Clinical significance of antibodies to native DNA as measured by a DNA binding technique in patients with articular features of rheumatoid arthritis

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SUMMARY The clinical significance of antinative DNA antibodies as measured by the Farr test was investigated in 10 patients with the articular features of rheumatoid arthritis. 5 of these patients also satisfied criteria for a diagnosis of systemic lupus erythematosus (SLE) and might be classified as rheumatoid/lupus overlap syndromes or as rheumatoids with systemic complications. None had evidence of renal disease and 3 of the 5 had Sjögren's syndrome. The sixth patient had aggressive peripheral arthritis, alopecia, and Sjögren's syndrome and developed anti-DNA antibodies after treatment with penicillamine. All of the 4 rheumatoid patients with no clinical features typical of SLE had some special disease feature. The first had subclinical liver disease and the other 3 had Sjögren's syndrome in addition to localized vasculitic skin ulceration (2) and pulmonary fibrosis (1).

The occurrence of antibodies to DNA in the sera of patients with systemic lupus erythematosus (SLE) had been known since 1957 (Cepellini *et al.*, 1957). Antibodies to native DNA but not those to denatured DNA have been said to be specific to SLE and to be of use in the diagnosis and management of patients with this condition (Hughes, 1971; Schur and Sandson, 1968; Pincus *et al.*, 1969; Koffler *et al.*, 1971). DNA antibodies have been shown using a number of techniques including complement fixation (Schur and Sandson, 1968), haemagglutination inhibition (Koffler *et al.*, 1971), gel diffusion (Arana and Seligmann, 1967), and the Farr test using an ammonium sulphate precipitation technique and radiolabelled native DNA antigen (Pincus *et al.*, 1969).

All these methods may be criticized on the grounds that native DNA is easily contaminated with single-stranded DNA, thus lowering the selectivity of the test. Purity of the DNA antigen has been improved by various laboratory techniques including the use of a supposedly pure native DNA from the mitochondria of flagellates (Aarden *et al.*, 1975). However, in view of its advantages, including sensitivity, ease of quantification, and availability, the Farr test using radiolabelled *E. coli* DNA as antigen, is the

method widely used in clinical practice to measure antibodies to native DNA. Previous studies using this technique have found raised DNA antibody levels unassociated with SLE in a few patients with Sjögren's syndrome (Hughes, 1971; Whaley *et al.*, 1973b), chronic active hepatitis (Hughes, 1975; Holian *et al.*, 1975), rheumatoid arthritis with pulmonary fibrosis or vasculitis (Holian *et al.*, 1975), and in one patient with Q fever endocarditis (Hughes, 1975). The present study examined the significance of raised DNA binding levels in patients with a primary diagnosis of rheumatoid arthritis.

Patients

Sera from 200 patients with the articular features of definite rheumatoid arthritis as defined by the ARA criteria (Ropes *et al.*, 1959) and who had positive antinuclear factors (1/16), cutaneous vasculitis, or subcutaneous nodules were examined. The 103 patients studied in detail with DNA antibodies all had DNA binding abnormalities shown in blood specimens collected on more than one occasion. Sjögren's syndrome was diagnosed when in addition to the articular disease there was either keratoconjunctivitis sicca or diminished salivary flow rates with grade 4 lymphocytic infiltration of labial salivary gland biopsy. The methods used here were as described previously (Whaley *et al.*, 1973a).

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Laboratory methods

DNA binding levels were measured using labelled *E. coli* native DNA as antigen (Radiochemical Centre, Amersham) and using methods previously reported (Webb, *et al.*, 1974). The normal range for the laboratory is 0–25%.

Serum antinuclear factor was measured by an indirect immunofluorescent antibody test using four-fold dilutions to obtain a titre (Beck, 1961). Serum rheumatoid factor was measured using the R3 titration kit (Denver laboratories). Antimitochondrial antibody and smooth muscle antibody were sought using an indirect immunofluorescent technique with rat kidney and rat stomach as substrates, respectively.

Results

The clinical features of the 10 patients studied are summarized in the Table. All these patients have had a symmetrical peripheral polyarthritis with synovial hypertrophy over a period of years, and radiological evidence of either loss of joint space or erosions.

The first 5 patients in the Table also had at some time manifested three of the clinical features included in the preliminary criteria of the ARA (Cohen *et al.*, 1971) for a diagnosis of SLE, and if the positive

antinuclear factor (ANF) (1/256 or 1/1000) of all these patients is accepted as the fourth criterion instead of LE cells then all 5 patients on these criteria qualified for a diagnosis of SLE.

