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

methods which allow them to detect most of the aromatic quaternary carbons in small proteins. By using a proton noise decoupling field slightly off-resonance for optimum proton decoupling only the quaternary carbons are efficiently decoupled and the sharp signals from these carbons can then be separated from the very broad bands from proton-bearing carbons using convolution difference methods (Campbell, *et al.*, *J. Magn. Res.*, **11**, 172; 1973). The resulting spectra feature only the quaternary carbon signals. Allerhand and coworkers have applied these techniques to several native proteins (lysozymes, cytochromes *c* and myoglobins) and using concentrated aqueous solution (10–20 mM) in the 20 mm diameter sample tubes at 15.18 MHz they are able to obtain high quality spectra in ~5 h of accumulation time.

Many assignments of the signals could be made by consideration of chemical shifts, relaxation behaviour in H_2O and D_2O, selective proton decoupling experiments, *p*H dependence, deuterium isotope effects on chemical shifts and line broadening induced by bound lanthanide ions. For hen egg white lysozyme the γ-carbons of the 6 Trp residues have chemical shifts over a range of ~5 p.p.m.; when the protein is denatured with guanidine the signals coalesce into a single absorption band. In the ^{13}C spectrum of horse heart cyanoferricytochrome *c* all 18 non-protonated aromatic carbon signals are resolved. When the diamagnetic forms of haem proteins such as horse heart ferrocytochrome *c* are examined, the haem carbons are also detected in the spectra but these disappear in the spectra of the paramagnetic form. In mixtures of ferro- and ferricytochromes *c* there is fast electron transfer which produces exchange effects in the averaged spectrum such that a 1 : 1 correspondence between non-protonated aromatic carbon signals from the two forms can be established.

Clearly one can use the assigned ^{13}C signals in the various proteins to monitor protein unfolding, ionisation states of specific residues and interactions between the protein and other molecules. It should be borne in mind, however, that studies of ^{13}C at natural abundance (1.11%) have the disadvantage that they require large amounts (50 mM per sample) of soluble protein.

An alternative approach is to enrich the protein selectively with ^{13}C-labelled amino acids. In this way, the concentration and solubility requirements (<1 nm solutions can be examined) are reduced and a much wider range of proteins can be studied. Several workers have demonstrated that ^{13}C-labelled amino acids can be incorporated into bacterial proteins from auxotrophic organisms. An elegant example is found in the work of Hunkapiller and coworkers (*Biochemistry*, **12**, 4732; 1973) who isolated α-lytic protease from *Myxobacter* 495 grown on a medium containing all the naturally occurring amino acids including 2-^{13}C-labelled histidine. There is only a single histidine residue in α-lytic protease (His-57) and thus there is no assignment problem for the intense signal obtained in the protein ^{13}C spectrum. The C-2 carbon is coupled to the directly bonded proton and the measured $^2J_{CH}$ coupling constants are characteristic of the ionisation states of the imidazole ring ($^2J_{CH}$=219 Hz when the ring is protonated and $^2J_{CH}$=206 Hz for the unprotonated ring). Thus they were able to show from such measurements that the *p*K of His-57 is very low in the enzyme (*p*K <4), a fact which has important implications in understanding the mechanism of action of serine proteases.

Usually the incorporated labelled amino acid occurs at several different sites in the protein and one is faced with the difficult task of assigning the ^{13}C signals. A general solution to the problem of observing and assigning ^{13}C signals from proteins will be available when semisynthetic proteins can be made by incorporating synthetically prepared fragments containing several different labelled amino acids in a region of interest in the protein structure.

Tumour viruses discussed at Copenhagen

from Ian Macpherson

The Seventh International Symposium on Comparative Research on Leukaemia and Related Diseases was held in Copenhagen on October 13–18.

ONE of the most important issues raised at the meeting concerned the true nature of the C-type viruses apparently isolated from the leukocytes of a human case of acute myeloid leukaemia by Gallagher and Gallo (*Science*, **187**, 350; 1975) also from the bone marrow cells of a child with lymphosarcoma by Nooter *et al.* (*Nature*, **256**, 595; 1975) and from human embryo lung cells passaged in culture by Panem *et al.* (*Science*, **189**, 297; 1975). These viruses were recognised as typical C-type particles like those known to cause animal leukoses and were found to have antigenic and reverse transcriptase affinities with simian sarcoma virus (SiSV). These relationships were not considered to be surprising or even unexpected but what raised a few eyebrows at the meeting were the observations made in several laboratories using competition radio-immunoassay analysis and molecular hybridisation, that both Gallagher and Gallo's and Panem's isolates each contain two distinct viruses with uncomfortably close affinities to SiSV and the baboon endogenous virus. At present it is not possible to say who got what from where and when. The forthcoming conference of the Virus Cancer Program at Hershey may help clarify these uncertainties.

Regardless of how these questions are answered there is evidence from other sources suggesting that man commonly encounters viruses antigenically related to the murine, feline and/or simian agents. H. W. Snyder (Sloan-Kettering, New York) found that most, if not all, adult human sera contain antibodies that combine with polypeptides from these C-type RNA tumour viruses. Placental cord sera are negative and sera from leukaemic patients reacted like normal sera. These results suggest that a virus or viruses with antigenic specificities common to the animal C-type viruses used as probes in this study are widespread in the human community. R. S. Metzgar (Duke University Medical School) reported antigenic relationship between RNA tumour viruses and surface antigens of leukaemic cells in cytotoxicity tests. He has shown that human leukaemic myeloblasts have antigens related to the p30 and gp70 proteins of Friend mouse leukaemia virus. R. A. Lerner (Scripps, La Jolla) has found that gp70 has a remarkable pleomorphism in the mouse. By immunofluorescence he has shown that this component of mouse leukaemia virus surface coat is also present in mouse lymphoid and epithelial cells and in the secretions of the epididymis. The system offers an excellent model for the study of host control of this gene's expression.

An interesting observation that may provide at least a partial explanation why C-type viruses are not as readily detectable in man compared with other animals also came from studies carried out at Scripp's: F. C. Jensen provided evidence that human complement, without the intervention of antibodies, was capable of lysing a wide range of C-type viruses. This may provide man with an important line of defence against infection with viruses of this type.

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