WHAT I THINK ABOUT ANCA

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Introduction

The development of an immunological test for vasculitis has been a very important advance in both the recognition and understanding of these complex and unusual diseases[1,2]. Undoubtedly, the recognition of this class of antibodies has increased the reported incidence of vasculitis in epidemiological studies[3]. The test was originally described by Davies et al 1982[1] and then later connected with the presence of Wegener's granulomatosis by Van der Woude and colleagues in 1985[2]. ANCA are directed against antigens present in the primary granules of neutrophils. These azurophilic granules contain a variety of different enzymes involved in the digestion of foreign material. These enzymes play an important role in regulating host defence against bacterial invasion[4,5,6,7,8,9,10]. The antibodies may be telling us important information about the possible causation of vasculitis. It has been suggested that measuring ANCA in patients with established vasculitis gives an indication of disease activity[11]. ANCA antibodies are present in diseases other than vasculitis[12,13], as well as in drug induced forms of vasculitis[14]. The measurement of these antibodies has been the subject of some controversy and the current guidelines will be reviewed. In this editorial, we will discuss some of the points mentioned above in more detail to determine whether or not there is good evidence to support the claims suggested for ANCA in each of these settings.

Quality control

The original ANCA test was an indirect immunofluorescence assay which identified a cytoplasmic granular staining pattern on normal human neutrophils when covered in the serum of patients with Wegener's granulomatosis[2]. Subsequently, a distinct staining pattern was described with perinuclear antibody on neutrophil preparations[15]. It emerged that this was simply an artefact of the preparation process of the neutrophil so that if the cells were prepared with ethanol fixation there was no migration of positively charged enzymes, such as myeloperoxidase, towards the nuclear membrane (which is negatively charged). Therefore on ethanol fixed preparations, both types of ANCA give similar appearances of cytoplasmic staining. However, this ability to distinguish between the two ANCA patterns is of significance since it does clearly separate ANCA antibodies directed against serine-protease-3 (a 29 kilodalton protein in primary granules) from other antigens which might generate ANCA. Almost all of the cANCA (cytoplasmic ANCA) are directed against serine-protease-3, whereas almost all of the pANCA are not directed against serine-protease-3. pANCA are directed against a variety of different enzymes, including myeloperoxidase as the most commonly recognised enzyme, but also including lactoferrin, elastase, enolase, cathepsin-G and bactericidal permeability inhibitor. pANCA are therefore a much more heterogeneous group of antibodies[16]. In order to further refine the specificity of ANCA, a subsequent development has been an ELISA test to try and identify the target antigens for ANCA[17]. Initial preparation of ELISA material was subject to considerable difficulty because of problems in the purification process, so that the target antigens being presented in the ELISA were contaminated by other relevant target antigens[18]. This led to early reports of ANCA testing perhaps being subject to the vagaries of the preparation process. It also revealed significant variation in the quality of ANCA testing in different centres. The development of a standardisation of ANCA testing was a direct result of this and in 1993 the EC/BCR project commenced in order to standardise ANCA testing across laboratories in Europe[19]. The essential and important results of this standardisation were that immunofluorescence assays could be standardised adequately between centres into very crude decisions on

whether the ANCA pattern was «c» or «p» or «indeterminate» or «negative». The ELISA preparations tested in the European standardisation allowed the development of standard recognition patterns for myeloperoxidase and serine-protease-3 specific ANCA. However, the standardisation did not address the lesser known forms of ANCA directed against other enzymes in primary granules. It is apparent from the large European data set that ELISA testing is not infallible and the gold standard still remains the immunofluorescence test. Therefore, immunofluorescence screening is regarded as the first important step, following which specificity of the ANCA may be achieved using an ELISA. If the immunofluorescence screening test is negative, it is clear that the ELISA test should not be performed and the ANCA result is regarded as negative. If on the other hand the ANCA is present on immunofluorescence screening, the serum should be subsequently tested for ELISA against myeloperoxidase and proteinase-3. If it is negative on either of these assays the ANCA is regarded as positive but non-specific[20]. In some research laboratories further specificity can be obtained by using ELISA's developed to detect less common antigens but these have not been standardised across a variety of centres as has been achieved with myeloperoxidase and serine-protease-3.

The pathogenesis of vasculitis

ANCA antibodies have been implicated in the pathogenesis of vasculitis, particularly that involving small vessel vasculitis, since the association is most apparent in this clinical setting [21,22]. The suggested mechanisms for ANCA being implicated in the pathogenesis of vasculitis have been studied in elegant experiments where primed neutrophils are able to engage ANCA antibodies on their surface because primed neutrophils express ANCA antigens on their surface. It is possible through endothelial interaction for a neutrophil engaged with ANCA antibodies to release reactive oxygen species, reactive nitrogen species and to discharge primary granule contents in the vicinity of the endothelial cell lining layer, thereby causing damage to it and stimulating influx of inflammatory cells and mediators which in turn exacerbate the vessel injury[23]. However, it is not clear how the anti-neutrophils cytoplasmic antibodies are generated in the first place and the mechanism does not appear to apply to all forms of vasculitis or even all forms of small vessel vasculitis. There is considerable controversy over the biological role of ANCA in vivo. A number of studies have reported significant variation in ANCA titre without any clinical consequence[24,25]. The proposed mechanisms for ANCA mediated damage in vasculitis are probably not universal to all forms of vasculitis. There are no associations between ANCA and large vessel vasculitis or medium vessel vasculitis.

