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Natural autoantibodies in systemic lupus erythematosus

PEGGY MATSIOTA, P. DRUET*, P. DOSQUET*, BRIGITTE GUILBERT & S. AVRAMEAS *Unité d'Immunocytochimie, Département d'Immunologie, Institut Pasteur, 75015 Paris, France and *INSERM U 28, Hôpital Broussais, 75674 Paris, France*

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SUMMARY

We have tested the sera of 25 patients with systemic lupus erythematosus (SLE) for antibody activity against a panel of six antigens: DNA, TNP, actin, tubulin, myosin, albumin. Eluates from renal biopsy tissue were also tested. Sera from patients with lupus nephritis were found to contain high titres of IgA antibodies directed against the antigens of the panel, and marked IgG anti-DNA and anti-TNP antibody activity. The IgG anti-TNP antibodies isolated from SLE serum by affinity chromatography on a TNP-immunoadsorbent, were also found to possess anti-DNA activity. Kidney eluates obtained from biopsy specimens of SLE patients contained IgG antibodies strictly specific for DNA in three out of the nine patients tested, while three eluates from the remaining six patients reacted with DNA and TNP and three with DNA and all the other antigens of the panel. These results strongly suggest that in SLE sera there are at least three populations of circulating anti-DNA antibodies: those strictly specific for DNA, those recognizing DNA and TNP and those recognizing DNA and other macromolecules. Furthermore, because six out of nine of the eluates contained antibodies with an absolute or restricted specificity for DNA, this suggests that these antibodies are more often pathogenic than the polyspecific ones recognizing DNA and other macromolecules.

Keywords systemic lupus erythematosus lupus nephritis natural autoantibodies anti-DNA anti-TNP

INTRODUCTION

Systemic lupus erythematosus (SLE) is a widespread disorder not specific to organs, and characterized by B cell hyperactivity involving hypergammaglobulinaemia (Blaese, Grayson & Steinberg, 1980) and the production of a variety of autoantibodies (Budman *et al.*, 1977): antibodies to the nuclear antigens, single and double-stranded DNA, ribonucleoproteins and histones, cytoskeletal proteins, cell surface constituents and plasma proteins have been described (Kunkel & Tan, 1964; Tan *et al.*, 1982). Some autoantibodies have direct biological effects such as anti-erythrocyte antibodies (Budman *et al.*, 1977), whereas the pathological effect of others may be mediated by the formation of immune complexes (Koffler, Schur & Kunkel, 1967). Immune complexes often circulate in the blood of SLE patients and of lupus-prone mice; DNA-anti DNA immune complexes seem to be deposited in the kidney and in other tissues (Dixon *et al.*, 1980; Siegel & Hayslett, 1981).

In previous studies, we have demonstrated the presence of natural autoantibodies in the sera of healthy individuals directed against common antigens (Avrameas, Guilbert & Dighiero, 1981;

Correspondence: Dr P. Matsiota, Unité d'Immunocytochimie, Départment d'Immunologie, Institut Pasteur, 25 rue du Docteur Roux, 75724 Paris Cédex, France.

Guilbert, Dighiero & Avrameas, 1982). Furthermore analysis of human monoclonal immunoglobulins (MIg) reveals a high incidence of MIg directed against cytoskeleton proteins which share antibody specificities with natural antibodies (Dighiero *et al.*, 1983).

Similar studies in the mouse indicated that the monoclonal antibodies secreted by hybridomas prepared from healthy non-immunized adults and newborn mice, have specificities very similar to those of natural antibodies found in normal human sera (Dighiero *et al.*, 1983; 1985).

