J Haematol* 1987, **66**: 411.
20. Leaker, B., Garcia, R., Cambridge, G. & Segal, A.W. Heterogeneous response of ANCA in vasculitis (Abstract). *Nephrol Dial Transplant*. In press.
21. Goldschmeding, R., van der Schoot, C.E., ten Bokkel Huinink, D. *et al.* Wegener's granulomatosis autoantibodies identify a novel diisopropylfluorophosphate-binding protein in the lysosomes of normal human neutrophils. *J Clin Invest* 1989, **84**: 1577–1587.
22. Gross, W.L., Ludermann, G., Kiefer, G. & Lehmann, H. Anti-neutrophil cytoplasmic antibodies in Wegener granulomatosis. *Lancet* 1986, **i**: 806.
23. Falk, R., Terrell, R., Charles, L. & Jennette, C. Neutrophil activation by ANCA: a potential pathogenetic mechanism of vascular injury (Abstract). *Proceedings of the Second ANCA Workshop, Leiden* 1989. In press.
24. Abbott, F., Jones, S., Lockwood, M. & Rees, A. Autoantibodies to glomerular antigens in patients with Wegener's granulomatosis. *Nephrol Dial Transplant* 1989, **4**: 1–8.
25. Palmer, A., Gjorstrup, P., Severn, A., Welsh & Taube D. Treatment of SLE by extracorporeal immunoadsorption. *Lancet* 1988, **ii**: 272.