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Failure to detect circulating DNA–anti-DNA complexes by four radioimmunological methods in patients with systemic lupus erythematosus

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SUMMARY

The presence of DNA–anti-DNA complexes in sera from patients with systemic lupus erythematosus (SLE) was investigated by two new radioimmunoassays (RIA) developed for this purpose and by measuring the C1q and DNA binding activity of serum before and after treatment with DNAse. Two direct RIA developed in this study were based on the reactivity of [^{3}H]actinomycin D ([^{3}H]ACT-D) or solid-phase methylated bovine serum albumin (mBSA) with DNA–anti-DNA complexes. DNA–anti-DNA complexes prepared *in vitro* could be efficiently detected at various antigen–antibody ratios by these two RIA.

Increased levels of circulating immune complexes as indicated by the C1q binding test were found in 52% of SLE sera. However, the frequency of specific DNA–anti-DNA complexes detected in SLE sera was very low. Only 6% of sera exhibited an increased value deviating by more than three s.d. from the normal mean when tested with the [^{3}H]ACT-D binding RIA or the solid-phase mBSA RIA. On the other hand, there was no significant difference in the serum C1q or DNA binding activity after treatment with DNAse. These results suggest that DNA–anti-DNA complexes do not occur frequently in circulating blood and represent only a very small portion of the immune complexes detected in serum from patients with SLE.

INTRODUCTION

Patients with systemic lupus erythematosus (SLE) produce a variety of antibodies which react with native or altered autologous antigens (Kunkel & Tan, 1964). These antibodies appear to be involved in the pathogenesis of SLE through the combination with soluble tissue antigens released in circulating blood or in extravascular spaces to form immune complexes resulting in tissue damage. Circulating immune complexes have been found in sera from SLE patients by various methods (Nydegger *et al.*, 1974; Zubler *et al.*, 1976; Theofilopoulos, Wilson & Dixon, 1976; Casali *et al.*, 1977). Of several antigen–antibody systems, the DNA–anti-DNA complexes have been shown to be the major component of immune complexes localized in renal and skin lesions (Koffler, Schur & Kunkel, 1967; Krishnan & Kaplan, 1967; Landry & Sams, 1973). A significant correlation exists between the occurrence of high titres of anti-DNA antibodies and the severity of clinical disease and immunoglobulin deposits in tissues (Tan *et al.*, 1966; Koffler *et al.*, 1967; Schur & Sandson, 1968). The direct detection of DNA–anti-DNA complexes has been attempted by Harbeck *et al.* (1973) by measuring the binding of ^{125}I-labelled DNA in serum treated with DNAse to release complexed antibodies. This assay was based on the fact that *in vitro* formed DNA–anti-DNA complexes are sensitive to the treatment with DNAse (Deicher, Holman & Kunkel, 1959). These authors found slightly increased levels of anti-DNA antibodies after treatment of serum with DNAse, but these results were not confirmed by others (Feltkamp, 1975).

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In the present study, two new methods for the detection of DNA–anti-DNA complexes have been developed. The first method was based on the reactivity of [^{3}H]actinomycin D ([^{3}H]ACT-D) with DNA (Reich & Goldberg, 1964). [^{3}H]ACT-D bound to DNA–anti-DNA complexes was separated from free or DNA-bound [^{3}H]ACT-D by precipitation with anti-human IgG antibodies (anti-HGG). In the second method, methylated bovine serum albumin (mBSA) has been used in a solid-phase system to recognize DNA–anti-DNA complexes in serum. DNA is known to react strongly with basic proteins such as mBSA by electrostatic forces (Sueoka & Cheng, 1967). DNA–anti-DNA complexes were quantified by the binding of ^{125}I-labelled anti-HGG to the mBSA-coated tubes previously incubated with sera from patients with SLE. The frequency of DNA–anti-DNA complexes in sera from patients with SLE was studied by using these two radioimmunoassays (RIA) and by comparing the effect of DNAse digestion of serum on the C1q and DNA binding activity.

MATERIALS AND METHODS

Sera. 200 sera from sixty patients with SLE and forty sera from healthy blood donors were used in this study. Sera were kept frozen at −70°C. In some experiments, fresh serum samples were tested immediately after collection in order to prevent the formation of cryoprecipitates.