In this work, we have studied the occurrence of antibodies with natural antibody activity to actin, tubulin, myosin, DNA, albumin and TNP in the sera of patients with lupus nephritis and we have compared these antibodies with those found in a pool of normal human serum. These antigens were selected because they are either highly conserved during evolution (actin, tubulin, myosin, DNA) or because they correspond to self (albumin) and non-self haptenic antigens (TNP), and finally because in the above-mentioned studies, most of the natural antibodies possessed activity against these antigens. In addition we have sought such antibodies in kidney eluates from the same SLE patients. The results demonstrate that sera from patients with lupus nephritis contain high titres of IgA antibodies directed against the panel of antigens tested, and very high titres of IgG antibody with anti-DNA and anti-TNP activity. The kidney eluates obtained from biopsy specimens from SLE patients contained IgG antibodies strictly specific for DNA in three out of nine patients, while three eluates from the six remaining patients reacted with DNA and TNP and three with DNA and all the other antigens of the panel.

MATERIALS AND METHODS

Reagents. Ninety-six-well flat-bottomed microtitre plates were obtained from CML (France). *Escherichia coli* β-galactosidase (spec. act. 500,000 U/ng) was kindly supplied by Dr A. Ullmann (Institut Pasteur, Paris). Gelatin was purchased from Prolabo (Paris) and Tween-20 was purchased from Merck. Glutaraldehyde 25% aqueous solution was purchased from TAAB Laboratories (Reading, England).

Antibodies. Sheep and goat antibodies to human immunoglobulins IgG, IgA and IgM isolated by passage of antiserum over immunoadsorbents were purchased from Institut Pasteur Production (Marnes la Coquette, France). β-galactosidase was coupled to antibodies by the one-step glutaraldehyde coupling technique (Avrameas, 1969).

Antigen sources. Pig brain tubulin was prepared following the method of Shelansky, Gaskin & Cantor (1973). Actin was purified from calf striated muscle as described by Spudish & Watt (1971). Myosin was prepared by the method of Whalen, Butler-Browne & Gros (1978). TNP_{25}/BSA was prepared by the procedure of Little & Eisen (1966). Native dsDNA was purchased from Sigma Chemical Co. and human albumin (100% pure) from Schwartz Mann (Cambridge, USA). All antigens were tested by SDS-polyacrylamide gel electrophoresis and each of them was found to be pure with little or no possibility of lipid contamination.

Patients. Twenty-five patients satisfying the 1982 revised criteria of the American Rheumatism Association (Tan *et al.*, 1982) for the classification of SLE, were included in this study (Table 1). All patients had renal involvement and routine percutaneous renal biopsy specimens were obtained in all cases. Serum samples were obtained from all patients at the time of renal biopsy. Diagnosis of renal involvement was based upon light microscopy and immunofluorescent findings. Assessment of disease activity was based upon clinical symptoms, low serum complement level, the presence of anti-nuclear antibodies (1/500), the presence of anti-DNA antibody in the Farr assay (30%) and the presence of an active nephritis (stages IIIa,b and IVa,b,c of the WHO classification: Churg & Sobin, 1982).

Screening of the sera and eluates by enzyme immunoassay (EIA). Polystyrene flat-bottomed plates were coated with the various antigens as described previously (Guilbert *et al.*, 1982). The antigen coated plates were washed thoroughly with phosphate-buffered saline (PBS) containing 0·1% Tween-20 (PBS-T). They were then incubated for 1 h at 37°C and overnight at 4°C with SLE serum, or with immunoadsorbent eluates diluted in PBS-T containing 0·5% gelatin (PBS-T-G), or with undiluted kidney eluates.

