Measurement of DNA Antibodies—A 3-Year Clinical Survey. By G. R. V. HUGHES (*Department of Rheumatology, Royal Postgraduate Medical School*)

The application of an immunoassay to the detection of anti-DNA antibodies (Pincus, Schur, Rose, Decker, and Talal, 1969; Hughes, Cohen, and Christian, 1971a) has led to its widespread use as a diagnostic and therapeutic guide in SLE. Since its introduction in this country, 2,780 tests have been performed in this unit, 690 of which were on sera sent from outside hospitals. Of 38 cases analysed for the A.R.A. criteria of SLE, 36 had raised anti-DNA antibody titres. Two, in whom the disease was clinically quiescent, gave normal titres.

262 sera with positive antinuclear factor tests were measured. Four false positive (non-lupus sera) results were obtained—3 cases of Sjögren's syndrome and one of chronic active hepatitis.

Serial studies were performed on 22 patients with SLE in order to determine the prognostic value of raised anti-DNA antibody titres in clinically quiescent patients. In 5, rising titres of anti-DNA antibodies preceded clinical flares of disease activity. In one patient, raised titres accompanied psychotic attacks as the sole manifestation of disease activity.

There was no relationship in serial studies between disease activity, as measured by DNA-binding, and the presence of complexes, as measured by C₁q precipitation. 'Upper normal' (20–30% binding) values were obtained in 28% of patients with RA, and 8% of normals. It is suggested that these values may represent low titres of anti-DNA antibodies in these patients rather than artefacts.

Discussion

DR. A. ST. J. DIXON (*Bath*) What is your evidence that in repeated testing of the same patient over many months variations in the results are due to variations in the patient rather than in the stability of the test?

DR. HUGHES We are trying to see if the conditions in any individual patient in which binding occurs do alter. We have no evidence at the moment that this is so.

DR. A. ST. J. DIXON (*Bath*) Well I really meant, was there some way of standardizing the tests so that you knew the results you got in 1972 were the same in 1974 for a given situation?

DR. HUGHES We use standard controls with each batch of tests. Even sera that have been stored frozen for 3 years give reproducibility with 5–8% on retesting.

DR. A. ST. J. DIXON So how do you propose to standardize it between Centres?

DR. HUGHES Obviously standard series are required with agreed standard conditions, so that between Centres similar binding is obtained on the same serum.

DR. E. J. HOLBOROW (*Taplow*) I would like to ask whether Dr. Hughes thinks a Farr binding test detects all anti-DNA antibodies. I ask because we have had some experience with an electro-immunodiffusion test and among sera giving positive precipitation lines, there were some that apparently gave no increase in DNA binding.

DR. HUGHES I cannot explain your findings.

DR. R. N. MAINI (*London*) I wonder if the DNA in the Farr and the electro-immunodiffusion tests were antigenically identical, for example, some so-called DNA preparations are contaminated by DNA-histone and RNA. Can I ask Dr. Hughes whether he had done any tests of purity on the DNA he uses? We feel purity of antigens should be well defined, as should storage conditions. These are some of the factors which could lead to conflicting results, and therefore require standardization.

DR. HUGHES I did not think this was the forum to go into details of standardization. We are working in collaboration with the Radiochemical Centre, Amersham, on standardization. Surprisingly, perhaps, the DNA is fairly stable on storage. Photo-oxidization of the DNA alters its structure and antigenicity and we thought this might have been a problem. Our own methods of assessment of the DNA include MAK columns and complement fixation against antinucleotide antibodies, which will bind denatured DNA but not native.

DR. D. GLASS (*London*) I would like to emphasize the need for standardization of assays for DNA antibodies. Our recent experience has drawn attention to a number of variables in addition to defining the nature of the DNA, as Dr. Maini has emphasized (Glass, Caffin, Maini, and Scott, 1973).

DR. HUGHES Can I just say that a lot of work has been done on standardization. Pincus (1971) has put in 3 years of work purely on this and his method is standard and does not vary, but DNA preparations vary enormously. The method developed by him is now being widely used in the U.S.A. and in a dozen or so centres here. I do not know the reason for your problems with it.

DR. D. GLASS (*London*) However well standardized an assay may be in a particular individual's laboratory, experience with radio-immunoassay in general has shown considerable inter-laboratory variation (Cotes, Mussett, Berryman, Ekins, Glover, Hales, Hunter, Lowy, Neville, Samols, and Woodward, 1969).

DR. I. M. ROITT (*London*) Now that the first triumphal flush of finding DNA antibodies is beginning to wane and you find that there are certain relationships between the presence of DNA antibodies and the clinical course of disease, am I right in assuming that you are turning your attention away from the Farr technique in an attempt to assess whether the distribution of anti-DNA antibody is not just in IgG but there is perhaps a distribution in the subclasses and to some extent other classes? This may have an important influence on the type of complex which is formed, and on the possible influence of affinity on the future outcome of the interaction between antibody and antigen.