Role in clinical diagnosis

The clinical diagnosis of vasculitis is certainly aided by the detection of ANCA antibodies but it is very unreliable to use ANCA antibodies as a screening tool for detecting vasculitis. In a large series of ANCA testing performed in one teaching hospital only in a small percentage of positive ANCA results was a diagnosis of vasculitis sustained[26]. Even in the setting of the pulmonary renal syndrome only approximately eleven percent of patients presenting in this way will have ANCA related vasculitis[27]. The specificity of ANCA directed against PR3, as compared to ANCA directed against MPO for particular forms of vasculitis is also less certain. Wegener's granulomatosis may present with either PR3 or MPO ANCA. Similarly for microscopic polyangiitis, patients may have either PR3-ANCA or MPO-ANCA. It is likely that the ANCA pattern may be a greater predictor for disease severity than the actual diagnosis itself. This has been reported for a large series of patients with microscopic polyangiitis where out of 107 patients, the risk of death was 3.78 fold greater for patients with cytoplasmic ANCA as compared to those with perinuclear ANCA[28].

The role of ANCA in clinical activity assessment

The value of ANCA is mainly in the establishment of a diagnosis of vasculitis. However, it has been suggested that serial measurements of ANCA may provide important information about disease activity. An early study proposed treatment on the basis of a rise in ANCA level to pre-empt the

development of a flare of vasculitis[11]. However, this is very controversial and further, larger studies have disputed the relationship between rise in ANCA titre and flare[24,25] and at present it be unwise to recommend treatment changes based wholly on the change in ANCA levels.

The role in other diseases

Undoubtedly there are a number of conditions where ANCA is present but usually the specificity is poor i.e., not directed against serine-protease-3 or myeloperoxidase and the role of ANCA in these conditions is uncertain. Perhaps it should be regarded as similar to the presence of antinuclear antibodies or rheumatoid factor in chronic diseases. Indeed ANCA is often present in patients with SLE and RA and may be found in a percentage of patients with chronic infections such as tuberculosis and leprosy[16]. Chronic stimulation of the immune system may generate epiphenomenona, such as ANCA or ANA and this should not be taken as an indication of pathogenic involvement. In chronic inflammatory bowel disease (Crohn's) and ulcerative colitis many patients have ANCA[29]. It is usually directed against BPI and has no known pathogenic role in the disease. Chronic infection, such as is found in patients with cystic fibrosis, also is associated with the presence of ANCA and this is a perhaps more intriguing association because of the finding of small vessel vasculitis in some biopsies from bronchoscopic studies of this disease[30].

The role of ANCA antigens in health and disease

The antigens to which ANCA is directed are often very destructive and are regulated by natural inhibitors such as α1-antitrypsin[31]. It is intriguing that patients who suffer Wegener's granulomatosis have an increased incidence of α1-antitrypsin deficiency[31] and that the amount of the deficiency does seem to correlate with disease activity; heterozygotes have a lesser disease severity than homozygotes who are deficient for α1-antitrypsin[32]. It may be that the lack of natural inhibitors to ANCA antigens allows their presence to be extended in the circulation and close to immunologically relevant cells, such as B-lymphocytes. These antigens have been shown to bind externally to endothelium and they may be directly responsible for the generation of ANCA antibodies[33].

Conclusion

The ANCA test has brought with it an important advance in our understanding and recognition of systemic vasculitis. However, the enthusiasm for making a diagnosis of vasculitis should be tempered by the recognition that ANCA antibodies are not present in all cases of vasculitis and nor are ANCA antibodies pathognomonic with a diagnosis of vasculitis. A diagnosis of systemic vasculitis must continue to depend upon basic clinical assessment supported by appropriate histological findings and further supported by the ANCA test. The ANCA test may alert us to the possibility of vasculitis or it may help us refine the type of vasculitis. It cannot be used as the sole indicator of vasculitis. Our greater understanding of the pathogenesis has undoubtedly been spurred on by the recognition of ANCA and this may lead to important changes in our current strategies to managing vasculitis by being able to be more specific in our intervention with the immune system in the future.

Keywords
Antineutrophil Cytoplasmic Antibodies (ANCA)
Vasculitis
Wegener's Granulomatosis (WG)
Microscopic Polyangiitis (MPA)
Serine protease-3 (PR3)

Acknowledgements:
Mrs Anne Houghton for secretarial support.

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