Table 1. Clinical, biological and histological findings of SLE patients

Patient	Sex	Age	Clinical activity	Biological activity: Complement CH50	C3	C4	Antibodies Nuclear	DNA	Classification of nephritis
1	F	28	No	L	N	L	No	No	Vc
2	F	19	No	N	N	N	No	No	IVd
3	F	40	No	N	N	N	Yes	No	IIIc
4	F	17	Yes	L	N	N	Yes	Yes	IVb
5	F	29	Yes	N	N	N	Yes	Yes	IIIa
6	F	30	No	L	N	L	No	No	V
7	F	53	No	N	N	N	No	No	IVa
8	F	16	Yes	L	L	L	Yes	Yes	IVb
9	F	41	No	L	N	L	Yes	Yes	IVa
10	M	20	Yes	L	L	L	Yes	Yes	I
11	F	44	No	L	N	L	No	No	IIIa
12	F	40	No	L	L	L	Yes	No	IVa
13	M	43	Yes	N	N	N	Yes	Yes	Vb
14	M	38	No	N	N	N	Yes	No	IVb
15	F	38	Yes	L	N	N	Yes	Yes	Va
16	F	56	Yes	N	L	L	Yes	No	IVb
17	F	33	Yes	L	L	L	Yes	Yes	IVb
18	M	76	No	N	N	N	Yes	ND	IVa
19	F	19	Yes	N	N	N	Yes	Yes	IIa
20	F	32	No	L	N	L	Yes	Yes	IIIa
21	F	39	No	N	N	N	No	No	IIb
22	M	30	Yes	N	N	L	Yes	Yes	Vb
23	F	27	Yes	L	L	L	Yes	Yes	IVb
24	F	21	Yes	L	L	L	Yes	Yes	IVa
25	F	30	Yes	L	N	L	Yes	Yes	IIIa

According to WHO classification (Churg & Sobin 1982).
L, low; N, normal, ND, not determined.

A pool of 70 normal sera was tested under the same conditions and at the same dilutions as the SLE sera and was considered to be the reference standard.

After incubation, the plates were washed five times, incubated for 2 h at 37°C with β-galactosidase-labelled sheep or goat antihuman IgG, IgA or IgM antibody (1 μg/ml in PBS-T-G). Plates were then washed, the enzyme substrate was added and the resultant optical density was determined at 414 nm using a Titertek Multiskan.

The IgA, IgG, IgM content of the sera and eluates was determined using an EIA similar to that described above, using plates coated with the corresponding specific antibody.

Expression of results. The values obtained by EIA with a given serum sample, at a given dilution and with a given antigen were compared to the values of a pool of 70 normal human sera examined under the same conditions. The results are expressed as the percentage absorbance of the test serum compared to the reference pool. Among these 70 normal sera, 25 were tested individually for antibody activity against the panel of antigens and for the three classes of immunoglobulins; their percent absorbance in relation to that found with the reference pool is presented in Fig. 1. In all the assays performed, only values 5-fold higher than background values were considered.

Elution procedure from kidney biopsies. The elutions were performed from nine cryostat sections of renal biopsy tissue from patients with lupus nephritis. The procedure used has been adapted from that described by Feltkamp & Boode (1970). In each case, four glass slides were covered at one end

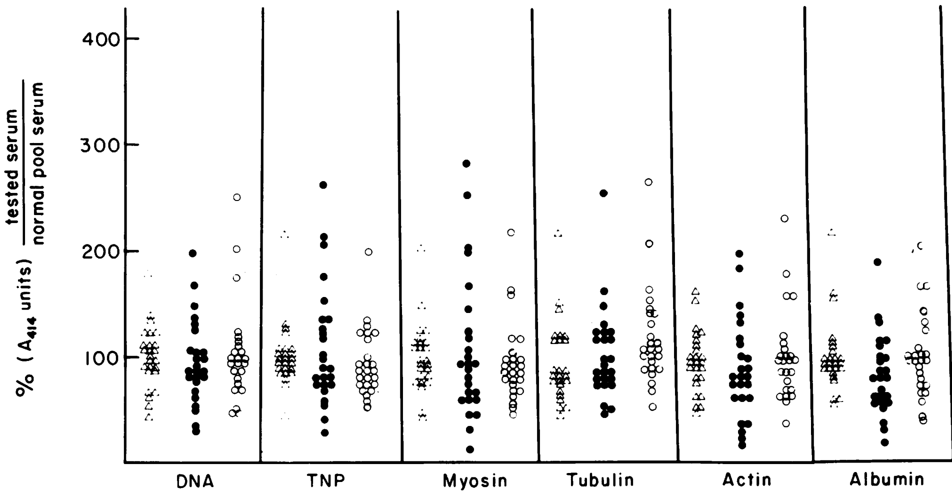

Fig. 1. Antibody titre of 25 normal human sera as compared to the antibody titre of a pool of 70 normal human sera. IgM (△), IgA (●), IgG (○).

with 10 sections. Each cryostat section contained at least six glomeruli. Two slides were then immersed back to back in 2 ml containers. The containers were filled twice with 2 ml PBS for 30 min at room temperature (PBS washing). The sections were then incubated for 45 min at room temperature with 2 ml of 0·02 M glycine buffer, pH 2·2. The eluates were neutralized with 2 M Tris base and dialysed overnight against PBS at 4°C (acid eluate).