[^{3}*H*]*ACT-D and immunological reagents.* [^{3}H]ACT-D (sp. act. 12·0 Ci/mmol) was purchased from the Radio Chemical Centre, Amersham, England. Highly polymerized calf thymus DNA (type V) was purchased from Sigma Chemical Co., St Louis, Missouri. Single-stranded DNA (ssDNA) was prepared by heating native double-stranded DNA (dsDNA) in phosphate-buffered saline (PBS), 0·01 M and pH 7·0, at 100°C for 10 min, and then transferring it directly to an ice bath. Synthetic RNA, polyriboinosinic–polyribocytidylic acid (poly I–poly C) was purchased from Grand Island Biological Co., Grand Island, N.Y. mBSA and BSA were obtained from Calbiochem, San Diego, California. HGG was prepared by chromatography through a DEAE–cellulose column equilibrated with 0·01 M phosphate buffer at pH 8·0. Anti-HGG antisera were raised in rabbits. IgG fractions of anti-HGG antisera were obtained by DEAE–cellulose column chromatography. C1q was purified from fresh human serum by the method of Yonemasu & Stroud (1971).

Radiolabelling procedures. The IgG fractions of anti-HGG antisera, mBSA and HGG were radiolabelled with iodine (^{125}I) according to the procedure of McConahey & Dixon (1966). DNA was labelled with ^{125}I by the method of Commerford (1971). ^{125}I-labelled dsDNA was purified by treatment with single-strand specific S1 nuclease (Shishido & Ando, 1972). C1q was labelled with ^{125}I according to Hausser *et al.* (1973).

Preparation of soluble DNA–anti-DNA complexes in vitro. DNA–anti-DNA soluble immune complexes were prepared by reacting dsDNA or ssDNA with the IgG fraction obtained from the serum of a patient with SLE. Quantitative precipitin reactions were performed with the anti-DNA IgG fraction and the amount of antibody against dsDNA or ssDNA was thus determined. The anti-DNA IgG preparation was found to contain 0·8 mg/ml of anti-dsDNA antibody and 1·0 mg/ml of anti-ssDNA antibody. The DNA–anti-DNA complexes were prepared at constant amounts of antibody with different amounts of DNA, ranging from ten times antigen excess to two times antibody excess when compared with the equivalent point. Both the antigen and the antibodies were prediluted in fresh normal human serum (NHS). The antigen–antibody mixtures were incubated at 37°C for 2 hr and centrifuged at 1200 *g* for 30 min at 4°C to obtain soluble DNA–anti-DNA complexes.

[^{3}*H*]*ACT-D binding RIA for detection of DNA–anti-DNA complexes.* To quantify DNA (free DNA and DNA bound to anti-DNA antibodies) in the serum, 0·1 ml of serum were first incubated with 0·1 ml of 1 N HCl for 30 min at room temperature before adding 0·1 ml of 1 N NaOH. Then, 0·1 ml of mBSA (1 mg/ml) and 0·2 ml of [^{3}H]ACT-D (5 ng/ml) in borate buffer (pH 8·4, ionic strength 0·1) were added. Immediately after mixing, the reaction mixtures were incubated with 1·2 ml of 7·5% polyethylene glycol (PEG; mean molecular weight 6000; from Siegfried, Zofingen, Switzerland) in borate buffer at 4°C overnight. After centrifugation (1200 *g*), precipitates were washed once with 3 ml of 5% PEG in borate buffer. The precipitates thus obtained were dissolved in 0·4 ml of 0·1 N NaOH and transferred into 15 ml of Dioxan scintillation fluid for counting in an LS 250 Beckman liquid scintillation counter. In preliminary experiments, it was found that mBSA did not interfere with the binding of [^{3}H]ACT-D to DNA but increased the insolubility of DNA in PEG.

To quantify the amounts of DNA bound to anti-DNA antibodies, 0·1 ml of serum tested were first incubated with 0·5 ml of hyperimmune rabbit anti-HGG antiserum at 37°C for 1 hr and at 4°C overnight. In the preliminary studies, the amount of anti-HGG antiserum necessary to precipitate most of the HGG in the reaction mixtures was determined using ^{125}I-labelled HGG as a trace marker. After centrifugation at 1200 *g* for 30 min and washing once with 3 ml of PBS, precipitated immunoglobulins were treated with 0·1 ml of 0·5 N HCl for 30 min at room temperature and 0·1 ml of 0·5 N NaOH were added. Then the same procedure as described for the quantification of DNA for the whole serum was carried out for determination of the amount of DNA present in the immunoglobulins precipitated. The results were expressed as a percentage of [^{3}H]ACT-D precipitated.

