THE IDENTIFICATION OF AUTOANTIGENS, AUTOANTIBODIES AND THEIR IDIOTYPES

Many of the inflammatory rheumatic diseases are accompanied by a characteristic spectrum of autoantibodies to intracellular antigens. In some diseases the unregulated production of these antibodies may be responsible for the pathogenesis of disease, e.g. where inflammation is caused by antibody-antigen complexes as in SLE. In other situations the autoantibody may play no part in disease causation but is a by-product of the disease process, e.g. anti-topoisomerase antibodies in the sera of some patients with systemic sclerosis [1]. In both of these situations there are good clinical reasons for us to be able to apply simple assays for autoantibody production. Detailed analysis of the autoantibody response may elucidate the prime cause of disease. Research workers in this field have divided into those who analyse the antigens and those who dissect the antibodies.

Several advances of clinical importance in recent years have been made in the assay of autoantibodies. These include the identification of anti-Ro and anti-La antibodies in patients with primary Sjögren's syndrome and in some patients with SLE; of anticentromere antibodies in the CREST syndrome [2]; of anti-Jo-1 antibodies in polymyositis with pulmonary fibrosis [3, 4]; of antineutrophil cytoplasmic antibodies in Wegener's granulomatosis [5, 6] and of the syndrome of thrombosis, recurrent abortions and thrombocytopenia associated with anticardiolipin antibodies [7, 8].

Striking progress has been made in the identification of many of the antigens recognized by these autoantibodies, accelerated by the interest of molecular biologists in the use of these naturally-occurring reagents for the analysis of intracellular physiology. Intracellular antigens that have been well characterized include the aminoacyl tRNA synthetase enzymes in some patients with polymyositis and pulmonary fibrosis [9]. Autoantibodies present in some SLE sera, in addition to polynucleotides, bind to a number of other antigens. These include cyclin (formerly PCNA, proliferating cell nuclear antigen) [10], La and Ro proteins which bind to the products of RNA polymerase III [11], and the U snRNP proteins which form the protein component of the small nuclear RNP particles (snRNP) recognized by anti-RNP and by anti-Sm [12, 13]. Autoantibodies present in sera of many patients with RA bind to the Epstein–Barr nuclear antigen (EBNA) [14, 15]. The list of such antigenic identifications grows monthly.

The major recent advances in techniques to identify autoantibodies in patients’ sera have been in the use of better-defined antigens and the wide availability of reference sera. The human epithelial cell line, HEp-2, gives much better morphological information than the traditional substrate of rat liver for the detection of autoantibodies by immunohistological techniques. Using this substrate several different autoantibodies can be identified: homogeneous nuclear reactivity suggesting the presence of antibodies to double-stranded DNA (dsDNA), speckled nuclear staining (anti-Sm or anti-RNP antibodies), antinuclear and anticientromere antibodies. Anti-cytoplasmic staining is found in the presence of anti-Ro, anti-Jo-1, antimitochondrial or anticytoskeletal antibodies. In this issue an immunoperoxidase technique, using HEp-2 cells as substrate, is described which obviates the need for a fluorescence microscope [16].

The technique for identification of antibodies to extractable nuclear antigens in widespread use is the precipitation in agarose gels of crude saline extracts of calf thymus or human spleen by autoantibodies derived from patients’ sera. Double immunodiffusion and counter-immunoelectrophoresis are both reliable and simple techniques; the key to success is the identification of precipitin lines by the formation of lines of identity with reference sera. As the antigens recognized by the autoantibodies are characterized and purified, so simple and precise microtitre plate assays can be established to quantitate autoantibody production. Most clinicians find anti-DNA antibody levels a helpful addition to their clinical assessment of SLE disease activity; similarly the quantitative assay of other autoantibodies, such as to La, may prove clinically useful [17].

The assay of autoantibodies is not without its pitfalls and this is well illustrated by consideration of assays to double-stranded DNA as recently reviewed by Emlen and colleagues [18].
Here the 'gold-standard' is the Farr assay in which antibodies of predominantly high avidity to dsDNA are measured. Antibodies binding with lower avidity and affinity to dsDNA may be found in other diseases as are antibodies to ssDNA; impure preparations of dsDNA contaminated by ssDNA may cause false-positive results. The development of ELISA assays to measure antibodies to dsDNA may allow the measurement of a different spectrum of anti-dsDNA antibodies whose clinical importance is as yet uncertain.

The study of the binding reactions of monoclonal antibodies has made very apparent the close light-chain sequence homology was found of many monoclonal antibodies and partly explains the divergent results from laboratories comparing the cross-reactivities of autoantibodies. Such cross-reactivities are favoured by low temperatures (a property of exothermic reactions), long incubations and high concentrations of antibody and antigen. Thus, although monoclonal antibodies reactive with both dsDNA and cardiolipin are described [19], the majority of naturally-occurring autoantibodies to these antigens do not cross-react at high affinity [20].

Much of the work published analysing the idiotypes of autoantibodies depends on cross-reactivity studies using anti-idiotypic antibodies. The interpretation of these studies is extremely difficult. In this issue, Isenberg and colleagues [21] report the absence of antibodies bearing the cross-reactive idiotype 16/6, found on some anti-DNA antibodies and on some anti-Klebsiella pneumoniae polysaccharide K30 antibodies, in sera from patients with ankylosing spondylitis. The 16/6 idiotype was previously defined on monoclonal anti-DNA antibodies raised from patients with SLE and the same idiotype with close light-chain sequence homology was found on a paraprotein reactive with K. pneumoniae from a patient with Waldenstrom's macroglobulinaemia [22]. Similarly, 16/6-bearing antibodies are found in the sera of patients with SLE [23] and with Klebsiella infections [24]. The 16/6 idiotype does not seem relevant to ankylosing spondylitis. Is it of aetiological importance in SLE? There are a number of grounds for doubting this. The anti-DNA response in SLE is not exclusively 16/6-positive, nor is there published evidence showing a high degree of cross-reactivity of anti-DNA antibodies from SLE sera with K. pneumoniae. More importantly, the autoantibody response in SLE usually includes several intracellular antigens; patients with anti-DNA antibodies often make antibodies to Sm and/or to La and Ro antigens.

The ultimate questions for experimenters studying the idiotypes of autoantibodies are: What germ-line genes are used? Are these disease susceptibility genes? What antigens trigger the production of antibodies formed from rearrangements of these germ-line genes? What are the physiological ligands for these antibodies in vivo? The answers to the first question are starting to be published [25]. The answer to the second is almost certainly no, since we probably all carry these genes [26], though more data are needed. The answer to the third and fourth may well be intracellular antigens themselves, which may be immunogens under certain circumstances [27]. Cross-reactivity with external antigens may be an interesting artefact.

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27. Reuter R, Luhrmann R. Immunization of mice with purified Ul small nuclear ribonucleoprotein induces a pattern of antibody specificities characteristic of the anti-Sm and anti-RNP autoimmune responses of patients with lupus erythematosus, as measured by monoclonal antibodies. *Proc Natl Acad Sci USA* 1986;83:8689-93.

SULPHOXIDATION ABILITY AND THIOL STATUS IN RHEUMATOID ARTHRITIS PATIENTS

It has been known for some time that polymorphisms exist in some metabolic pathways. Furthermore adverse reactions to certain drugs occur predominantly in the minority of individuals who are poor metabolizers of these drugs. As a consequence the risk of side-effects may be predicted from knowledge of patients' metabolic status. For example, by using a compound such as debrisoquine as a probe-drug the hydroxylation status can be determined. If impaired, there is an increased risk of toxicity with drugs such as phenformin or perhexiline which are metabo-