Both PBS washing and acid eluates were concentrated to 0·4 ml on an Amicon cell equipped with a Diaflo PM 10 membrane and were tested by EIA as described above, and the ratio of the optical density in the EIA of the acid eluate to the optical density of the PBS washing was determined.

Isolation of anti-TNP antibodies from SLE and normal sera. TNP was immobilized on a Sepharose column equilibrated with 0·2 M boric acid–NaCl buffer, pH 8, as already described (Jaffe *et al.*, 1969).

The sera were applied at a flow rate of 20 ml/h at 25°C. The column was extensively washed with the borate at the same flow rate. When the absorbance of the effluent at 280 nm reached zero, the proteins absorbed were eluted at room temperature with 0·1 M DNP-glycine (Sigma) in borate buffer, pH 8·6. All fractions with an absorbance higher than 0·05 were pooled and dialysed overnight at 4°C against the above buffer until the yellow color disappeared. This solution was then concentrated to approximately 2 ml on an Amicon cell equipped with a Diaflo PM 10 membrane, passed through a 0·45 μm Millipore membrane and tested for optical density at 280 nm.

Statistical tests. Student's one-tailed, two-tailed and paired *t*-tests were used for comparisons between groups with the level of sigificance fixed at 0·05.

RESULTS

Screening of lupus sera. Twenty-five sera from patients with lupus nephritis were tested for activity against actin, tubulin, myosin, albumin, TNP and DNA. All sera contained antibodies of all immunoglobulin classes against all the tested antigens.

The IgM titres in SLE sera did not differ from those observed in normal sera. Moreover, IgM antibody activity against a given antigen did not differ significantly from that observed with any other antigen (Fig. 2).

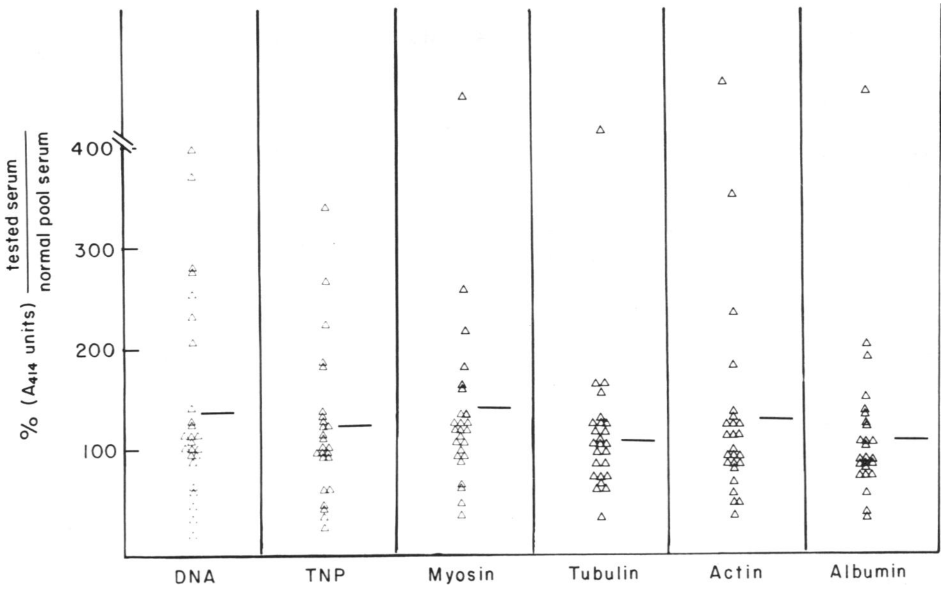

Fig. 2. IgM antibody titre of 25 sera from SLE patients as compared to the antibody titre of a panel of 70 normal human sera.