To control the specificity of the binding of [^{3}H]ACT-D, sera which exhibited an increased binding of [^{3}H]ACT-D were treated with DNAse prior to the incubation with [^{3}H]ACT-D. 0·1 ml of serum were incubated with 0·05 mg of DNAse

(deoxyribonuclease I: 2600 u/mg, Worthington Biochemical Corp., Freehold, N.J.) in 0·1 ml of veronal-buffered saline containg Mg^{2+} (VBS), pH 7·2, at 37°C for 3 hr. 0·01 ml of 0·1 M EDTA (pH 7·0) was added to stop the enzyme reaction. For undigested control, EDTA was added before DNAse.

Determination of the binding capacity of solid-phase mBSA for DNA. mBSA (10 mg/ml in distilled water) was diluted to desired concentrations in borate buffer. Disposable polypropylene tubes (Beckman, Palo Alto, California) were incubated with 0·25 ml of mBSA at 37°C for 3 hr. mBSA-coated tubes were washed three times with 1 ml of PBS containing 0·05% Tween 20 (PBS–Tween) (Sigma Chemical Co.). Various concentrations of dsDNA or ssDNA with a trace amount of ^{125}I-labelled DNA were prepared in fresh NHS. 0·05 ml of sample and 0·2 ml of PBS–Tween were incubated for 2 hr at room temperature in mBSA-coated tubes. Then, after washing three times with PBS–Tween, the radioactivity was measured. The amounts of DNA bound to mBSA-coated tubes were calculated from radioactivity measurement.

Solid-phase mBSA radioimmunoassay for detection of DNA–anti-DNA complexes. In a first step, the mBSA-coated tubes were incubated with 0·25 ml of the test serum diluted 1:5 in PBS–Tween containing 1% BSA for 2 hr at room temperature. BSA was added in order to prevent the binding of anti-albumin antibodies in the serum to solid-phase mBSA. In a preliminary experiment, it was found that 1% of BSA in the serum samples completely inhibited the binding of high titres of anti-BSA antibodies to solid-phase mBSA without any inhibition of the binding of DNA–anti-DNA complexes to solid-phase mBSA.

In a second step, after washing three times with PBS–Tween, 0·25 ml of ^{125}I-labelled anti-human IgG fraction (6 μg/ml) in PBS–Tween were added to each tube. The tubes were left for 4 hr at room temperature and washed three times with PBS–Tween. The amount of radiolabelled anti-IgG antibodies was calculated from radioactivity measurement. The results were expressed as the absolute amounts of ^{125}I-labelled anti-IgG bound per tube.

To control the specificity of the test, serum samples or *in vitro* prepared DNA–anti-DNA complexes were digested with DNAse in VBS. 0·12 ml of the serum samples were incubated with 0·12 mg of DNAse in 0·12 ml of VBS at 37°C for 3 hr. Then 0·06 ml of 0·15 M EDTA (pH 7·0) and 0·3 ml of PBS containing 0·1% Tween and 2% BSA were added. 0·25 ml of DNAse-treated samples were tested for the detection of DNA–anti-DNA complexes. For the undigested control, EDTA was added before DNAse in order to prevent its enzyme action.

Other methods for the detection of DNA–anti-DNA complexes. The Farr DNA binding radioimmunoassay using DNAse for detection of DNA–anti-DNA complexes was carried out according to the method of Harbeck *et al.* (1973). Using the antigen non-specific method, the presence of immune complexes in circulating blood was examined by the ^{125}I-labelled C1q binding test (Zubler *et al.*, 1976). The effect of DNAse treatment on the serum C1q binding activity was determined as follows: 0·05 ml of serum samples were treated with 50 μg of DNAse in 0·05 ml VBS at 37°C for 3 hr followed by the incubation with 0·2 ml of 0·2 M EDTA (pH 7·0) at 37°C for 30 min. Then 0·05 ml of ^{125}I-labelled C1q and 1·75 ml of 3% PEG in borate buffer were added and incubated at 4°C for 1 hr. In the control tubes, EDTA was added before DNAse in order to prevent its enzyme action.

Statistical analysis. The Students *t*-test was used for the statistical analysis.

