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Characterization of DNA in polyethylene glycol precipitated immune complexes from sera of patients with systemic lupus erythematosus

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SUMMARY

The nature and the quantity of DNA present in the circulating immune complexes (ICs) from 30 patients with systemic lupus erythematosus (SLE) was characterized. DNA was extracted from IC enriched material prepared by polyethylene glycol precipitation of serum and the extracted DNA was labelled with ^{32}P-phosphate. The size and the nature of DNA was determined by polyacrylamide gel electrophoresis and autoradiography. The quantity of DNA in the PEG precipitates from sera of 10 clinically active SLE was found to be significantly higher (mean 159×10^4 ct/min, range 49·9–807×10^4 ct/min) than 10 normal controls (mean $24{\cdot}7 \times 10^4$ ct/min, range 8·7–$47{\cdot}8 \times 10^4$ ct/min). Four different sizes of DNA fragments were detected: 370–470, 150–240, 30–40 and 20 base pairs (bp). DNA of 30–40 bp and 20 bp were frequently present in both SLE and normals, but the other two large sized DNA fragments were particularly prominent in SLE patients. In the majority of samples, DNA fragments appeared double stranded.

Keywords immune complexes systemic lupus erythematosus polyethylene glycol DNA serum

INTRODUCTION

Circulating immune complexes (ICs) are found in sera from patients with systemic lupus erythematosus (SLE) (Nydegger *et al.*, 1974; Huston *et al.*, 1978). A correlation of the levels of ICs with disease activity in SLE has been demonstrated (Huston *et al.*, 1978), but information on the antigens participating in the formation of such ICs is fragmentary. Evidence that ICs might be present in the circulation and that such complexes may involve DNA and antibodies to DNA was the demonstration of the sequential appearance of antibody followed by antigen in SLE patients (Tan *et al.*, 1966). There is evidence to show that DNA–anti-DNA ICs are one of the major components localized in skin and renal lesions (Landry & Sams, 1973; Koffler, Agnello & Kunkel, 1974). Recently, some reports have suggested that DNA–anti-DNA ICs may also be present in the circulation of SLE patients (Bruneau & Benveniste, 1979; Sano & Morimoto, 1981), but little information is available concerning quantities and the nature of DNA in circulating ICs.

The present study quantitated and characterized DNA in the circulating ICs which were obtained by polyethylene glycol (PEG) precipitation of sera from the patients with SLE. DNA was extracted from the PEG precipitates, radiolabelled by a polynucleotide kinase reaction and

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analysed by polyacrylamide gel electrophoresis (PAGE) and autoradiography. Small DNA fragments of four different sizes were found but DNA of 150–240 bp and 370–470 bp were particularly noticeable in SLE patients. Enzyme digestion studies confirmed that the fragments were double stranded DNA in the majority of samples.

MATERIALS AND METHODS

Patients and blood samples. Samples of sera and plasma were collected from 30 patients who fulfilled the 1982 classification criteria for SLE proposed by the American Rheumatism Association (Tan *et al.*, 1982). Ten normal healthy adults were also studied. The sera and plasma were stored at −20°C until used. Active disease was defined by the presence of at least two of the following clinical signs: active dermal manifestations, mouth ulcers, synovitis, serositis, myositis and active visceral organ inflammation such as pneumonitis, hepatitis, cerebritis or glomerulonephritis. Activity was defined as the presence of an actively changing clinical status involving the above organ systems and not on the evidence of previously active but currently quiescent disease.

PEG precipitation method for quantitation and enrichment of immune complexes. The PEG method described by Digeon *et al.* (1977) was used with slight modifications. A serum sample was diluted 1:25 with 0·1 M borate buffer, pH 8·4 and mixed in equal portions with PEG (mol. wt 6,000), at a final concentration of 4·0%. The mixture was incubated at 4°C for 18 h. Precipitates were centrifuged at 2,300*g* for 1 h at 4°C, washed with 4·0% PEG and then redissolved in Tris buffer/20 mM Tris-HCl, 0·15 M NaCl, pH 7·4. The quantity of precipitated protein was measured by a Bio-Rad protein assay (Bio-Rad Laboratories, Richmond, California, USA).