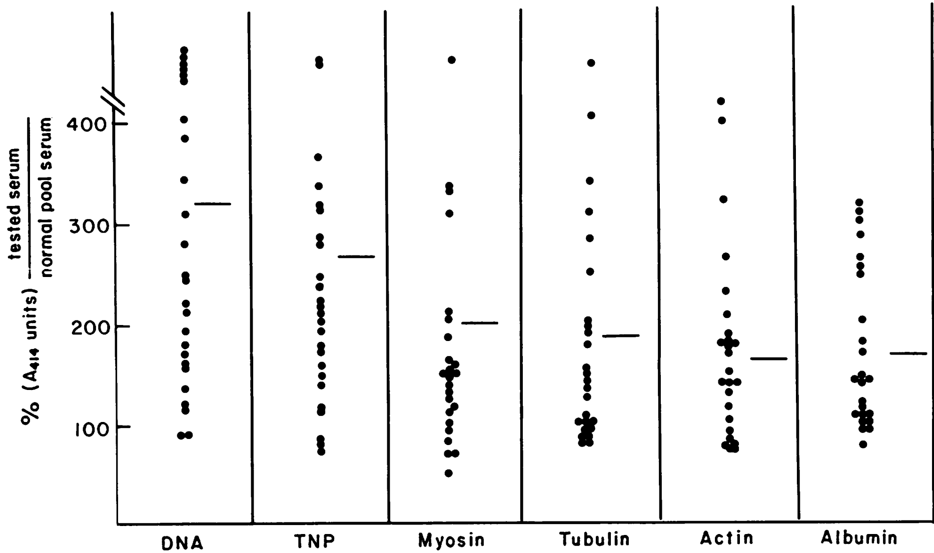

Fig. 3. IgA antibody titre of 25 sera from SLE patients as compared to the antibody titre of a panel of 70 normal human sera.

In contrast, IgA antibody activity for all the panel antigens was significantly increased when compared to normal human sera. When the IgA antibody activity for one antigen was compared to that observed for the other antigens it was found that both the anti-DNA and anti-TNP titres were significantly higher as compared to the other activities. No significant differences between anti-DNA and anti-TNP activity were observed (Fig. 3).

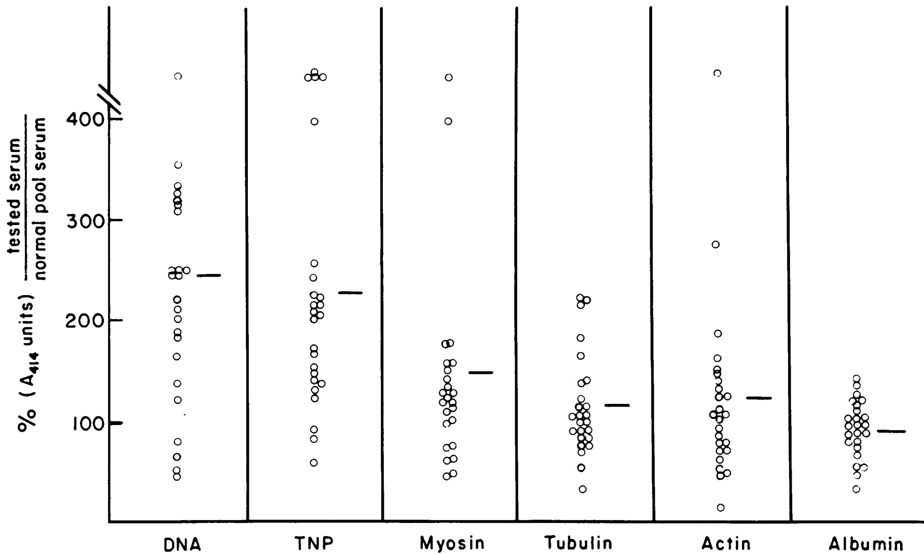

Fig. 4. IgG antibody titre of 25 sera from SLE patients as compared to the antibody titre of a panel of 70 normal human sera.