RESULTS

Detection of in vitro prepared DNA–anti-DNA complexes by [^{3}H]ACT-D binding RIA

The property of [^{3}H]ACT-D which specifically binds DNA was applied for detection of DNA and DNA–anti-DNA complexes. The precipitation of [^{3}H]ACT-D at a concentration of 5% PEG was 72% in the presence of normal serum containing 50 μg/ml ssDNA, but lower than 10% of [^{3}H]ACT-D was precipitated in the absence of DNA. The percentage of [^{3}H]ACT-D precipitated was correlated with the concentration of DNA in the reaction mixtures, and [^{3}H]ACT-D precipitated with ssDNA ten times more efficiently than with dsDNA (Fig. 1). However, the precipitation of [^{3}H]ACT-D in samples containing dsDNA could be increased to the levels observed with ssDNA after denaturation of dsDNA by treating the samples with 0·5 N HCl. There was no specific precipitation of [^{3}H]ACT-D in samples containing a synthetic RNA, poly I–poly C, even after treatment with HCl.

The reactivity of [^{3}H]ACT-D with dsDNA–anti-DNA complexes was studied by incubation of a constant amount of dsDNA (10 μg/ml in serum) with various dilutions in NHS of a lupus patient's serum with a high titre of anti-DNA antibodies. The concentration of DNA in serum was determined after HCl treatment of serum by precipitation of [^{3}H]ACT-D in the presence of PEG. The concentration of DNA complexed with anti-DNA antibodies was measured by similar quantification of DNA on immunoglobulin fractions specifically obtained from the same serum samples by precipitation with anti-HGG antibodies. In order to control the specificity of the binding of [^{3}H]ACT-D, DNAse-treated samples were used. It was found that the percentage of [^{3}H]ACT-D precipitated in the presence of whole serum remained constant after the addition of various amounts of anti-DNA antibodies (Fig. 2).

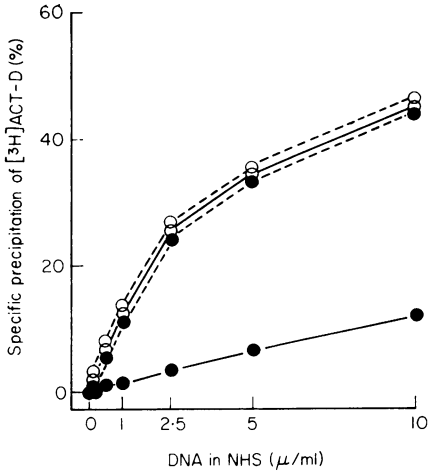

FIG. 1.

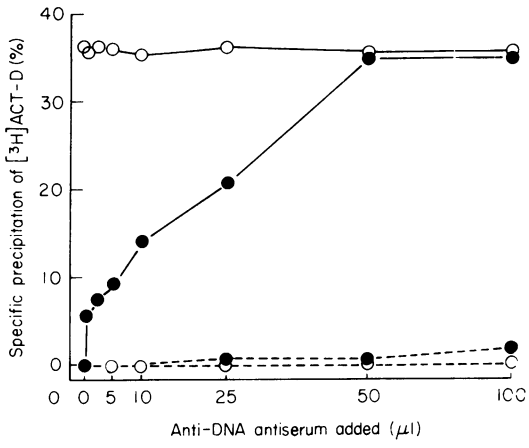

FIG. 2.

FIG. 1. The precipitation curve of [^{3}H]ACT-D in the presence of various concentrations of ssDNA or dsDNA in NHS with 5% PEG. (——) Represents the percentage of [^{3}H]ACT-D precipitated in the presence of untreated serum samples containing ssDNA (○) or dsDNA (●). (– – – –) Represents the percentage of [^{3}H]ACT-D precipitated in the presence of HCl-treated samples containing ssDNA (○) or dsDNA (●).

FIG. 2. The binding of [^{3}H]ACT-D to free and complexed DNA. (——) Represents the percentage of [^{3}H]-ACT-D precipitated in the presence of whole serum containing a constant amount of dsDNA (10 μg/ml) and various amounts of anti-DNA antibodies (○) or in the presence of corresponding immunoglobulin fractions (●). (– – – –) Represents the percentage of [^{3}H]ACT-D precipitated after treatment of serum samples with DNAse, with anti-DNA antibodies (○) or immunoglobulin fractions (●).

In corresponding immunoglobulin fractions, the percentage of [^{3}H]ACT-D precipitated was correlated with the addition of anti-DNA antibodies. Therefore, the values obtained on whole serum and on immunoglobulin precipitates allowed for an estimation of amounts of free and complexed DNA. The pretreatment of these serum samples with DNAse completely abolished the precipitation of [^{3}H]ACT-D.

