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Detection and characterization of DNA–anti-DNA complexes in a patient with systemic lupus erythematosus

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SUMMARY

DNase digestion of SLE serum, with consequent release of bound DNA antibody has been proposed as a method for the direct demonstration of circulating DNA–anti-DNA complexes. In the present studies on the serum of a girl with active SLE nephritis, circulating DNA–anti-DNA complexes were demonstrated at the precise time of relapse of SLE nephritis. Ultracentrifugation showed that these complexes were of low molecular weight.

INTRODUCTION

While glomerular-elution studies have suggested a role for DNA–anti-DNA complexes in the pathogenesis of SLE nephritis (Koffler, Agnello & Kunkel, 1974) direct evidence of circulating DNA–anti-DNA complexes has proved difficult to obtain.

In 1973, Harbeck *et al.* (1973) found a significant increase in titres of antibody directed against DNA after digestion by deoxyribonuclease in eleven of fifteen cases of SLE with active renal disease. They reported a similar finding in the cerebro-spinal fluid in one case of lupus meningitis (Keefe *et al.*, 1974).

We wish to report our findings in one patient where circulating DNA–anti-DNA complexes were detected by this method and characterized by analytical ultracentrifugation on sucrose density gradients.

CASE HISTORY

In September, 1971, a 9-year-old Egyptian girl presented with fever, polyarthritis, malar erythema, photosensitivity, alopecia, vasculitis of the finger tips and anaemia. Investigations revealed an ESR of 129 mm/hr, positive LE cells and a 24-hr urinary protein concentration of 0·7 g/day. The creatinine clearance was normal. Treatment with Prednisone 10–15 mg daily was initiated. In July, 1973, she was referred to the Hammersmith Hospital for assessment. She was asymptomatic and there were no abnormal findings except for Cushingoid facies and proteinuria of 1·7 g/day. In October, 1973, reassessment revealed that the proteinuria had increased to 5·1 g per 24 hours. This was accompanied by a fall in C3 to 20% of normal and of total haemolytic complement (CH_{50}) to unmeasurably low levels. No change in therapy was made apart from an increase in Prednisone dosage from 10–15 mg/day. By the time of her return to Egypt in December the proteinuria had decreased to 2·4 g per day and CH_{50} and C3 levels were normal. Prednisone dosage had been reduced to 10 mg on alternate days.

The immunological studies reported in this paper were carried out during this period of observation.

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METHODS

Collection of sera. All tests were performed on sera which were immediately frozen in aliquots at −20°C.

DNA binding capacity. This was measured by the Farr assay as modified by Pincus *et al.* (1969) using 0·1 μg of ^{14}C-labelled *E. coli* DNA. Sera were pretreated at 56°C for 30 min. The method was modified for density gradient studies by the addition of 800 μg of normal HGG to act as a carrier protein.

Anticomplementary activity. This was performed on sera as described by Mowbray *et al.* (1973). According to a complement-titration curve, the loss in haemolytic complement activity was expressed as units of complement lost. The upper limit of normal established at a level of confidence of 99% was 0·3 u.

Anticomplementary activity of the density-gradient fractions was read in microtitre plates. Fractions were incubated with guinea-pig complement for 1 hr at 37°C and 16 hr at 4°C, when sensitized sheep RBC were added. Cell lysis was read after a further incubation of 1 hr at 37°C.

DNase digestion of sera. This was performed as described by Harbeck *et al.* (1973). Two samples of 50 μl were taken from each serum to be assayed. DNase I (10 μg) and $MgCl_2$ (0·3 μmol) in borate-saline buffer (0·15 M, pH 8·0) was added to one of these. An equal volume of buffer was added to the undigested control sample. Both samples were incubated for 1 hr at 37°C. EDTA 0·01 M in borate-saline buffer was added to the digested sample and EDTA 0·01 M, DNase 10 μg and $MgCl_2$ 0·3 μmol to the control to obtain a final dilution of sera of 1:10 or, if necessary, to a further dilution of serum binding 40–50% of the added labelled DNA. Results were expressed as percent increase in DNA-binding capacity above that of the undigested sample.