As expected, the majority of sera expressed IgG anti-DNA activity. Surprisingly, most of the sera also exhibited high anti-TNP activity. The antibody activity against other antigens did not differ from that observed in normal human sera. There were significant differences between the IgG anti-DNA and anti-TNP activity and the other IgG antibody activities (Fig. 4). A significant correlation was observed between the IgG anti-DNA and IgG anti-TNP antibody activity ($r = 0{\cdot}65$).

The main clinical and biological manifestations and the renal biopsy findings are listed in Table 1. No significant correlation could be found between the presence of IgG anti-DNA or anti-TNP antibodies and disease activity.

Screening of eluates obtained from DNP-lysine Sepharose adsorbent. Two SLE sera with high anti-TNP antibody activity, one SLE serum with low anti-TNP antibody activity and the pool of 70 normal sera were passed through a DNP-lysine Sepharose adsorbent. The same volume (approximately 3 ml) of each serum was passed through the immunoadsorbent and concentrated to the same extent. The eluates were tested by EIA for the presence of IgA, IgM and IgG antibodies directed against the panel of the antigens. All eluates from SLE sera contained IgG antibodies directed simultaneously against TNP and DNA (Fig. 5). Eluates also contained IgA and IgM antibodies reacting mainly with TNP and DNA but also with all the antigens of the panel. In contrast, the eluates obtained from normal serum contained IgM and IgA but also IgG antibodies which reacted only with TNP.

Screening of the kidney eluates. The PBS washings and acid eluates from the nine renal biopsy specimens from patients with lupus nephritis were tested by EIA for IgG activity against the six antigens. It was checked that the immunofluorescence picture was not affected after washing with PBS, and that after acid treatment there was little or no immunofluorescence remaining.

The antibody activity found in the acid eluate is shown in Table 2. Patients could be divided into three groups. In one group (three eluates), only anti-DNA activity was recovered, in the other (three eluates), an anti-DNA and anti-TNP activity was found while in the last group (three eluates), the antibodies eluted recognized all the antigens of the panel. No correlation between the IgG specificity of the eluates and disease activity was found. The limited quantity of eluted material did not permit more extensive studies.

