Clin. exp. Immunol. (1991) **85**, 66–69

ADONIS 0009910491001955

Antigen DNA isolated from immune complexes in plasma of patients with systemic lupus erythematosus hybridizes with the *Escherichia coli* lac Z gene

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(Accepted for publication 17 January 1991)

SUMMARY

Antigen DNA was isolated from immune complexes in plasma of three patients with active systemic lupus erythematosus (SLE) using affinity column. The antigen DNA thus obtained was subjected to hybridization experiments in order to investigate its origin. Unexpectedly, plasmid pUC18 used as a probe was found to hybridize with the antigen DNA. pUC18 was then cleaved into three fragments with the restriction enzyme *Hae*II. A 445-bp fragment containing lac Z DNA hybridized with the antigen DNA. Finally, the lac Z DNA itself was found to hybridize with the antigen DNA. These data strongly suggest that the antigen DNA obtained from three patients is of bacterial origin.

Keywords Systemic lupus erythematosus immune complexes antigen DNA hybridization *Escherichia coli* lac Z

INTRODUCTION

There is a large body of literature concerning anti-DNA autoantibodies in serum of patients with systemic lupus erythematosus (SLE) (Tan *et al.*, 1966; Stollar & Papalian, 1980; Schwartz & Stollar, 1985). Although these anti-DNA autoantibodies serve as a marker of diagnostic significance in SLE (Miniter, Stollar & Agnello, 1979; Swaak *et al.*, 1979; Tan, 1982), no investigators have succeeded in producing anti-ds DNA antibodies in animals (Madio *et al.*, 1984). Recently, Gilkeson *et al.* (1989) presented an interesting report that anti-dsDNA antibodies were elicited in mice by immunizing them with dsDNA from *E. coli*, implying that the *E. coli* DNA used contained antigenic determinants required for the production of anti-dsDNA antibodies. From this evidence, we anticipated that some foreign DNA might be present in the circulatory blood of SLE patients, and that this might be connected with the pathogenesis of SLE. Although some investigators have reported the properties of antigen DNA in sera of SLE patients (Raptis & Menard, 1980; Sano & Morimoto, 1981, 1982; McCoubrey-Hoyer, Okarma & Holman, 1984; van Helden, 1985), the antigen DNA they isolated was derived from total serum or the gamma-globulin fraction. Therefore, our aim was to separate antigen DNA from immune complexes present in the plasma of patients with active SLE using an affinity column. We report here that the antigen DNA thus obtained was found to be homologous with *E. coli* lac Z DNA on the basis of the Southern blot analysis.

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MATERIALS AND METHODS

Separation of plasma

Ten milliliters of each blood sample were immediately diluted with an equal volume of equilibrated salt solution (0·126 M NaCl/$5{\cdot}0 \times 10^{-6}$ M $CaCl_2$/$9{\cdot}8 \times 10^{-5}$ M $MgCl_2$/0·01% anhydrous D-glucose/0·014 M Tris–HCl, pH 7·6), and then carefully layered upon 20 ml of Ficoll–Paque. After centrifugation (400 ***g***, 20°C, 30 min), the upper layer (plasma) was saved.

Preparation of anti-human IgG antibody-bound Sepharose 4B affinity column

According to the method of Kitagawa & Okuhara (1981), 6 mg of purified anti-human IgG antibody were bound to 1 ml of Sepharose 4B activated with CNBr.