The [^{3}H]ACT-D RIA was examined for detection of DNA–anti-DNA complexes prepared at various antigen–antibody ratios ranging from ten times antigen excess to two times antibody excess using a constant concentration (100 μg/ml) of human Ig-containing anti-DNA antibody in fresh NHS. The [^{3}H]ACT-D binding RIA efficiently detected DNA formed in the presence of 3·125 μg/ml of anti-DNA antibodies (absolute amount: 0·3125 μg) and of 0·875 μg/ml of DNA (absolute amount: 0·0875 μg) were still detectable (Table 1).

Detection of in vitro prepared DNA–anti-DNA complexes by solid-phase mBSA RIA

The binding capacity of polypropylene tubes coated with mBSA to DNA was investigated. It was found that solid-phase mBSA efficiently bound acidic substances and particularly DNA. A maximum binding of both ssDNA and dsDNA was observed using mBSA at a concentration of 100 μg/ml. Under this condition it was found, using ^{125}I-labelled mBSA, that 16 μg of mBSA were actually absorbed on the polypropylene tubes. Solid-phase mBSA fixed two times more ssDNA than dsDNA.

Soluble complexes were prepared *in vitro* with anti-DNA human antibodies (100 μg/ml) and dsDNA (56 μg/ml) or ssDNA (70 μg/ml) at two times antigen excess. These complexes were incubated for 2 hr at room temperature in tubes coated with mBSA (100 μg/ml). After washing, ^{125}I-labelled anti-HGG was added, incubated for 4 hr at room temperature, and the radioactivity bound to the tubes was then measured: there was a high binding of ^{125}I-labelled anti-HGG which was completely abolished after treatment of DNA–anti-DNA complexes with DNAse.

In the solid-phase mBSA RIA, the detection of soluble DNA–anti-DNA complexes was very efficient

TABLE 1. Minimum concentration of detectable DNA–anti-DNA complexes by various assays

Assay	DNA–anti-DNA complexes*	
	Anti-DNA antibody†	DNA‡
[^{3}H]ACT-D binding RIA	3·125 μg/ml	0·875 μg/ml
Solid-phase mBSA RIA	12·5 μg/ml	3·5 μg/ml
C1q binding test	12·5 μg/ml	3·5 μg/ml
DNA binding RIA	6·25 μg/ml	1·75 μg/ml

* DNA–anti-DNA soluble complexes were prepared by incubating dsDNA and anti-DNA antibodies in fresh NHS at equivalence point. Complexes were serially diluted in fresh NHS.

† IgG fraction from the serum of a patient with SLE.

‡ Calf thymus dsDNA.

in the range of equivalence to five times antigen excess (Fig. 3). Lower, but still significant, values were obtained for all other antigen concentrations. At equivalence, complexes formed in presence of 12·5 μg/ml of anti-DNA antibodies (absolute amount: 0·625 μg) and of 3·5 μg/ml of DNA (absolute amount: 0·175 μg) could be detected (Table 1).

Detection of in vitro prepared DNA–anti-DNA complexes by C1q binding test and DNA binding RIA

Similar DNA–anti-DNA complexes prepared at various antigen–antibody ratios were also tested for