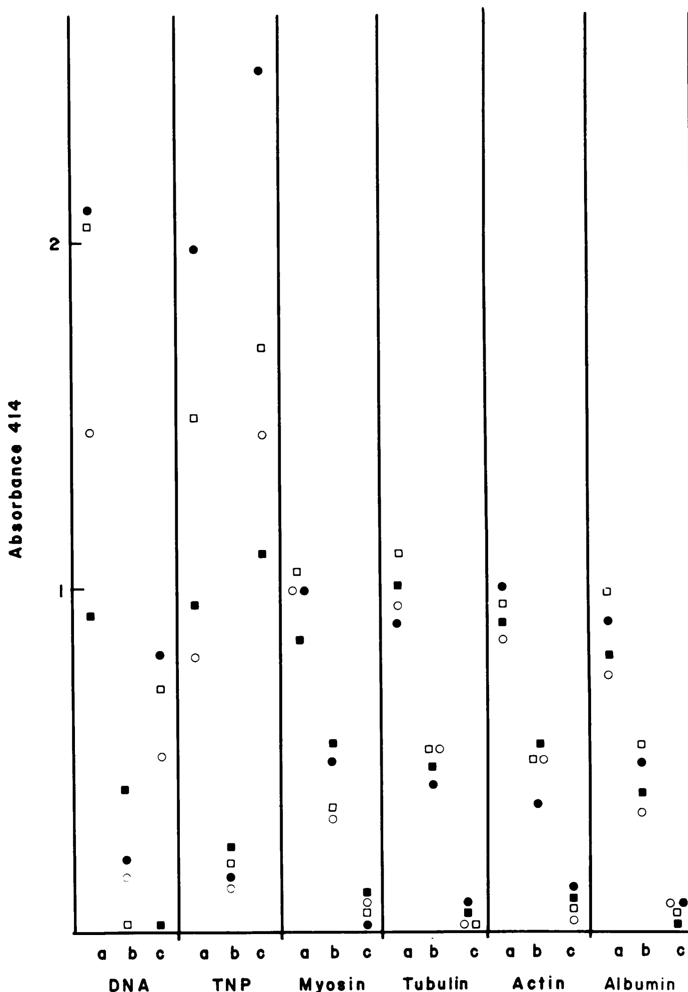

Fig. 5. Isolation of anti-DNP antibodies on TNP-immunoadsorbent. Three millilitres of serum from SLE patients and from a pool normal serum were passed through a DNP-Sepharose column. The anti-TNP antibodies in the whole sera (a), in the effluent (b) and in the adsorbed and eluted fraction (c) were titrated on plates coated with various antigens. (■) normal pool serum origin; (○,●,□) SLE serum origin.

DISCUSSION

In this study, we demonstrate that sera from patients with lupus nephritis contain high levels of IgA against the panel of antigens tested. The presence in SLE sera of IgA anti-nuclear antibodies has been reported a long time ago (Svec, Blair & Kaplan, 1967). Furthermore these sera contained marked IgG anti-DNA and anti-TNP antibody activity. The IgG anti-DNA activity was found to be associated with the specifically eluted anti-TNP antibodies obtained by passage of SLE serum over a DNP immunoadsorbent. Finally we found that three out of nine kidney eluates obtained from biopsy specimens from SLE patients contained IgG antibodies strictly specific for DNA; three recognized both DNA and TNP and three reacted with all the antigens of the panel.

SLE in humans and mice is characterized by the production of various autoantibodies as a consequence of polyclonal B cell activation. Among these, anti-DNA antibodies are thought to play a major role in the appearance of renal lesions. Anti-DNA antibodies may circulate as part of

Table 2. Antibody activity in kidney eluates

Patient	Antibody to: DNA	TNP	Actin	Tubulin	Myosin	Albumin
17	0·320* (3·2)	1·130 (8·0)	1·100 (5·6)	1·460 (8)	1·310 (8·7)	0·530 (11·8)
18	0·185 (7·1)	0·780 (22·9)	0·630 (52·3)	0·125 (1·4)	0·315 (4·4)	0·130 (4·3)
19	0·645 (7·8)	0·225 (1·5)	0	0	0	0
20	0·125 (1·4)	0	0	0	0	0
21	0·270 (6·4)	0	0	0	0	0
22	0·245 (3·3)	0	0	0	0	0
23	0·210 (5·9)	0·200 (1·4)	0	0	0	0
24	0·345 (23)	0·350 (2·3)	0·160 (2·2)	0·165 (2·1)	0·185 (1·8)	—
25	0·255 (2·5)	0·500 (1·5)	0	0	0	0

* Values are given as absorbance (A_{414} unit) of the acid eluates. Numbers in parentheses represent the ratio of absorbance of the acid eluate to the PBS washing. Values given as 0 correspond to values ≤five times the background values.

immune complexes and may be deposited in the kidney. Alternatively free antibodies may be responsible for *in situ* immune complexes formation. It has been reported that mouse monoclonal anti-DNA antibodies from autoimmune mice also react with many other determinants such as polynucleotides, phosphilipids, cytoskeletal proteins, platelets, membrane proteins of Raji cells, T lymphocytes, glomeruli, neurocytes and erythrocytes (Lafer *et al.*, 1981; Jacob & Tron, 1982; Shoenfeld *et al.*, 1983; Koike *et al.*, 1984; Schwartz & Stollar, 1985). More recently and while the present work was carried out, it has been reported that monoclonal anti-DNA antibodies also react with 2,4,6-trinitrophenyl determinants (Serban *et al.*, 1985). These reactions have been attributed to cross-reactions between DNA and phospholipids or DNA and proteins. Such antibodies reacting with DNA and proteins have also been shown to be present in unimmunized normal mice (Dighiero *et al.*, 1983) and normal humans (J. M. Seigneurin, B. Guilbert, M. J. Bourgeat & S. Avrameas, unpublished results). Taking into account these latter studies the present results would indicate that the polyspecific antibodies reacting with DNA in SLE situation correspond to a polyclonal activation of B cells producing polyspecific natural autoantibodies.