DNA extraction from PEG precipitates. DNA was extracted from the PEG precipitates with phenol followed by chloroform-isoamyl alcohol (24:1) extraction (Kirby, 1968). The aqueous nucleic acid phase was dialysed extensively against 20 mM Tris-HCl, pH 7·6, 10 mM NaCl, 5 mM Na_2-EDTA, and digested with 10 μg of pancreatic RNAase (Sigma Chemical Co., St Louis, Missouri, USA) at 37°C for 30 min. The RNAase stock had been previously heated at 80°C for 10 min in 1 mM acetate buffer, pH 4·5, in order to destroy any contaminating DNAases. The reaction mixture was phenolyzed, followed by chloroform-isoamyl alcohol extraction. After ethanol precipitation, the DNA pellet was dissolved in 50 μl of distilled water, and an aliquot of 25 μl was radiolabelled.

Radiolabelling of DNA with ^{32}P-phosphate. Extracted DNA was radiolabelled according to the method of Maxam & Gilbert (1977). A 50 μl reaction mixture containing 50 mM Tris-HCl, pH 9·5, 10 mM $MgCl_2$, 1 mM spermidine, 6 mM dithiothreitol, 80 pmol ^{32}P-ATP (specific activity 3,100–1,000 Ci/mmol, New England Nuclear, Boston, Massachusetts) and 40 u polynucleotide kinase (P-L Biochemicals, Inc., Milwaukee, Wisconsin, USA) was incubated at 37°C for 30 min. The reaction was stopped by adding 200 μl of ammonium acetate. Fifty micrograms of tRNA (Sigma) was added as carrier, and the mixture was precipitated by 66% ethanol. After centrifugation, the pellet was dissolved in 250 μl of 0·3 M sodium acetate, precipitated by 75% ethanol, and centrifuged again. The final pellet was washed with 95% ethanol, dissolved in 100 μl Tris buffer and deproteinized by chloroform-isoamyl alcohol extraction. Radioactivity was assayed by adding 5 μl of sample to 10 ml of Aquasol (New England Nuclear) and counting in a liquid scintillation counter.

Preparation of low molecular weight DNA fragments. Low molecular weight DNA fragments were prepared by limited micrococcal nuclease digestion (Marmur, 1961) of calf thymus DNA (Worthington Biochemical Corp., Freehold, New Jersey, USA). DNA solution (2 mg/ml in 6 mM Tris-HCl, pH 8·0, 2 mM $CaCl_2$, 0·1 M NaCl) was digested with micrococcal nuclease (40 u/mg DNA) at 37°C for 4 min. The reaction was stopped by the addition of 1/10 vol. of 0·2 M EDTA, pH 8·0. DNA was extracted twice with chloroform-isoamyl alcohol and precipitated by ethanol. Precipitates were centrifuged, and dissolved in 0·01 M PBS, pH 7·2 at a concentration of 4 mg/ml. Five millilitres of this solution was passed through a 3 × 0·50 cm column of Bio-Gel A0·5M (Bio-rad Laboratories), and eluated with 0·01 M PBS (0·5 ml/min). Fractions of 3 ml were collected. The size of DNA fragments in each fraction was determined by 5% PAGE and ethidium bromide staining.

The fraction containing small DNA fragments of 25–125 bp was used as the standard DNA for the polynucleotide kinase reaction.

PAGE. ^{32}P-labelled DNA (about 30,000 ct/min) in Tris buffer was mixed with 10 μl of 0·025% bromophenol blue in 50% glycerol, heated at 60°C for 1 min, and loaded on 10% polyacrylamide gel equilibrated with 40 mM Tris, 5 mM sodium acetate, 1 mM EDTA, pH 7·9. Electrophoresis was done at 20 mA for 3 h and the gel was dried and exposed to X-ray film (Kodak, XR-P5) for 2–3 days. For the determination of molecular size of DNA, 5% PAGE was used, and Hae III digest of ØX 174 RF DNA served as mol. wt marker recognized by staining with 0·5 μg/ml ethidium bromide.