DNA preparation

Individual plasma samples from patients with active SLE and normal individuals were subjected to anti-human IgG antibody-bound Sepharose 4B column chromatography. After washing with phosphate-buffered saline (PBS), 1 M NaCl and PBS in order, DNA was eluted with 20 mM $NaHCO_3$, pH 10·5/5% DMSO. The eluate was passed through a DEAE-cellulose column, and then the DNA fraction was eluted with 2 M NaCl. This fraction was treated with RNAse A, extracted with phenol and treated with ether. Then it was desalted and concentrated

using a NENSORB-20 column (New England Nuclear, Cambridge, MA). The final eluate obtained with 50% methanol was evaporated under a nitrogen flow and stored at −20°C. The amount of DNA was measured by nick-translation labelling according to the method of Fournié *et al.* (1986) with minor modifications. Portions of DNA samples were incubated at 37°C for 2 h in 50 μl of a mixture containing 50 mM Tris–HCl, pH 7·4/5 mM $MgCl_2$/10 mM 2-mercaptoethanol/50 μg/ml bovine serum albumin (BSA)/10 pmol α-^{32}P-dCTP (111 TBq/mmol, ICN, Costa Mesa, CA)/500 pmol each of dATP, dGTP and dTTP/0·5 U DNA polymerase I/100 pg DNAse I. The reaction was terminated by addition of 2·5 μl of 0·5 M EDTA followed by addition of 0·5 ml of cold 10% TCA. The precipitates collected on nitrocellulose filter discs were washed with 5 ml of 7% TCA and dried, and then the ^{32}P radioactivity incorporated into the DNA was measured. The amount of DNA was calculated from the radioactivity in DNA and the specific radioactivity of [α-^{32}P]dCTP.

Preparation of DNA probes

Plasmid pUC18 DNA was labelled with α-^{32}P-dCTP (111 TBq/mmol) by nick-translation (Rigby *et al.*, 1977) to a specific activity of $1·0 \times 10^8$ ct/min per μg. Three fragments of 1871 bp, 445 bp and 370 bp produced from pUC18 by digestion with the restriction enzyme *Hae*II, as well as other *E. coli* chromosomal DNA fragments, i.e. the 391-bp *Sal*I–*Hin*fI fragment of rps A, the 302-bp *Hae*III–*Hae*III fragment of rec A, the 485-bp *Bam*HI–*Bam*HI fragment of dna Q and the 205-bp *Eco*RI–*Eco*RI fragment of lac Z, were labelled with α-^{32}P-dCTP using the random primers (Feinberg & Vogelstein, 1983, 1984).

The range of specific activity of these fragments was 0·5–$8·8 \times 10^8$ ct/min per μg. DNA fragments prepared from rsp A, rec A, dna Q and lac Z of *E. coli* were all kindly provided by Dr S. Hirose, NIG, Mishima, Japan.

Southern hybridization

Southern hybridization was performed according to Southern (1975) with minor modifications. Each DNA sample was subjected to 1% agarose gel electrophoresis and then transferred to a nylon membrane (Gene Screen Plus, New England Nuclear). After u.v. irradiation for 1 h, the membrane was prehybridized in 10 ml of a solution containing 50% formamide/1 M NaCl/1% SDS at 42°C for 15 min. To the solution were added ^{32}P-labelled probe DNA and salmon sperm DNA (100 μg/ml) which had been thermally denatured in advance, and hybridization was then carried out at 42°C for 20 h. Following this, the membrane was washed twice in 2 × SSC/0·1% SDS at room temperature for 5 min, once in 1 × SSC/0·1% SDS at 42°C for 1 h and twice in 0·1 × SSC/0·1% SDS at 42°C for 30 min. Autoradiography was performed at −80°C for 5 days or longer. The used membrane was recovered by washing once in 0·4 *N* NaOH at 42°C for 30 min, and once in 0·1 × SSC/0·1% SDS/0·2 M Tris–HCl, pH 7·5, at 42°C for 30 min, followed by rehybridization.

RESULTS

Quantity of DNA from plasma of SLE patients

Trace amounts of antigen DNA isolated from the immune complexes present in plasma of three SLE patients were measured by nick-translation. In 1 ml of plasma, 273–1044 ng of DNA were present: the quantity of DNA from five healthy controls was 170–402 ng/ml (Table 1).

