Antinucleic acid antibodies

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The discovery of the LE cell phenomenon (Hargraves *et al.*, 1948) has had particularly far-reaching effects beyond the immediate clinical field of rheumatology. It was, firstly, a timely spur urging research on the underlying causes of the systemic diseases of connective tissue into the then untrodden immunological paths that are now highways accommodating an ever-increasing army of investigators with ever-increasing expectations. It was also the seed (to change the metaphor) that engendered in the minds of those connected with laboratory investigation of patients the conviction that the more precisely the specificity of autoantibodies such as the LE cell factor can be defined the greater their discriminant value for diagnosis and prognosis and the better the chance of gaining insight into the underlying immunopathology, not to say aetiology. The extent to which these expectations about the role of antinuclear antibodies have been realised in the intervening decades is ripe for consideration.

Immunofluorescence test

We may begin by assessing the effects of the now widespread practice of substituting the much less time-consuming immunofluorescence test (Holborow *et al.*, 1957; Holborow, 1978) for antinuclear antibodies (ANA) for the LE cell test of Hargraves *et al.* (1948). At first this seemed to entail an unacceptable loss of diagnostic specificity, since many patients who do not have systemic lupus erythematosus (SLE), and also some normal people, have positive immunofluorescent ANA tests. The practical advantage is largely recovered, however, if sera giving positive immunofluorescence tests are titrated to their end points, and especially if the end point dilution is compared with that given by a readily available reference preparation (WHO International Reference Preparation for Antinuclear Factor 66/233 (homogeneous)) so that ANA results may be expressed in units/ml. This allows universal comparability. Most SLE sera, especially in active disease, have high ANA concentrations, often greater than 1000 U/ml. Most normal sera have less than 25 U/ml.

The international reference preparation 66/233 contains chiefly IgG antibody giving a homogeneous

nuclear staining pattern on, say, rat liver cryostat sections. The specificity it is measuring has not been formally determined, although it is probably deoxyribonucleoprotein (DNP), like the LE cell factor (Holman and Kunkel, 1957) predominantly reactive with the insoluble DNA-protein complex in such nuclei. The LE cell factor is the complement-fixing version of this specific IgG antibody. It is probably true to say that the single most useful result of an immunofluorescence ANA test is a negative one, since this virtually excludes a diagnosis of SLE. A positive test, on the other hand, poses the questions whether, and which, additional tests are likely to determine more precisely the antigenic specificities involved and to be of most help with diagnosis or management, or both.

The mention of nuclear staining pattern leads to the question whether particular patterns betoken particular nuclear antigenic specificities. This was, and is, a controversial matter on which different exponents of the art have different views. It should be remembered, however, that the immunofluorescence ANA test is properly a screening test, the results of which should be taken to indicate the need to perform additional tests against identified nuclear antigens. The number of nuclear antigens more or less well-defined and/or extractable continues to increase and the present complement is shown in the Table.

Table *Specificities of antinuclear antibodies*

Deoxyribonucleoprotein (Holman and Kunkel, 1957)
Deoxyribonucleic acid (Robbins <i>et al.</i> , 1957; Seligmann, 1957)
Histone (Holman <i>et al.</i> , 1959)
Ribonucleic acid (Munroe, 1969)
Nucleolar antigens (Beck, 1969; Finnas <i>et al.</i> , 1973; Miyawaki <i>et al.</i> , 1978)
'Extractable nuclear antigens'
Sm (Tan and Kunkel, 1966)
Ribonucleoprotein (Matteoli and Reichlin, 1971)
Ha (Akizuki <i>et al.</i> , 1977)
SS-A, -B, -C (Alspaugh and Tan, 1975)
PM-1 (Wolfe <i>et al.</i> , 1977)
RANA (RAP antigen) (Alspaugh and Tan, 1976)
Granulocyte-specific nuclear antigen (Elling <i>et al.</i> , 1968)