Enzyme treatment. ^{32}P-labelled DNA (about 30,000 ct/min) in 40 mM Tris-HCl, pH 7·4, 8 mM $MgCl_2$, 2 mM $CaCl_2$ was digested with 500 μg/ml of bovine pancreatic DNAase I (Worthington Biochemical) at 37°C for 24 h. After deproteinization by chloroform-isoamyl alcohol extraction, the remaining DNA was analysed by PAGE and autoradiography. For digestion with S1 nuclease, 25 μl of DNA solution extracted from PEG precipitates was incubated with 2 units of S1 nuclease (Bethseda Research Lab, Maryland, USA) in a 50 μl reaction mixture containing 30 mM sodium acetate, pH 4·6, 1 mM $ZnCl_2$, 50 μg/ml bovine serum albumin at 37°C for 3 h. Thereafter, the pH of the reaction mixture was adjusted to 7·4 by adding adequate amounts of 250 mM Tris base, and treated further with 5 μg of alkaline phosphatase (Worthington Biochemical) at 37°C for 30 min. The reaction mixture was then deproteinized and the DNA was extracted, electrophoresed and autoradiographed.

Measurement of anti-dsDNA antibody activity. Anti-dsDNA antibody activity was measured by Millipore filter assay (Ginsberg & Keiser, 1973).

RESULTS

Quantitation of DNA in the PEG precipitates of sera from SLE patients

To quantitate DNA in the PEG precipitates, the method of radiolabelling DNA at the 5′ end by polynucleotide kinase was employed. To determine the quantitative relationship of the radioactivity incorporated in DNA by this reaction, varying amounts of DNA fragments of 25–125 bp were radiolabelled. The radioactivity incorporated in DNA showed a linear relationship in the range of 10–500 ng of DNA, whereas at higher concentrations of DNA, a plateau was reached (Fig. 1).

Nucleic acid was extracted from IC enriched materials obtained by PEG precipitation from serum or plasma samples of 30 SLE patients and 10 normal controls, and after RNAase treatment was subjected to 5′ end labelling. Ten samples from normal controls had a mean radioactivity of $24{\cdot}7 \times 10^4$ ct/min (s.d. 14·4, range 8·7–47·8) and 20 samples from SLE patients with inactive disease showed radioactivity within this range. However, 10 samples from SLE patients with active disease showed a significantly higher radioactivity and four of these had very high counts (mean, 316×10^4 ct/min, range $123–807 \times 10^4$ ct/min) (Fig. 2).

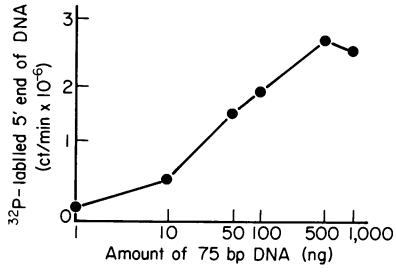

Fig. 1. Polynucleotide kinase reaction reference curve. Increasing amounts of limited micrococcal nuclease digest of calf thymus DNA (size 25–125 bp) was labelled by polynucleotide kinase reaction and incorporated ^{32}P radioactivity was counted. A linear relationship between radioactivity incorporated and the amount of DNA in the range of 10–500 ng of DNA was observed.

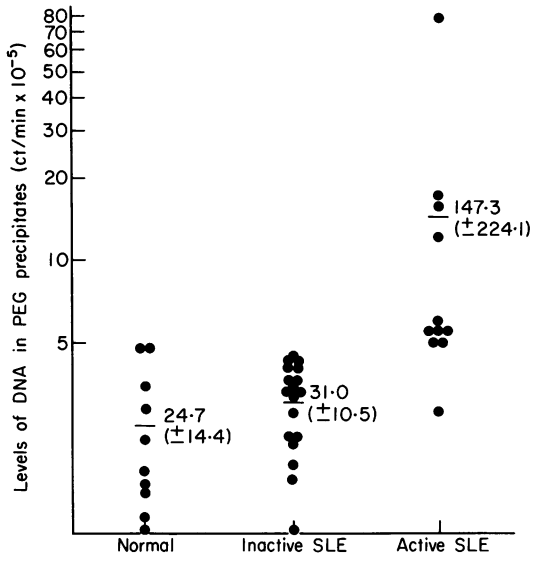

Fig. 2. ^{32}P-labelled DNA from PEG precipitates of sera from normals and SLE. Patients with inactive SLE and normal controls had the same amounts of DNA in PEG precipitates. However, clinically active SLE patients had significantly higher amounts.