Three of these 5 patients had Sjögren's syndrome. All have had repeatedly normal urine microscopy, creatinine clearance, and quantitative proteinuria estimations. Cases 1–4 at present are well on low doses of steroids, while the fifth patient is developing breathlessness because of interstitial pulmonary involvement.

The sixth patient had a 13-year history of rheumatoid arthritis with symptomatic Sjögren's syndrome and a 3-year history of Raynaud's phenomenon and alopecia. Before treatment with penicillamine she had active joint disease which had been treated with indomethacin and salicylates, her ANF was positive at 1/1000, R3 was positive at 1/2048, and DNA binding was within normal limits at 10%. Penicillamine was begun at 250 mg daily and increased gradually 1 g/day over a 4-month period. No side effects were noted and there was no proteinuria, but after 6 months the DNA binding was 37% and 1 month later 64%. At this stage her joint symptoms had improved marginally if at all and penicillamine therapy was stopped.

Case 7 had a 16-year history of a deforming, peripheral polyarthritis. Her serum was persistently

Table Salient features of 10 patients with abnormal DNA binding

Case no.	Sex	Age at onset (years)	Disease duration (years)	Sjögren's syndrome		Raynaud's syndrome
				Ophthalmological	Oral	
1	F	43	10	+	—	+
2	F	18	10	—	—	+
3	F	38	4	+	+	+
4	F	41	10	—	—	+
5	F	55	3	+	+	—
6	F	19	14	+	—	+
7	F	39	16	—	—	—
8	F	43	25	—	+	—
9	F	40	20	+	—	—
10	M	61	2	+	—	—

Case no.	Systemic features	ANF	RF	AMA	SMA	DNAB (%)
1	Facial rash, pleurisy	1/1000	1/256	Neg	Neg	74
2	Facial rash, pleurisy	1/256	1/64	"	"	80
3	Pericarditis, leucopenia	1/1000	1/1024	"	"	60
4	Facial rash, alopecia	1/1000	1/1024	"	"	47
5	Pleurisy, alopecia, leucopenia	1/1000	Neg	"	"	70
6	Alopecia	1/256	1/1024	"	"	63.8 RA/penicillamine
7	Alk phos 40 KA units; SGOT 51 units/l	1/256	Neg	1/128	Strong +	84 RA/liver
8	Vasculitic ulcerative pulmonary fibrosis	1/256	1/2048	Neg	Neg	63
9	Vasculitic ulcer	1/1000	1/512	"	"	45
10	Nodules	1/1000	1/256	"	"	50

ANF=antinuclear factor; RF=rheumatoid factor; AMA=antimitochondrial antibody; SMA=smooth muscle antibody; DNAB=DNA binding.

negative for rheumatoid factor while the ANF was strongly positive, as were smooth muscle antibody and antimitochondrial antibody tests. Urea and electrolytes were normal and intravenous pyelography showed a left renal calculus. Serum alkaline phosphatase was raised at 65 King-Armstrong units and serum aspartate aminotransferase was raised at 59 units/l (normal 0–60) while the bilirubin level was normal. Liver biopsy has not yet been carried out.

Cases 8, 9, and 10 all have had strongly positive ANF and rheumatoid factor. Cases 8 and 9 have had vasculitic leg ulcers, while Case 8 has had radiographic evidence of pulmonary fibrosis, pulmonary function tests showing the diffusion capacity to be reduced at 58% of the predicted value by the single breath technique. Case 10 has had no evidence of cutaneous vasculitis nor of pulmonary fibrosis to date but has several subcutaneous nodules. Cases 8–10 have no clinical or biochemical evidence of renal disease; all have Sjögren's syndrome.

Discussion

Ten patients had the joint features of rheumatoid arthritis in association with repeated high antinative DNA antibody levels in their sera as measured by a DNA binding technique. 9% contamination of the native DNA test antigen with single-stranded DNA occurs (Radiochemical Centre, Amersham data), and it seems unlikely that this degree of contamination alone would account for the raised DNA binding levels observed. Accepting the fact that this point is unproved, it still seems important to examine the significance of an abnormal result produced by this widely used technique in patients with apparent rheumatoid joint disease.