Some of these antinuclear antibodies give recognisably consistent nuclear staining patterns on

appropriate tissue or cell substrates. One of these is distinct nucleolar staining, quite commonly but not exclusively seen in progressive systemic sclerosis (Miyawaki and Ritchie, 1973). Another is 'speckled' staining of the nuclei in rat liver sections. Among the nuclear antigens which give this pattern are two extractable nuclear antigens (ENAs)—ribonucleoprotein (RNP) and the Sm antigen described by Tan and Kunkel (1968). The specificity of 'speckled' ANA for RNP, it has been claimed, may be demonstrated by the differential susceptibilities of the antigens to RNAase treatment. This has been shown by effects on both immunofluorescence and on passive haemagglutination titres (Sharp *et al.*, 1976; Cohen *et al.*, 1979). High-titre (1:1000 or more) speckled ANA, high-titre passive haemagglutination with ENA-coated erythrocytes, and double diffusion precipitin lines with ENA have all been thought (Sharp *et al.*, 1972, 1976; Parker, 1973; Farber and Bole, 1976; Leibfarth and Persellin, 1976; Cohen *et al.*, 1979) to identify a group of patients with 'mixed' connective tissue disease (MCTD) showing features of SLE, progressive systemic sclerosis, polymyositis, and especially arthritis, Raynaud's phenomenon, myositis, and swollen hands with only mild or minimal accompanying renal disease (Cohen *et al.*, 1979). Speckled ANA of anti-RNP specificity, however, is also reported in both clinically defined SLE (Reichlin and Matteoli, 1972; Cohen *et al.*, 1979) and in scleroderma alone (Parker, 1973; Cohen *et al.*, 1979), and it is perhaps still an open question whether laboratory inquiry into the specificity of ANAs giving 'speckled' patterns is for rheumatologists a clinically conclusive exercise. For the sake of completeness I should add that the other 'speckled' ANA is directed at an RNAase-resistant ENA, is usually of much lower titre, and is virtually confined to patients with SLE, about 30% of whom produce it for reasons at present unknown.

The coexistence of two or more antinuclear specificities in a given serum is not uncommon and may confuse nuclear staining patterns. In SLE the Sm speckling may be concealed by the homogeneous nuclear staining given by the coexistent anti-DNP antibodies. There have been several claims that anti-DNA antibodies give 'shaggy' or 'rim' staining of nuclei under given conditions, but we have never been able to demonstrate this consistently using the common rat liver substrate.

Anti-DNA antibodies

It was shown in the late 1950s that while only nucleoprotein is effective in absorbing the LE cell factor from lupus sera the latter may give precipitin

reactions with and often also fix complement with DNA. Since then anti-DNA antibody measurements have come to play an important part in the investigation of patients with positive ANA tests on immunofluorescence. The generally held view is that antibodies reactive with native, or double-stranded (ds) DNA are found almost exclusively in SLE, and that the amount of such anti-dsDNA antibodies correlate with disease activity. Antibodies to single-stranded (ss) DNA, on the other hand, are reported to be often present in other connective tissue diseases, and changes in their amount are considered to be of less clinical significance. These considerations are of immunopathological relevance in SLE because the immune complex deposits in the glomeruli of patients with lupus nephritis have been shown to include DNA as antigen (Koffler *et al.*, 1967).

Establishing the specificity of serum antibodies for dsDNA and measuring their amounts have posed problems. The most commonly used methods for anti-DNA antibody are radioimmunoassays by Farr's ammonium sulphate precipitation method (Wold *et al.*, 1968) or by the Millipore filter method (Ginsberg and Keiser, 1973). In both the radio-labelled antigen is native dsDNA from *Escherichia coli* or from tissue culture cells. Both tests appear to measure the primary interaction between DNA and anti-DNA antibodies, the 'DNA binding capacity'. That this is so has been confirmed by Aarden *et al.* (1976a). They first showed a linear relationship between the molecular weight of DNA preparations and the degree of binding of antibody, and then showed that the circular form of dsDNA (PM2 from bacteriophage >95%, MW 5.9×10^6) needed only one molecule of antibody to render each ring precipitable in the Farr test (Aarden *et al.*, 1976b). Circular DNA has the presumed advantage of being less susceptible to exposure of single-stranded regions which could bind antibodies reactive with ssDNA.