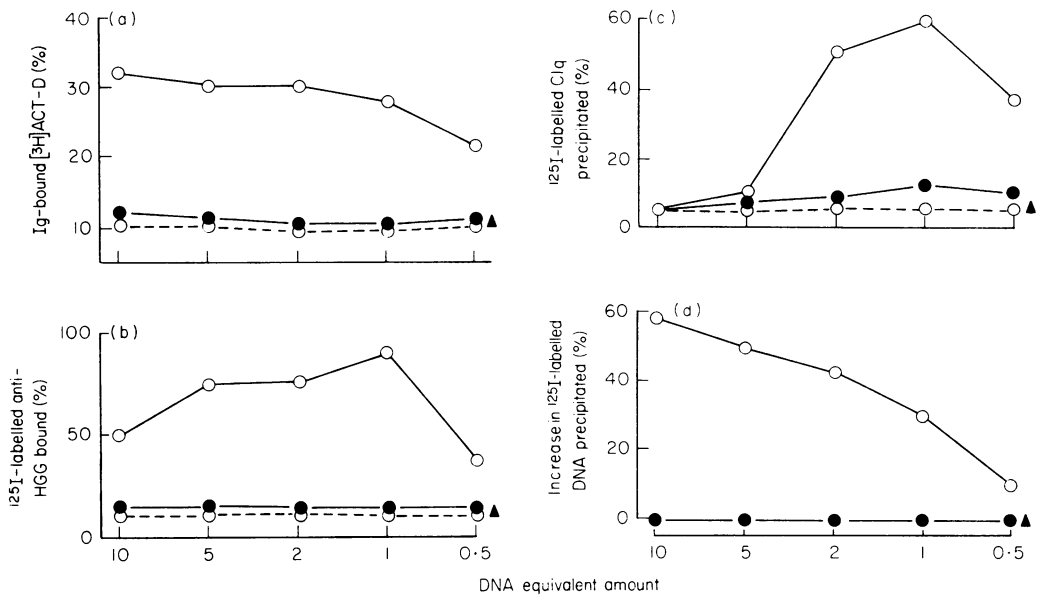

FIG. 3. Detection of dsDNA–anti-DNA complexes formed at various antigen–antibody ratios by four radioimmunological methods. The amount of antigen corresponding to equivalence is expressed as 'equivalent amount'. In the [^{3}H]ACT-D binding RIA (a), solid-phase mBSA RIA (b) and C1q binding test (c), the (——) represents the values obtained in the presence of DNA–anti-DNA complexes (○) or DNA alone (●). (○- - -○) Represents the values obtained after treatment of complexes with DNAse. In the DNA binding RIA (d), the lines represent the increased percentage of ^{125}I-labelled dsDNA binding activity after treatment of sera containing DNA–anti-DNA complexes (○) or DNA alone (●) with DNAse. The values obtained in the presence of sera containing only anti-DNA antibodies are indicated at the edge of each figure (▲).

their C1q binding activity. The C1q binding test was efficient for detection of DNA–anti-DNA complexes prepared in the range of two times antigen excess to two times antibody excess (Fig. 3). At more than five times antigen excess, the C1q binding test could not detect DNA–anti-DNA complexes. Complexes formed at equivalence in presence of 12·5 μg/ml of anti-DNA antibodies (absolute amount: 0·625 μg) and of 3·5 μg/ml of DNA (absolute amount: 0·175 μg were still detectable (Table 1). The increased C1q binding activity in the presence of DNA–anti-DNA complexes was completely inhibited by prior treatment with DNAse.

The same DNA–anti-DNA complexes were tested by the DNA binding RIA combined with DNAse treatment. Treatment of sera containing DNA–anti-DNA complexes with DNAse greatly increased their DNA binding activity in the presence of excess amounts of DNA (Fig. 3). A smaller, but still significant, increase in the DNA binding activity was observed in the presence of excess amounts of anti-DNA antibodies after treatment with DNAse. At equivalence, complexes formed in presence of 6·25 μg/ml of anti-DNA antibodies (absolute amount: 62·5 ng) and of 1·75 μg/ml of DNA (absolute amount: 17·5 ng) could be detected (Table 1).

Detection of DNA–anti-DNA complexes in SLE sera

The presence of DNA–anti-DNA complexes in sera of SLE patients was investigated by using the [^{3}H]ACT-D binding RIA and the solid-phase mBSA RIA, and by measuring the C1q and DNA binding activity of serum before and after treatment with DNAse.

In the [^{3}H]ACT-D binding RIA, the mean values obtained in the presence of sera or immunoglobulin precipitates from SLE patients were not significantly increased when compared to those of normal blood donors; mean ±1 s.d. of [^{3}H]ACT-D precipitate for sera: SLE, 11·7±1·9%; NHS, 10·0±3·0%; $P>0·02$; immunoglobulin fractions: SLE, 7·3±2·0%; NHS, 6·6±1·3%; $P>0·05$. Only 4% of sera and 6% of immunoglobulin precipitates exhibited an increased precipitation of [^{3}H]ACT-D which deviated from the normal means by more than 3 s.d. values. DNAse treatment of these positive samples from SLE patients did not change the percentage of [^{3}H]ACT-D precipitated (Fig. 4).