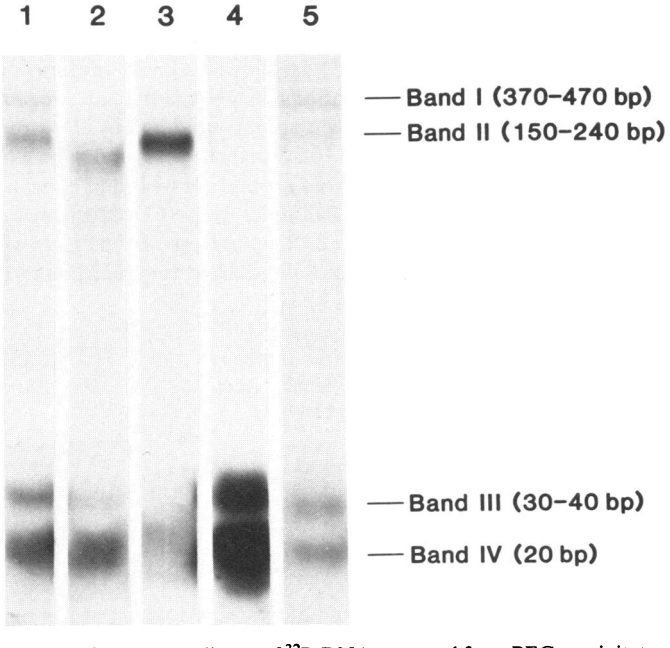

Fig. 3. Size determination of DNA. An aliquot of ^{32}P-DNA extracted from PEG precipitates containing 30,000 ct/min was electrophoresed in 10% polyacrylamide gels and autoradiographed. Hae III nuclease digest of ØX 174 RF DNA was used as a standard size marker recognized by ethidium bromide staining. (Sample in lane (1) patient SJ; (2) patient EV; (3) patient PJ; (4) patient KO; (5) normal subject.)

Analysis of the size of DNA extracted from PEG precipitates

To determine the size of DNA in PEG precipitates, radiolabelled DNA was analyzed in 10% PAGE. Autoradiograms revealed four bands of somewhat discrete size: 370–470 bp (band 1), 150–240 bp (band II), 30–40 bp (band III), and 20 bp (band IV) (Fig. 3). In normal controls, the smaller DNA fragments (bands III and IV) were the main components, but in eight SLE samples a prominent band II (150–240 bp) was noticed in addition to the smaller DNA fragments. However, we found differences among samples in regard to the number of bands and the density of each band. For example, patient S.J. (Fig. 3) showed all four bands (lane 1), patient E.V. three bands (bands II, III and IV, lane 2) patient J.P. two bands (bands II and IV, lane 3) and patient O.K. two bands (bands III and IV, lane 4).

DNA in ammonium sulphate precipitated serum samples

We employed PEG precipitation to enrich ICs from serum or plasma, but there was the possibility that PEG precipitation might have caused co-precipitation of DNA complexed with non-immunoglobulin DNA binding proteins such as low density lipoprotein (Smeenk, Lelij & Aarden, 1982). Therefore DNA was also extracted from ammonium sulphate precipitates of five samples and compared to that from PEG precipitates since the former has been used by other investigators (Sano & Morimoto, 1981). The amount of DNA detected in the ammonium sulphate precipitates was quite comparable to that in the PEG precipitates (data not shown). Also, the size of DNA fragments in the PEG precipitates was similar to those detected in the ammonium sulphate precipitates.

DNAase and S1 nuclease treatment

To confirm that ^{32}P-labelled materials were indeed DNA, their sensitivity to DNAase was studied. On DNAase treatment of samples, the previously observed bands I and II were not detected, but instead increased radioactivity was detected in the regions of the gel of lower than 20 bp (Fig. 4). Bands III and IV were not affected by DNAase digestion.

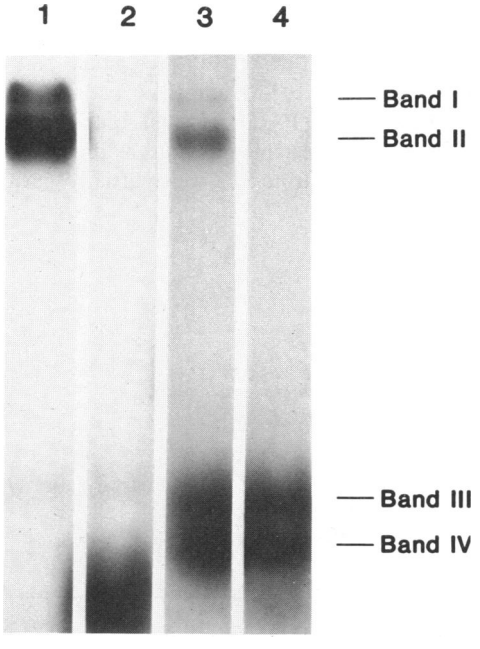

Fig. 4. Effect of DNAase treatment. ^{32}P-labelled samples were treated with bovine pancreatic DNAase I and applied to 10% polyacrylamide gels and then autoradiographed. Lanes 1 and 2: patient HT; lanes 3 and 4: normal subject. Samples in lanes 2 and 4 were treated with DNAase I. After DNAase I treatment, the bands I and II disappeared and increased radioactivity at the lower position (<20 bp) was noticed.