In DNA binding assays anti-DNA activity should be expressed quantitatively rather than as percentage-binding of DNA added. According to Swaak *et al.* (1977) this is especially important in determining antibody profiles in SLE in relation to disease activity, since in patients with renal involvement they have demonstrated in this way a sharp rise in anti-dsDNA just before recurrence of proteinuria and a sharp fall accompanying its onset, heralded by an antecedent depletion of serum complement components. This pattern of events appears consistent with the suggestion of Izui *et al.* (1977) that anti-DNA antibodies form immune complexes locally with DNA already bound at the glomerular basement membrane.

Crithidia lucilliae

A useful method for anti-dsDNA which avoids the preparation of DNA characterised for molecular weight and double-strandedness exploits the fact that the haemoflagellate *Crithidia lucilliae* carries a large mitochondrion in which the mitochondrial DNA forms a prominent feature, the kinetoplast. Smears prepared from *Crithidia* cultures and treated with lupus sera show brilliant, readily recognisable kinetoplast staining by indirect immunofluorescence, and titres correlate well with anti-dsDNA antibody levels by Farr assay (Aarden, 1977). This relatively simple test provides a welcome addition to the range of antinuclear antibody tests available in clinical immunology laboratories, and our own experience agrees with the conclusions of a recent study that results compare very favourably with those obtained by radioimmunoassays (Chubick *et al.*, 1978). The *Crithidia* test has the further advantage that, as an immunofluorescent procedure, it is readily adapted to measure the complement-fixing properties of the antibody, which correlate both with high titre and with the presence of severe renal disease (Sontheimer and Gilliam, 1978).

Anti-ds v. anti-ss

Most workers agree with Seligmann and Arana's (1967) proposition that SLE sera contain anti-DNA antibodies of three sets of specificities: (1) antibodies reacting exclusively with ssDNA and comprising specificities directed at the nucleic acid bases, nucleosides, nucleotides, and polynucleotides, determinants on all of which are accessible in ss-DNA; (2) antibodies that recognise sites common to both ds- and ssDNA; and (3) rare antibodies reactive only with dsDNA. The so-called anti-dsDNA antibodies include both the last two categories. There is little doubt that antibodies of such specificity are predominantly found in SLE, but they may also be present in chronic active hepatitis and in a few patients with rheumatoid arthritis. It is of some interest that we have occasionally found them also in rheumatoid patients treated with D-penicillamine. This contrasts with the generally single-stranded-only specificity of anti-DNA antibodies induced by hydralazine and procainamide therapy (Blomgren *et al.*, 1972), and raises the question whether there is a connection with the nephrosis which it is well known may complicate penicillamine therapy in rheumatoid arthritis.

Most SLE sera have antibodies reactive with nucleic acid bases of ssDNA (category 1 above) as well as antibodies reactive with dsDNA (categories 2 and 3) (Alarcón-Segovia *et al.*, 1975). Using

constant amounts (in picomoles) of ds- and ssDNA as antigens to test a large panel of SLE sera, Steward *et al.* (1977) found that antibodies preferentially reactive with ssDNA predominated in all cases studied. The clinical significance of anti-ds as compared with anti-ss reactivity and titres has been recently reassessed by several workers. Using a double antibody solid phase assay, Lange (1978) found anti-dsDNA in a majority of SLE patients' sera, especially during active disease with multi-system involvement, but an even higher incidence of anti-ssDNA. In patients with possible SLE, with rheumatoid arthritis, and with other connective tissue disease the incidence of anti-DNA was considerably lower and mostly anti-ss. Similar findings are reported by Locker *et al.* (1977), who at the same time underline the fact that false positive tests for anti-dsDNA antibodies may result from contamination of dsDNA with molecules having ss regions. This practical difficulty of providing a stable native DNA antigen for clinical immunology service testing has not yet been overcome. For this reason Barnett (1979) persuasively urges that a reproducible quantitative test with relatively stable ssDNA as antigen is as sensitive an indicator of SLE and disease activity as the more widely used but less specifically characterised tests using dsDNA.

It is certainly true that ssDNA, suitably coupled, is experimentally immunogenic while dsDNA is not. As Locker *et al.* (1977) point out, whether the presence of anti-dsDNA antibodies in SLE and their relative scarcity in other conditions denotes a real qualitative difference between SLE and other connective tissue diseases remains a fundamental question. We may predict with cautious confidence that an answer is unlikely to be forthcoming until the relevant aetiological factors are better understood.

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