Using the solid-phase mBSA RIA, there was no significant difference in the level of immunoglobulins bound to mBSA tubes between sera from SLE patients and sera from normal blood donors (mean

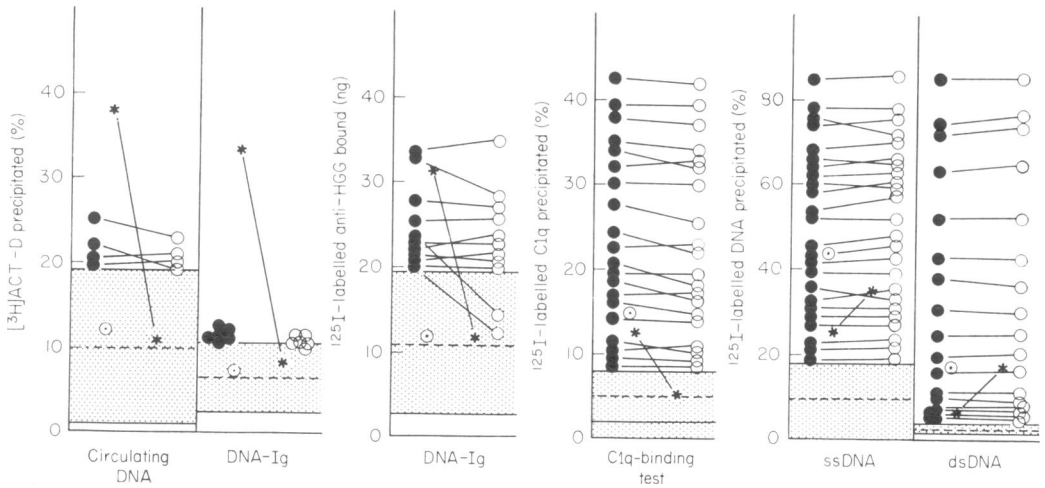

FIG. 4. Effect of DNAse treatment of sera in four radioimmunological methods. In [^{3}H]ACT-D binding RIA (a) and solid-phase mBSA RIA (b), all the positive samples were tested before (●) or after (○) treatment with DNAse. In C1q binding test (c) and DNA binding RIA (d), samples were selected at random to be tested before (●) or after (○) treatment with DNAse. The shadowed areas represent the mean ±3 s.d. values for NHS. Among sera samples of SLE patients, the following numbers of negative results, i.e. results located within the shadowed areas, were obtained in the various groups: ninety-eight for circulating DNA, ninety-six for DNA IgG, 189 for DNA Clq, eighteen for Clq binding, three for ssDNA and ten for dsDNA. *In vitro* prepared dsDNA–anti-DNA complexes using 5 μg/ml of dsDNA and 18 μg/ml of anti-DNA antibodies were tested in each test before or after treatment with DNAse (*).

± 1 s.d. of ^{125}I-labelled anti-HGG bound to mBSA tubes: SLE, $11{\cdot}3 \pm 7{\cdot}5$ ng; NHS, $11{\cdot}0 \pm 2{\cdot}9$ ng; $P > 0{\cdot}3$). 6% of serum samples from SLE patients exhibited an increased binding deviating from the normal mean by more than 3 s.d. values, but only two out of eleven positive sera showed a decreased binding to mBSA tubes after the treatment with DNAse (Fig. 4). The presence of anti-albumin antibodies in such false positive samples was ruled out since the binding of immunoglobulins to mBSA tubes did not increase in the absence of 1% BSA in the reaction mixtures.

To prevent the formation of cryoprecipitates, twelve serum samples from SLE patients were tested immediately after the collection of blood. None of the sera exhibited an increased binding of immunoglobulins to mBSA tubes (mean ± 1 s.d. of ^{125}I anti-HGG bound: SLE, $9{\cdot}6 \pm 2{\cdot}9$ ng; NHS, $9{\cdot}4 \pm 3{\cdot}7$ ng; $P > 0{\cdot}4$).

52% of sera from patients with SLE exhibited an increased C1q binding activity which deviated from the normal mean by more than 3 s.d. values. However, there was not any significant difference in the serum C1q binding activity after treatment with DNAse (mean ± 1 s.d. of C1q binding activity: DNAse-treated, $20 \pm 7\%$; non-treated, $21 \pm 8\%$) (Fig. 4).

An increased binding activity of ^{125}I-labelled ssDNA or ^{125}I-labelled dsDNA was observed in 89% and 61% respectively of untreated SLE sera. None of the sera exhibited a significant increase in the DNA binding activity after treatment with DNAse (mean ± 1 s.d. of DNA binding activity; ssDNA, DNAse-treated, $58 \pm 22\%$; non-treated, $57 \pm 22\%$; dsDNA, DNAse-treated, $18 \pm 27\%$; non-treated, $17 \pm 27\%$) (Fig. 4).