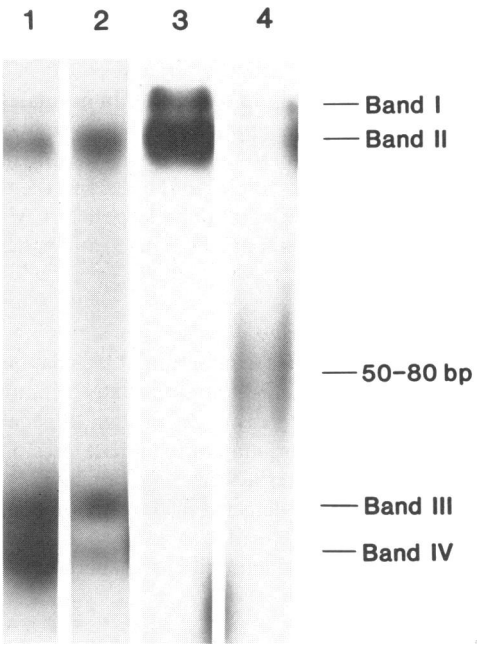

Fig. 5. Effect of S1 nuclease treatment. DNA extracted from PEG precipitates was treated with S1 nuclease and alkaline phosphatase and then labelled with ^{32}P and analysed in PAGE. Samples were from two patients with SLE. Samples in lanes 1 and 3 were without S1 nuclease treatment. Samples in lanes 2 and 4 were after S1 nuclease treatment. Lanes 1 and 2 were representative of the majority of SLE and normal sera i.e. DNA was resistant to S1 nuclease. Exceptions are included in lanes 3 and 4 where bands I and II were susceptible to S1 nuclease but the size was reduced to material giving a wide zone of approximately 50–80 bp.

To determine whether DNA extracted from PEG precipitates was double stranded or single stranded, extracted DNA from samples of 10 SLE and 10 normals was treated with S1 nuclease and after radiolabelling, DNA was analysed in PAGE. In eight samples from SLE, DNA was resistant to S1 nuclease. They showed bands at the same positions as those obtained without S1 nuclease treatment (Fig. 5). However, two samples from SLE were partially sensitive to S1 nuclease. Instead

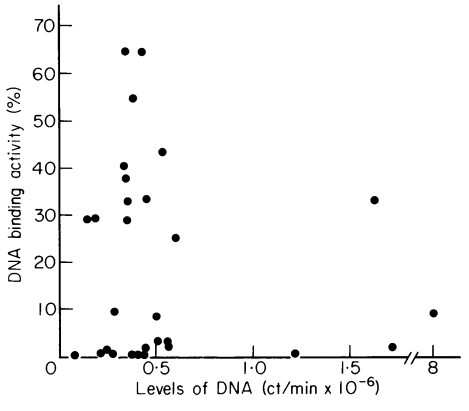

Fig. 6. Correlation between levels of DNA from the PEG precipitates and DNA binding activity of corresponding sera from SLE patients. DNA extracted from PEG precipitates was labelled with ^{32}P and expressed as radioactivity incorporated (ct/min $\times 10^{-6}$). DNA binding activity was determined by the millipore method and expressed as % of normal binding activity. No significant correlation was found.

of two dense bands (I and II) seen without S1 nuclease treatment, we noticed a faint band at these positions and an appearance of a wide zone at 50–80 bp region (Fig. 5, lane 3 and 4). In normals, DNA was found resistant to S1 nuclease as well.

