Antibodies to polynucleotides in SLE

from Graham R. V. Hughes

JUST under two decades ago, four groups reported almost simultaneously the presence of antibodies against DNA in the serum of patients with systemic lupus erythematosus (SLE). While early studies used double stranded (ds) DNA, it was found that many, if not all, SLE sera also reacted against single stranded (ss) DNA. Indeed it is now apparent that a heterogeneous collection of anti-DNA antibodies exists, binding to a variety of sites on the polynucleotides (Arana and Seligmann, *J. clin. Invest.*, **46**, 1867; 1967; Cohen, *et al., Clin. exp. Immun.*, **8**, 551; 1971). During the past six years, the measurement of DNA antibodies by the ammonium sulphate precipitation technique (Farr) has become a standard clinical tool in the diagnosis and management of SLE. While antibodies against ds DNA occur almost exclusively in SLE, anti-ss DNA antibodies are not specific for this disease, occurring in rheumatoid arthritis, drug-induced lupus and a variety of other conditions, as well as in some normal sera.

The agent most commonly used in DNA binding tests is ^{14}C-labelled bacterial or mammalian double stranded DNA, and therein lies the main obstacle to standardisation of results. It has proved difficult using these preparations to assess the contribution to 'native' DNA binding of single stranded regions in the molecule. A recent study by Samaha and Irwin (*J. clin. Invest.*, **56**, 446; 1975) used a variety of techniques in an attempt to further characterise the antigenic regions in mammalian DNA. Using MAK chromatography followed by enzyme removal of single stranded regions, they showed that purified mammalian DNA is a mixture of almost homogeneous ds DNA, and ds DNA with significant single stranded regions. The latter were found to be rich in thymine, complementing the earlier studies of Koffler and his colleagues (*J. exp. Med.*, **134**, 294; 1971) who showed that sera with antibody to ds DNA are inhibited by the synthetic single stranded polymer poly(dA), less so by poly(dT), and least of all by poly(dC).

These studies suggest that some antibodies in SLE are directed at the ends of the molecule containing short thymine-rich single stranded portions and possibly also at areas of bifurcation between ds DNA and AT-rich single stranded regions in ds DNA. Thus it seems that the variable binding activity reported with various SLE sera may have more complex explanations, not simply reflecting specificity towards ss or ds nucleic acid, but possibly also pointing to differential binding of antibody to specific regions of structurally heterogeneous DNA. Two possible ways of bypassing the problem of ss DNA binding might be the use of circular ds DNA (such as SV40 DNA) or the use of synthetic self replicating polymers such as poly (dAT). Neither of these antigens has yet received adequate clinical trial. In the meantime, the clinical significance of slightly raised DNA binding values in some non-SLE sera remains uncertain.

Apart from clinical considerations, delineation of the various polynucleotide antigen–antibody systems has more than academic interest. The renal disease in SLE results largely from immune complex deposition, with DNA–anti-DNA antibody complexes being of considerable pathological importance. Less clear, however, is the relative contribution and possible interaction of the various polynucleotide complexes. Discrepancies appear to exist, for example, between the presence of anti-ss DNA antibodies in rheumatoid arthritis and scleroderma, and the lack of glomerular immune complex deposition in the majority of patients with these diseases.

One of the earlier known manifestations of circulating immune complexes in SLE was the presence of cold precipitating globulins (Christian, Haltfield and Chase, *J. clin. Invest.*, **42**, 823; 1975) and in a recent study, Winfield, Koffler and Kunkel (*J. clin. Invest.*, **56**, 563; 1975) have returned to cryoprecipitation in an attempt to determine the specific concentration of anti-polynucleotide antibodies in cryoprecipitates relative to serum levels. Using haemagglutination techniques, they found that antibody against ds DNA and ss DNA were both enriched up to a 100-fold in some SLE cryoprecipitates, as compared with serum. Antibody to ribonucleoprotein (thought to be associated with milder or absent renal disease) was only occasionally concentrated in cryoprecipitates. In contrast, anti-ds-RNA, which was commonly detectable in high titre in SLE serum, was only minimally concentrated, and only in a minority of precipitates. These data are in broad agreement with earlier elution studies of SLE glomeruli, where again anti-ds-RNA was not detected.

The findings again indicate that different anti-polynucleotide antibodies have differing pathogenetic properties. While this has, in essence, been known for over a decade, the dissection of these antigen–antibody systems appears now to be gaining momentum, the clinical goal being more precise management of SLE.

it may be necessary to use anti-inflammatory drugs during the early stages of treatment. In immune deficiency states there is a suggestion that uncontrolled lymphoid cell proliferation and autoimmune disease may be additional complications (Gelfand, E. W. *et al., New Engl. J. Med.*, **289**, 1385; 1973, and Ballow, M. *et al., J. Pediatr.*, **83**, 772; 1973). Lawrence (*Clin. Immunobiol., loc cit*) noted that "therapy with transfer factor (TF_D) as presently employed is empirical, since the precise mechanism via which this small molecule confers or uncovers a specific antigen–receptor site on the recipient's circulating lymphocytes remains to be clarified."

Immunotherapy with transfer factor for the persistent carrier of hepatitis B surface antigen or antigen carriers with chronic liver damage requires a cautious and carefully considered approach. The safety and effectiveness of such preparations should be evaluated as far as possible in animal model systems (*WHO Techn. Rep. Series*, No. 570, 1975). There is also, of course, a need to establish provisional standard reference preparations of transfer factor and studies on specificity are urgently required.

Carbon-13 NMR of proteins

from J. Feeney

IMPROVEMENTS in NMR instrumentation over the last few years have largely overcome the sensitivity problems which had previously retarded ^{13}C NMR studies of medium-sized molecules (molecular weight >1000). By using a combination of Fourier transform and proton noise decoupling techniques, high sensitivity natural abundance ^{13}C spectra can now be obtained for small peptides such as oxytocin, angiotensin and LHRH in which each carbon features as a single sharp resonance signal. For larger peptides there is considerable overlap of the ^{13}C spectral lines and when a small protein such as lysozyme is examined relatively few single ^{13}C resonances can be resolved.

Professor A. Allerhand and his coworkers (Oldfield *et al., J. biol. Chem.*, **250**, 6381; 1975) have now reported

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