Table 1. Quantification of DNA from immune complexes

Subject no.	Concentration of DNA from immune complexes (ng/ml plasma)
SLE	
1	703·2
2	1044·1
3	273·2
Control	
4	243·4
5	169·6
6	401·9
7	243·4
8	222·9

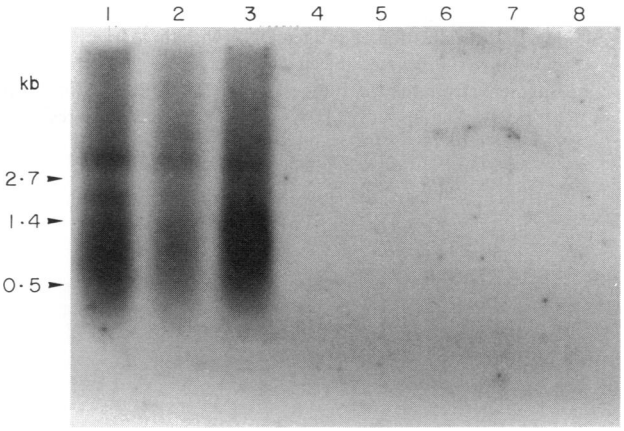

Fig. 1. Hybridization of antigen DNA with plasmid DNA. DNA fragments from three SLE patients (lanes 1–3) and five normal individuals (lanes 4–8) were subjected to 1% agarose gel electrophoresis, transferred to a nylon membrane and hybridized with ^{32}P-labelled pUC18 by nick-translation ($1·0 \times 10^8$ ct/min per μg). Autoradiography was performed at −80°C for 9 days. The amount of DNA used for the electrophoresis was 150 ng.

Hybridization of antigen DNA with plasmid pUC18

Plasmid pUC18 was used arbitrarily as a probe. The antigen DNA was found to hybridize with the probe as revealed by smears in lanes 1–3 of Fig. 1. The smear patterns suggest that DNA fragments carrying the sequence homologous to pUC18 DNA are not discrete in size. DNAs from normal individuals never hybridized with the probe (Fig. 1, lanes 4–8). It was thus revealed that the antigen DNA had a sequence homologous with some region of pUC18.

Hybridization of antigen DNA with restriction enzyme digests

pUC18 was cleaved using the restriction enzyme *Hae*II, giving rise to three fragments: a 1871-bp fragment containing the Amp^r