The first 5 patients all had three clinical features included in the preliminary criteria of the ARA for a diagnosis of SLE (Cohen *et al.*, 1971) in association with high titre positive antinuclear factors. None of them has had biochemical or clinical evidence of renal disease though none has had renal biopsy. It might be argued that they may develop florid renal disease in the future, but in the 3 patients with a disease duration of 10 years this seems unlikely. Whether these patients should be regarded as having a rheumatoid/lupus overlap syndrome ('rheupus'), or whether they should be regarded as having rheumatoid disease with systemic complications is debatable. The potentially benign nature of this type of disease is illustrated by the current good health of Cases 1–4, although the fifth patient is becoming increasingly breathless because of interstitial pulmonary involvement. In all 4 patients, as in the others discussed here, except Case 5, the joint

complaint has become the major factor to be considered in their management.

The sixth patient had a destructive arthritis, Sjögren's syndrome, and two features commonly occurring in SLE, alopecia and Raynaud's phenomenon, before treatment with penicillamine. The rise in DNA antibody followed 6 months' treatment with penicillamine and it is likely that this drug was responsible for producing the antibodies. Penicillamine has been reported as inducing a lupus-like syndrome in patients treated for cystinuria (Oliver *et al.*, 1972), and Wilson's disease (Walshe, 1975). 2 patients out of 4 with rheumatoid arthritis who developed a myasthenic syndrome after treatment with penicillamine also had Sjögren's syndrome (Bucknall *et al.*, 1975) and these patients are more likely to develop side effects after penicillamine therapy than after treatment with antibiotics (Whaley, 1973a). Penicillamine therapy in our patient probably precipitated anti-DNA antibody production in a predisposed individual.

None of the last 4 patients had had any of the clinical features usually regarded as typical of SLE. All, however, had some other finding with which their raised DNA binding levels may have been associated. Thus Case 7 had definitely positive tests for mitochondrial and smooth muscle antibodies as well as biochemical evidence of liver disease. DNA antibodies have been described in association with chronic active hepatitis and biliary cirrhosis (Holian *et al.*, 1975; Hughes, 1975) and it is likely that subclinical liver disease was a significant feature in this case. 2 of the last 3 patients had localized vasculitic skin ulceration and one had asymptomatic pulmonary fibrosis, both of which features have been described in association with abnormal DNA binding levels in patients with rheumatoid arthritis (Holian *et al.*, 1975). All 3 also had Sjögren's syndrome, in which DNA antibodies have also been reported (Whaley *et al.*, 1973b; Hughes, 1971). None of these last 4 patients had clinical or biochemical evidence of renal lupus, although we did not regard it as justifiable to carry out renal biopsy.

It has been shown that DNA antibodies may be induced by treatment with phenylbutazone (Grayson, *et al.*, 1975). None of the patients reported here had received this or related drugs although most of them had received treatment with indomethacin or salicylates before anti-DNA antibodies were shown in their sera. Gold therapy in the last patient was started after DNA antibodies had been shown. Thus, apart from Case 6, previous drug therapy could not be clearly implicated as a mechanism for DNA antibody production.

This study again emphasizes the relationship between Sjögren's syndrome and both DNA anti-

bodies and rheumatoid/SLE overlap syndromes, a factor that was ignored in two recent studies of patients with rheumatoid/SLE overlap syndromes (Go and Lockshin 1975; Astapenko *et al.*, 1975). Thus 7 out of the 10 patients described here, 4 with rheumatoid/SLE overlap syndromes and 3 with rheumatoid arthritis, had Sjögren's syndrome. If the concept of a spectrum of connective tissue diseases with typical rheumatoid arthritis and typical SLE at the poles is accepted, it may be that rheumatoid patients with Sjögren's syndrome tend to lie near the middle. This concept may have clinical significance as many Sjögren's patients have multiple drug allergy and unwise antibiotic therapy may precipitate florid active SLE in patients with previously benign disease (Grennan *et al.*, 1975).

In some but not all patients with typical SLE the DNA binding level may provide a guide to disease activity (Schur and Sandson, 1968). In only one of our patients (Case 3) has the DNA binding level been found to rise and fall in parallel with changes in disease activity and in her it has been extra-articular features of the disease and pyrexia which in particular have paralleled DNA binding levels. In the other patients DNA binding has shown no clear-cut relationship to either articular or extra-articular features of disease activity.

We conclude that (1) antinative DNA antibodies occur in diseases other than typical SLE; (2) patients with rheumatoid arthritis in whom they occur have either clinical features suggestive of SLE, Sjögren's syndrome, or some other disease feature but often have no evidence of renal disease; (3) anti-DNA antibody levels often do not correlate with disease activity.

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