DISCUSSION

Immune complexes consisting of DNA and anti-DNA antibodies have been shown to be the major component of the immune complexes localized in tissue lesions of patients with SLE (Koffler *et al.*, 1967; Krishnan & Kaplan, 1967; Landry & Sams, 1973). It has been assumed that such DNA–anti-DNA complexes are formed in the circulation and are then deposited in filtering membranes or in perivascular areas. Indeed, unidentified immune complexes have often been detected by various methods in sera from patients with SLE (Nydegger *et al.*, 1974; Zubler *et al.*, 1976; Theofilopoulos *et al.*, 1976; Casali *et al.*, 1977). The possible involvement of DNA–anti-DNA complexes in these circulating immune complexes has been suggested by Harbeck *et al.* (1973), who observed a slight increase of the DNA binding activity in SLE serum treated with DNAse.

These results have not been confirmed by the present study, which used four methods to detect DNA–anti-DNA complexes. It was shown that these methods were capable of a sensitive detection of DNA–anti-DNA complexes which had been preformed *in vitro*. Firstly, the [^{3}H]ACT-D binding RIA was capable of detecting $0{\cdot}9\,\mu$g/ml of DNA, either free or in the form of immune complexes. The system used was more sensitive for ssDNA than for dsDNA. Although a few samples from SLE patients exhibited an increased ACT-D binding activity which suggested the presence of small amounts of complexed DNA, this binding was not inhibited by pretreatment with DNAse. One should consider that these results are due to a binding of ACT-D to other substances than DNA, possibly to immunoglobulins or to other types of immune complexes. Secondly, mBSA in solid-phase has been shown to efficiently bind acidic substances such as DNA and this principle could be used for the detection of DNA–anti-DNA complexes. This test could detect $3{\cdot}5\,\mu$g/ml of DNA in complexes formed at equivalence, but it is not specific for such complexes and any positive results require further analysis of DNAse-digested samples. There was an increase in the binding of immunoglobulins to such solid-phase mBSA in 6% SLE sera, but a significant decrease of this binding after DNAse treatment was observed only in two out of eleven positive samples. The binding of immunoglobulins to mBSA in the other nine samples may be due to a reactivity of mBSA with other acidic substances, possibly within the complexes, or with antibodies to mBSA. Thirdly, the C1q binding test has been shown to react efficiently with *in vitro* formed DNA–anti-DNA complexes (minimum amount of DNA: $3{\cdot}5\,\mu$g/ml) and this reaction is completely inhibited by a prior treatment with DNAse. Although the C1q binding activity was high in the majority of samples from SLE patients, no decrease was observed after DNAse treatment of serum and no evidence

for circulating DNA–anti-DNA complexes was obtained. Fourthly, the effect of DNAse on the DNA binding activity of serum samples was confirmed on DNA–anti-DNA complexes prepared *in vitro*, but such an effect was not seen in serum samples from SLE patients.

It is clear that the relative antigen–antibody ratios may influence to various degrees the method used. A high excess of DNA would only influence the C1q binding assay, while a large excess of anti-DNA antibodies would only inhibit the detection of complexes by solid-phase mBSA RIA or by an increase of DNA binding activity. *In vitro* experiments did not show an insusceptibility to DNAse of DNA complexed with antibodies in the conditions used for the four radioimmunoassays.

These data suggest that the presence of large amounts of DNA–anti-DNA complexes in circulating blood is unlikely and that most of the immune complexes detected in serum from patients with SLE involve other antigen–antibody systems. However, it is possible that circulating DNA–anti-DNA complexes would be cleared very rapidly by the mononuclear phagocytic system since DNA has an extremely short half-life in circulating blood (Tsumita & Iwanaga, 1963; Chused, Steinberg & Talal, 1972). Such a short half-life of complexes should not favour their deposition in filtering membranes. Therefore, on one hand, DNA–anti-DNA complexes may be the major component in lesions associated with immune complexes in SLE, but, on the other hand, they would represent not more than a minor component of immune complexes in circulating blood. Some particular mechanism may be responsible for their selective localization in tissues. Indeed, it has been demonstrated that, *in vitro*, DNA has a particularly high affinity for collagen or collagen-containing structures such as the glomerular basement membrane (Izui, Lambert & Miescher, 1976). Anti-DNA antibodies can react with DNA-bound collagen or basement membrane, thus forming immune complexes locally. There is evidence which suggests that this hypothetical mechanism is involved in the deposition of DNA–anti-DNA complexes in mice injected with bacterial lipopolysaccharides (Izui *et al.*, 1977).

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