Effect of DNase digestion on sera containing DNA–anti-DNA complexes formed in vitro. The DNA-binding capacity of three SLE patients' sera was inhibited significantly by the addition of 1 μg of calf thymus DNA. After treatment with DNase, the DNA-binding capacity was restored to over 90% of that shown prior to the addition of DNA (Table 1).

TABLE 1. Effect of DNase on SLE sera

Serum	Per cent DNA binding	Per cent DNA binding after addition of cold DNA	Per cent DNA binding after DNase digestion
A	86	30	80
B	89	28	88
C	97	36	95

$C1_q$ precipitation. This was performed in 0·6% to agarose gel in a 0·01 M EDTA veronal buffer pH 7·2, ionic strength 0·09 as described by Agnello, Winchester & Kunkel (1970).

Sucrose density-gradient studies. Sera were analysed on isokinetic sucrose gradients prepared by the slow addition of 32·9% sucrose in PBS in a mixing chamber of constant volume. Serum 0·1 ml was applied to a 11·6 ml gradient and centrifuged in a SW 41 rotor at 36,000 rev/min for 16 hr. IgG and IgM were analysed with an automatic immunoassay on a technicon autoanalyser II. Fifty microlitres of each fraction was tested for DNA binding by the Farr assay, as described above.

RESULTS

Serological events are shown on Fig. 1. There was an increase in DNA-binding capacity after DNase digestion of 73% and 27% in early October This phenomenon coincided with an increase in proteinuria from 2·1 g to 5·1 g per hr, a fall in C3 to 20% of normal values, an unmeasurably low CH_{50} and high anticomplementary activity. A decrease in DNA-binding activity and the presence of $C1_q$-reactive precipitins were also observed.

At this point the patient appeared to improve spontaneously. There was a decrease in urinary protein concentration and in anticomplementary activity, a rise in C3 and CH_{50} to normal values and an increase in DNA binding capacity to 70%. No increase in DNA-binding activity of DNase-treated sera was observed. There was also a transient disappearance of C1q precipitins but there were otherwise present throughout the follow-up period.

Ultracentrifugation studies of serum collected in early October revealed that the DNA-binding activity was confined to the IgG peak (Fig. 2). Prior to DNase digestion, the DNA-binding capacity in this

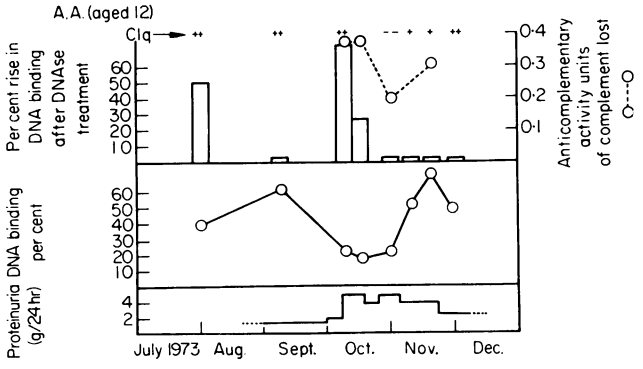

FIG. 1. Serological events in SLE patients. Increases in DNA binding after treatment with DNase are shown by the vertical columns. DNA-binding activity results are recorded on sera diluted 1:100 (as opposed to the normal 1:10 dilution).