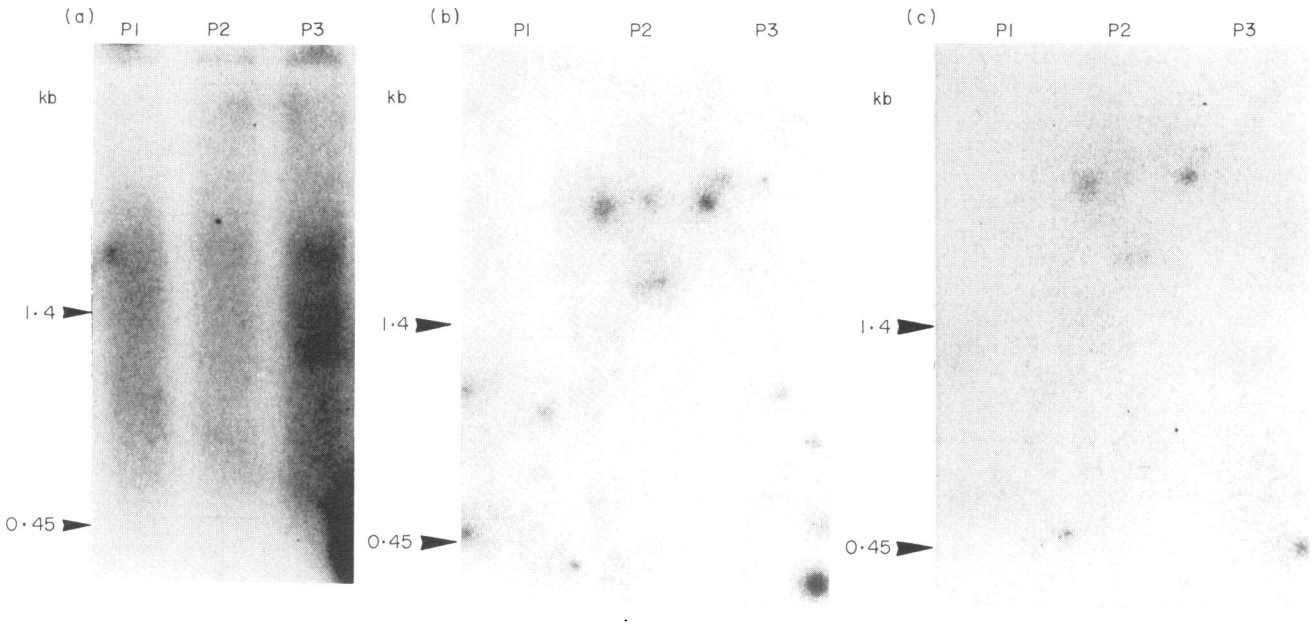

Fig. 2. Hybridization of antigen DNA with three fragments from the *Hae*II digest of plasmid pUC18. DNA fragments from three SLE patients (P1–P3) were subjected to 1% agarose gel electrophoresis, transferred to a nylon membrane and hybridized with a ^{32}P-labelled fragments from the *Hae*II digest of pUC18 by the random-primed DNA labeling method. Autoradiography was performed at −80 C for 8 days. The amount of DNA used for the electrophoresis was 80 ng. a 445 bp ($1{\cdot}7 \times 10^8$ ct/min per μg); b 1871 bp ($1{\cdot}5 \times 10^8$ ct/min per μg); c 370 bp ($3{\cdot}7 \times 10^8$ ct/min per μg).

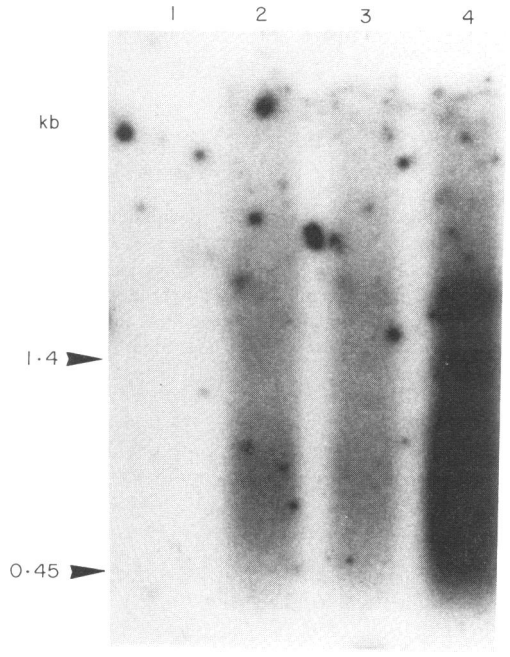

Fig. 3. Hybridization of antigen DNA with *E. coli* lac Z fragment. Antigen DNAs from three SLE patients (lanes 2–4) and a normal individual (lane 1) were subjected to 1% agarose gel electrophoresis, transferred to a nylon membrane and hybridized with the 205 bp *E. coli* lac Z fragment labelled with ^{32}P by the random-primed DNA labelling method (3×10^8 ct/min per μg). Autoradiography was performed at −80 C for 5 days. The amount of DNA used for the electrophoresis was 80 ng.