Relationship between levels of DNA in the PEG precipitates and anti-dsDNA antibody activity of sera Thirteen patients' sera had significantly elevated levels of DNA binding activity (normal levels $< 10\%$). Only three of the 13 patients showed high levels of DNA in their PEG precipitates. These three patients had clinically active disease. The other 10 patients were clinically active 3–6 months prior to the examination of their samples but were classified as inactive at the time of this study. In this group of patients, the mean level of DNA ($32{\cdot}3 \times 10^4$ ct/min) in the PEG precipitate was higher but not significantly different than normals. When patients were analysed based on significantly high levels of DNA in the PEG precipitates, seven of the 10 patients were found to have low levels of DNA binding activity in the corresponding sera. Thus, in general, we found no correlation ($r = 0{\cdot}1$) between the levels of DNA in the PEG precipitates and anti-dsDNA antibody in sera (Fig. 6).

DISCUSSION

The direct demonstration that DNA is present in circulating ICs of patients with SLE has been difficult due to the paucity of appropriate methods to isolate ICs from sera. Our finding of significantly elevated levels of DNA in the PEG precipitates of ten SLE patients who were clinically active supports the findings by Sano & Morimoto (1981) that small DNA fragments are involved in IC formation. Earlier, evidences of circulating DNA–anti-DNA ICs have been proposed by demonstrating a rise in the titre of anti-DNA antibody activity in sera of SLE patients after either DNAase treatment (Harbeck *et al.*, 1973) or acid dissociation (Bruneau & Benveniste, 1979). Others have found DNA like substances from cryoprecipitates after pronase treatment (Davis, Godfrey & Winfield, 1978), In this study, we have demonstrated a direct relationship between active disease, and the amounts of DNA fragments isolated from circulating ICs.

DNA fragments of four different sizes: 370–470 bp, 150–240 bp, 30–40 bp and 20 bp were found in circulating ICs of SLE. Two fragments of related sizes to the 150–240 bp and 30–40 bp were reported initially by Sano & Morimoto (1981). In a subsequent paper, a large fragment related to the 370–470 bp fragments was also observed (Morimoto *et al.*, 1982). We also found that these DNA fragments exist in normal sera albeit in low levels. In most of the normal controls, smaller DNA fragments (bands III and IV) were the main components, which were not digested by DNAase 1. These DNA fragments may be the final degradation products of circulating DNA. The production of anti-DNA antibodies in normals may be playing a role to clear these DNA fragments, which is not susceptible to circulating DNAase by forming ICs to be cleared by the reticuloendothelial system. In the majority of patients, we found an inverse relationship between the levels of DNA in the PEG precipitates and anti-dsDNA antibody activities of the corresponding sera. The samples showing high DNA levels in the PEG precipitates but low anti-DNA antibodies of the sera may represent the condition in which most anti-DNA antibodies have formed ICs and little anti-DNA antibodies are in the free form. Other samples showing low DNA levels and high DNA binding activities are, perhaps, from those patients who may have low release of DNA in the circulation and most DNA antibodies exist in the free form. These results are in agreement with the previous finding which showed alternate appearance of free DNA and anti-DNA (Tan *et al.*, 1966). Only three samples showed high DNA levels and high DNA binding activities. The significance of this result is difficult to explain. It is possible that these samples contain anti-DNA antibodies of different specificities from those forming ICs.

We isolated ICs by PEG precipitation, a method which has been utilized for the measurement and enrichment of ICs. We used PEG at the final concentration of 4% based on the observations of Chia *et al.* (1979) that a lower concentration of PEG does not precipitate ICs efficiently from SLE sera. Sano & Morimoto (1981) used 40% saturated ammonium sulphate precipitation to separate DNA– anti-DNA ICs from free DNA. However, it is suggested that ICs of low avidity antibodies are dissociated in this condition by its high salt concentration (Smeenk *et al.*, 1982). PEG

precipitation does not suffer from this drawback. It is possible that PEG may precipitate DNA complexed to proteins in serum other than anti-DNA antibodies (Smeenk *et al.*, 1982). However, Riley, McGrath & Taylor (1979) have shown that this type of binding is not present when small DNA fragments (about 5×10^5 daltons) are used as antigen. The DNA fragments we detected in the PEG precipitates were of smaller size. Also, we observed that a significant amount of DNA was not precipitated by PEG when 1 μg of small DNA fragments (25–125 bp) were added to the normal serum.