DR. HUGHES Schur, Monroe, and Rothfield (1972) did some work on this. They found an association with G1 and G3 subclasses.

DR. K. WHALEY (*Glasgow*) I am interested that you didn't find a relationship between the presence of C₁q precipitations and DNA binding in the acute phase; this is contrary to our experience; and following on from

that observation I notice that you found C₁q precipitins in one patient where the DNA binding was going down. Was this patient on corticosteroid therapy?

DR. HUGHES Yes.

DR. K. WHALEY (*Glasgow*) Could this relate to the presence of free DNA in the serum, as this binds with C₁q under the same conditions as it binds to aggregated gamma globulin?

DR. HUGHES Yes. We have looked at it; as you know we described this effect that steroids have of releasing DNA into serum (Hughes, Cohen, Lightfoot, Meltzer, and Christian, 1971b) and we wondered if the fall might be related to release of DNA and subsequent complexing. We haven't been able to show that in any of the 6 patients in whom C₁q studies were performed serially. Our results conflict with those of Agnello who did claim a close association between disease activity and C₁q precipitins. I cannot explain the difference except perhaps C₁q precipitins do not relate to the main disease entity in SLE.

DR. K. WHALEY (*Glasgow*) Yes; could I ask one more question? Have you followed the course of titrations for the total DNA binding capacity by the standard Farr technique and the antigen binding capacity as a measure of disease remission? We find in the acute sera it is a far better index of response to therapy, it falls far quicker than the DNA binding capacity and in our experience, which is somewhat limited, patients who are going to respond to corticosteroid therapy appear to reduce their total DNA binding capacity much faster than those who don't reduce it.

DR. HUGHES I agree.

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Antibodies to RNA in the Connective Tissue Diseases.
By P. DAVIS and G. R. V. HUGHES (*Department of Rheumatology, Royal Postgraduate Medical School, London*)

The finding of antibodies to double stranded RNA in SLE (Koffler, Carr, Agnello, Thoburn, and Kunkel, 1971) has led to speculation concerning the possible role of RNA viruses in the aetiology of this disease (Talal, 1970). This report describes the application of an immunoassay for the measurement of these antibodies and its use in SLE and other connective tissue diseases.

Methods

Antibodies to polynucleotides were measured by the modified Farr technique using the synthetic polynucleo-

tides poly I:poly C:poly A:poly U, and viral RNA, previous studies having shown cross-reaction between viral-RNA and poly I:C antibodies. Optimum experimental conditions were determined using polynucleotide conc. 0.01 µg/50 µl and serum diluted 1:10. Precipitation was maximal with 50% saturated ammonium sulphate. Reproducibility within 5% was obtained. Binding was inhibited by an excess of cold RNA. Inhibition studies with sera of patients with virus infections are in progress.

Cases

104 serum specimens from 75 patients with SLE have been measured. Serial studies have been performed on 4 of them. RNA antibodies have also been measured in 20 patients with RA, 20 with DLE, 20 with chronic active hepatitis, and 40 normals. Parallel DNA antibody measurements were performed in all patients.

Results

Viral RNA antibodies were found in 21 of the 75 patients with SLE (36%). In the serial studies RNA-Ab were not found to be as sensitive a clinical guide as DNA-Ab measurements and 60% of the active SLE group had no RNA antibodies. High titres of viral RNA-Ab were not found in patients with RA, though there was a high incidence of antibodies to poly I:C.

Discussion

DR. G. S. PANAYI (*Guy's Hospital*) The interest of these antibodies is that they may reflect persistent infection with an RNA virus and yet people who have recovered from diseases with RNA virus do not show any increase in these antibodies. Can you explain this?

DR. DAVIS Yes. The majority of antibodies in the recovery stages of virus infections are not directed against the nuclear core of RNA or DNA which they may contain, but the protein which surrounds them. The suggestion has been made that with persistent infection some breakdown in the protein coat may occur exposing the antigenic sites on the RNA.

DR. K. WHALEY (*Glasgow*) I would like to point out that human RNA has a very well-defined secondary structure with extensive double-stranded regions, and until you perform inhibition studies it is rather dangerous to assume that this is an antibody to any sort of viral antigen.

DR. DAVIS Yes, I agree. We are not supporting the concept that SLE is a virus infection or that these antibodies are directed necessarily against an RNA virus. Our interest in RNA antibodies is firstly in their clinical usefulness and secondly in their possible role in immune complex formation.

DR. R. N. MAINI (*London*) Can we also correct for the record the impression you created about the possible aetiological significance of raised antibodies to viruses in SLE? The work of Phillips and Christian (1973) has emphasized that the antibody rise in SLE to certain viruses is correlated to the rise in gamma globulin also observed, and does not indicate that a virus is implicated as a specific aetiological factor.