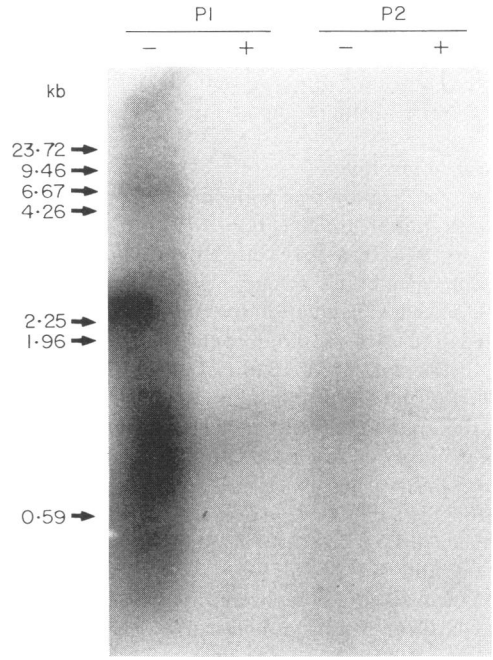

Fig. 4. Hybridization of antigen DNA treated with or without DNase I to radioactive *E. coli* lac Z fragment. Antigen DNAs from two SLE patients (P1 and P2) were treated with (+) or without (−) DNAse I, then subjected to 1% agarose gel electrophoresis, transferred to a nylon membrane and hybridized with the 205 bp *E. coli* lac Z fragment labelled with ^{32}P by the random-primed DNA labelling method (3×10^8 ct/min per μg). Autoradiography was performed at −80 C for 5 days. The amount of DNA used for the electrophoresis was 80 ng.

region, a 445-bp fragment containing lac Z, and a 370-bp fragment containing the replication origin. Each fragment was used as a hybridization probe after labelling with ^{32}P. As shown in Fig. 2a, the smear hybridization signals were observed when the 445-bp fragment was used as a probe. However, no hybridization occurred with the other DNA probes (Fig. 2b, c). These results suggested that the antigen DNA had sequence homology with part of the 445-bp fragment containing lac Z.

Hybridization of antigen DNA with E. coli *DNA fragments*

Subsequently, hybridization was done using a DNA fragment carrying the *E. coli* lac Z gene alone as a probe in order to confirm whether this was the part of the 445-bp fragment homologous with the antigen DNA. As shown in Fig. 3, the antigen DNA clearly hybridized with the lac Z DNA probe, suggesting that antigen DNA from SLE immune complexes contains a sequence homologous with *E. coli* lac Z DNA. Additionally, as shown in Fig. 4, the antigen DNA digested with DNAse I did not hybridize with the lac Z DNA. Finally, we addressed the possibility that contamination with *E. coli* had taken place during the preparation of antigen DNA from the SLE patients. For this, hybridization was similarly performed using different DNA probes, such as rps A, rec A, and dna Q from *E. coli*. None of these probes hybridized with the antigen DNA (data not shown), thus confirming that the antigen DNA was not due to contamination with *E. coli* DNA.

DISCUSSION

The aetiology of autoimmune diseases in unknown, although genetic, hormonal and environmental factors have all been postulated to play roles in triggering immune dysfunction. Molecular mimicry or immunologic cross-reactivity of an infecting agent with the host has long been considered to be the trigger which provokes some autoimmune diseases (Oldstone, 1987). It has been suspected for some time that foreign DNA released into the circulatory blood after infection by some micro-organism might be involved with the production of anti-dsDNA antibodies in SLE patients. Our present evidence that immune complex antigen DNA contains a sequence homologous with the lac Z gene in *E. coli* may support this notion. However, it seems to be strange that rsp A, rec A and dna Q having their origin in *E. coli* do not hybridize with the antigen DNA. In this connection, we interpret that these *E. coli* DNAs do not have sequences which form base pairs with the antigen DNA.

More extensive research is required to explain why foreign DNA was present in plasma of SLE patients. It is now of great interest to investigate whether the antigen DNA has any association with the pathogenesis of SLE.

This is the first study to have isolated antigen DNA from immune complexes present in plasma of three SLE patients using an affinity column, and to have shown the antigen DNA to be of bacterial origin by Southern blot hybridization. Further details of the cloning and sequencing of this antigen DNA will be described elsewhere.

ACKNOWLEDGMENTS

We are grateful to Dr S. Hirose for providing various DNAs of *E. coli* and for valuable advice.

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