The mechanism by which the small DNA fragments of unique size are formed is unclear. The circulating DNA may be derived either from nuclei of degenerating or dead cells, or secretion by living cells (Olsen & Harris, 1974; Stroun *et al.*, 1977). In mice, Izui *et al.* (1977) showed the appearance of small DNA fragments in the circulation following injection with lipopolysaccharides and Pancer *et al.* (1981) have found spontaneous secretion of 150 bp DNA fragments from spleen cells of NZB/NZW (F_1) strain. Also, increased levels of DNA secretion from human peripheral lymphocytes have been found as a result of mitogen and antigen stimulation (Rogers *et al.*, 1972; Anker, Stroun & Maurice, 1975). The circulating DNA may be further degraded into smaller subunits of chromatin (nucleosome) of about 200 bp by DNAase normally present in sera. As a result, DNA fragments of single strand lengths in multiples of ten nucleotides will form (Sollner-Webb & Felsenfeld, 1977), but some of the DNA fragments may remain protected from the digestion by binding to antibody molecules (Fidelus, Lightfoot & Jones, 1981). The finding of different size DNA fragments in the study is probably due to the presence of a heterogeneous group of anti-DNA antibodies present in SLE sera reacting with various sizes of DNA fragments (Papalian *et al.*, 1980). The important finding was that high levels of large DNA fragments (150–240 bp and < 300 bp) were present in SLE and lower levels in normals whereas the smaller fragments (30–40 bp and 20 bp) were present equally in both groups. This is an important difference between our findings and those of Sano & Morimoto (1981) who did not find DNA in normal sera. Notwithstanding, the finding of higher levels of large DNA fragments in SLE may be of interest in relationship to the report of Bell, Milazzo & Singhal (1982) that DNA fragments of 180 bp activated T helper cells in responder mice of NZB/NZW (F1) and DBA strains. The question may be asked whether circulating DNA fragments of such sizes may have a relationship to the activity of disease observed in the group of patients reported here.

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REFERENCES

ANKER, P., STROUN, M. & MAURICE, P.A. (1975) Spontaneous release of DNA by human blood lymphocytes as shown in an *in vitro* system. *Cancer Res.* **35,** 2375.

BELL, D.A., MILAZZO, M. & SINGHAL, S.K. (1982) Immunogenic effects of low molecular weight DNA (LM DNA) *vs* high molecular weight DNA (HM DNA) in normal and preautoimmune NZB/W mice. *Arthrit. Rheum.* **25,** 542.

BRUNEAU, C. & BENVENISTE, J. (1979) Circulating DNA: anti-DNA complexes in systemic lupus erythematosus. Detection and characterization by ultracentrifugation. *J. clin. Invest.* **64,** 191.

CHIA, D., BARNETT, E.V., YAMAGATA, J., KNUTSON, D., RESTIVO, C. & FURST, D. (1979) Quantitation and characterization of soluble immune complexes precipitated from sera by polyethylene glycol (PEG). *Clin. exp. Immunol.* **37,** 399.

DAVIS, J.H., GODFREY, S.M. & WINFIELD, J.B. (1978) Direct evidence for circulating DNA/anti-DNA complexes in systemic lupus erythematosus. *Arthrit. Rheum.* **21,** 17.

DIGEON, M., LAVER, M., RIZA, J. & BACH, J.F. (1977) Detection of circulating immune complexes in human sera by simplified assays with polyethylene glycol. *J. Immunol. Meth.* **16,** 165.

FIDELUS, R.K., LIGHTFOOT, R.W. & JONES, J.V. (1981) Role of anti-DNA in protection of immune complexes against DNAase digestion in SLE. *Arthrit. Rheum.* **24** (Suppl.), S111.

GINSBERG, B. & KEISER, H. (1973) A millipore filter assay for antibodies to native DNA in sera of patients with systemic lupus erythematosus. *Arthrit. Rheum.* **16,** 199.

HARBECK, R.J., BARDANA, E.J., KOHLER, P.F. & CARR, R.I. (1973) DNA: anti-DNA complexes: their detection in systemic lupus erythematosus sera. *J. clin. Invest.* **52,** 789.

HUSTON, K.A., GUPTA, R.C., DONADIO, J.V., MCDUFFIE, F.C. & ILSTRUP, D.M. (1978) Circulat-

ing immune complexes in systemic lupus erythematosus: association with other immunologic abnormalities but not with change in renal function. *J. Rheum.* **5,** 423.