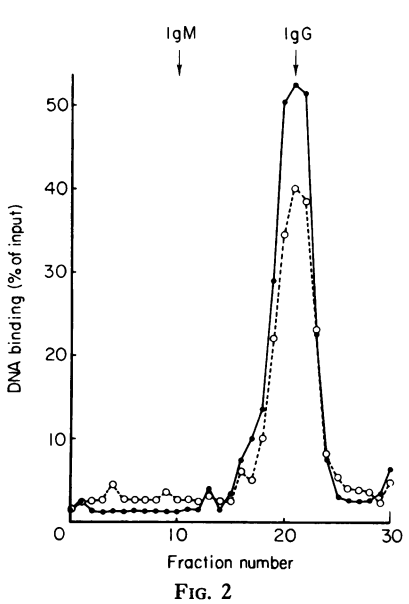

FIG. 2

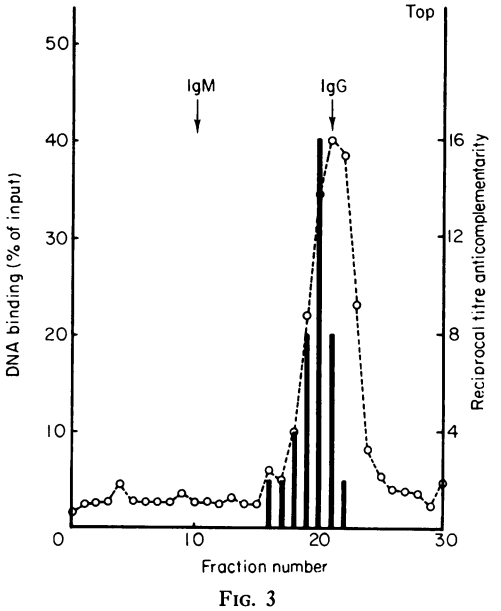

FIG. 3

FIG. 2. Ultracentrifugation studies of SLE serum (1). The rise in DNA binding following DNase digestion is confined to the IgG peak. (○- - -○) No DNAse; (●——●) with DNAse.

FIG. 3. Ultracentrifugation studies of SLE serum (2). Anticomplementary activity is localized to the heavy part of the IgG peak.

peak reached 40%. It increased after digestion to 52%, a rise of 30% above the undigested value. There was no IgG detected above 13S. Anticomplementary activity was localised to the heavy part of the IgG peak between 9S and 13S (Fig. 3). It was resistant to treatment with DNase.

DISCUSSION

A rise in DNA-binding capacity after digestion of sera by deoxyribonuclease suggests that DNA has bound *in vivo* to anti-DNA antibody to form circulating immune complexes. In our experience the finding of such a rise in DNA-binding capacity is unusual. Only in this patient among twenty-nine

studied with SLE and renal disease could we document such a rise (Hughes, 1975). It is noteworthy that this patient had aggressive renal disease with minimal signs of other organ involvement.

The finding of such a rise in DNA-binding capacity after DNase digestion accompanied by an increase in proteinuria, a low CH_{50} and C_3, and high anticomplementary activity suggests an association of circulating DNA–anti-DNA complexes with clinical exacerbation of the renal disease. The fall in titre of anti-DNA antibody immediately preceding the increased proteinuria may also support the presence and role played by these complexes.

$C1_q$ precipitins were present at the time of relapse, disappeared briefly and reappeared. Their significance is difficult to assess (Agnello *et al.*, 1971).

The ultracentrifugation studies reported here showed that the DNA-binding activity was confined to the IgG peak. There was no Ig heavier than 13S and immune complexes identified by determination of anticomplementary activity were small in size, 8–13S. While the DNA-binding activity increase after DNase digestion suggests the presence of DNA–anti-DNA complexes, the persistence of anticomplementary activity after DNase digestion suggested the presence of other types of circulating complexes or the inability of DNase to attack small strands of DNA particularly in large Ab excess. This may explain our failure by this method to detect DNA–anti-DNA immune complexes in more cases of active SLE. The use of other methods of dissociation of complexes, such as low pH or high molarity may test this hypothesis.

The finding of low mol. wt. DNA–anti-DNA complexes is open to a number of interpretations. There is considerable evidence that anti-DNA antibody reacts mainly with native helical DNA and not with denatured regions (Arana & Seligman, 1967; Cohen, Hughes & Christian, 1971; Aarden, De Groot & Feltkamp, 1975) and that one or two IgG molecules with both antigen-binding sites of the IgG molecule attached to the same DNA strand may form small cyclic complexes. Aarden *et al.* (1975) have found evidence for this type of high affinity binding between DNA and anti-DNA antibody in 6M caesium chloride gradients.

In summary, we have presented here data confirming the presence of preformed circulating DNA–anti-DNA complexes at the time of relapse of SLE nephritis and suggested that these may be small cyclic complexes constituting small strands of helical DNA and of one or two IgG antibody molecules. These complexes, especially in antibody excess, may be less available to DNase digestion, explaining the frequent failure of the method to detect DNA–anti-DNA complexes in SLE.

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