IZUI, S., LAMBERT, P.H., FOURNIE, G.J., TURLER, H. & MIESCHER, P.A. (1977) Features of systemic lupus erythematosus in mice injected with bacterial lipopolysaccharides. Identification of circulating DNA and renal localization of DNA–anti-DNA complexes. *J. exp. Med.* **145,** 1115.

KIRBY, K.S. (1968) Isolation of nucleic acids with phenolic solvents. In *Methods in Enzymology XII, Part B* (ed. by L. Grossman & K. Moldave) pp. 87–99. Academic Press, New York.

KOFFLER, D., ANGELLO, V. & KUNKEL, H.G. (1974) Polynucleotide immune complexes in serum and glomeruli of patients with systemic lupus erythematosus. *Am. J. Path.* **74,** 109.

LANDRY, M. & SAMS, W.M. (1973) Systemic lupus erythematosus. Studies of the antibodies bound to skin. *J. clin. Invest.* **52,** 1871.

MARMUR, J. (1961) A procedure for the isolation of deoxyribonucleic acid from micro-organisms. *J. mol. Biol.* **3,** 208.

MAXAM, A.M. & GILBERT, W. (1977) A new method for sequencing DNA. *Proc. Natl. Acad. Sci. USA.* **74,** 560.

MORIMOTO, C., SANO, H., ABE, T., HOMMA, M. & STEINBERG, A.D. (1982) Correlation between clinical activity of systemic lupus erythematosus and the amount of DNA in DNA/anti-DNA antibody immune complexes. *J. Immunol.* **129,** 1960.

NYDEGGER, U.E., LAMBERT, P.H., GERBER, H. & MIESCHER, P.A. (1974) Circulating immune complexes in the serum in systemic lupus erythematosus and as carriers of hepatitis B antigen. Quantitation by binding to radiolabeled C1q. *J. clin. Invest.* **54,** 297.

OLSEN, I. & HARRIS, G. (1974) Uptake and release of DNA by lymphoid tissue and cells. *Immunol.* **27,** 973.

PANCER, L.B., MILAZZO, M.F., MORRIS, V.L., SINGHAL, S.K. & BELL, D.A. (1981) Immunogenicity and characterization of supernatant DNA released by murine spleen cells. *J. Immunol.* **127,** 98.

PAPALIAN, M., LAFER, E., WONG, R. & STOLLAR, B.D. (1980) Reaction of systemic lupus erythematosus antinative DNA antibodies with native DNA fragments from 20 to 1,200 base pairs. *J. clin. Invest.* **65,** 469.

RILEY, R.L., MCGRATH, H. & TAYLOR, R.P. (1979) Detection of low avidity anti-DNA antibodies in systemic lupus erythematosus. *Arthrit. Rheum.* **22,** 219.

ROGERS, J.C., BOLDT, D., KORNFELD, S., SKINNER, S.A. & VALERI, C.R. (1972) Excretion of deoxyribonucleic acid by lymphocytes stimulated with phytohemagglutinin or antigen. *Proc. Natl. Acad. Sci. USA.* **69,** 1685.

SANO, H. & MORIMOTO, C. (1981) Isolation of DNA from DNA/anti-DNA antibody immune complexes in systemic lupus erythematosus. *J. Immunol.* **126,** 538.

SMEENK, R., LELIJ, G. & AARDEN, L. (1982) Avidity of antibodies to dsDNA: comparison IFT on *Crithidia luciliae*, Farr assay and PEG assay. *J. Immunol.* **128,** 73.

SOLLNER-WEBB, B. & FELSENFELD, G. (1977) Pancreatic DNase cleavage sites in nuclei. *Cell,* **10,** 537.

STROUN, M., ANKER, P., MAURICE, P. & GAHAN, P.B. (1977) Circulating nucleic acids in higher organisms. *Int. Rev. Cytol.* **51,** 1.

TAN, E.M., SCHUR, P.H., CARR, R.I. & KUNKEL, H.G. (1966) Deoxyribonucleic acid (DNA) and antibodies to DNA in the serum of patients with systemic lupus erythematosus. *J. clin. Invest.* **45,** 1732.

TAN, E.M., COHEN, A.S., FRIES, J.F., MASI, A.T., MCSHANE, D.J., ROTHFIELD, N.F., SCHALLER, J.G., TALAL, N. & WINCHESTER, R.J. (1982) The 1982 revised criteria for the classification of systemic lupus erythematosus. *Arthrit. Rheum.* **25,** 1271.