Compared however to normal humans and mice where polyspecific natural autoantibodies are almost exclusively of the IgM isotype in SLE situation, it appears that there is a significant increase of polyspecific IgG and IgA autoantibodies. The reason why we did not find an increased serum level of IgG natural antibodies other than anti-DNA and anti-TNP autoantibodies is unclear. However, it is possible that as suggested recently (Cohen, Rapoport & Eisenberg, 1985) such antibodies are present but complex with their corresponding antigens and are thus masked. Such an interpretation, however, does not explain why the IgA natural autoantibodies are increased. This may reflect preferential activation of different clones depending upon the antigen.

An important question in SLE is whether or not antibodies reacting with both DNA and other determinants are a pathogenic significance. It has been suggested that anti-DNA antibodies may be pathogenic because they react with heparin sulphate which is present in the GBM (Faaber *et al.*, 1984) or, alternatively, with other antigens expressed on glomerular visceral epithelial cells (Jacob *et al.*, 1985) but the specificity of the circulating antibodies and that of antibodies deposited in the kidney has not been compared. We have carried out such a study comparing the specificity of circulating antibodies with that of antibodies deposited in the kidney and eluted at acid pH. It would appear that the latter correspond to truly kidney-bound antibodies for two reasons. Firstly, kidney immunofluorescence was still positive after PBS washing and negative after acide elution. Secondly, in all cases in a positive reaction the ratio of the optical densities in EIA of the acid eluates versus PBS washer was at least equal to 1·4 and in most cases much higher. We have found that at

least in some patients only antibodies specific for DNA are recovered from kidney sections, although anti-DNA antibodies which also react with TNP have been isolated from the sera of these patients. The suggestion that anti-DNA antibodies are responsible for lupus nephritis has been made several years ago (Winfield, Faiferman & Koffler, 1977). However in three out of nine patients we have examined, antibodies with polyspecific natural autoantibody activity were recovered from kidney sections. Because of insufficient amounts of eluates, we were unable to determine if only polyspecific antibodies or both polyspecific and monospecific antibodies were present in these three cases. For the same reasons, the electric charge and affinity of the eluted antibodies could not be examined.

All the results obtained in the present work in addition to those reported in the studies mentioned above suggest that in SLE there are at least two distinct kinds of anti-DNA antibodies: those reacting with DNA and also with other macromolecular antigens which may or may not be involved in the pathogenesis of lupus nephritis, and those that are more DNA specific which seem to be more involved in kidney lesions.

REFERENCES

AVRAMEAS, S. (1969) Coupling of enzymes to proteins with glutaraldehyde. Use of the conjugates for the detection of antigens and antibodies. *Immunochemistry* **6,** 43.

AVRAMEAS, S., GUILBERT, B. & DIGHIERO, G. (1981) Natural antibodies against tubulin, actin, myoglobin, thyroglobulin, fetuin, albumin and transferrin are present in normal human sera and monoclonal immunoglobulins from multiple myeloma and Waldenström's macroglobulinemia may express similar antibody specificities. *Ann. Immunol. (Inst. Pasteur)* **132C,** 231.

BLAESE, R.M., GRAYSON, J. & STEINBERG, A.D. (1980) Elevated immunoglobulin secreting cells in the blood of patients with active systemic lupus erythematosus: correlation of laboratory and clinical assessment of disease activity. *Am. J. Med.* **69,** 345.

BUDMAN, D.R., MERCHANT, E.B., STEINBERG, A.D., DOFT, B., GERSHWIN, M.E., *et al.* (1977) Increased spontaneous activity of antibody forming cells in the peripheral blood of patients with active systemic lupus erythematosus. *Arthritis Rheum.* **20,** 829.

BUDMAN, D.R. & STEINBERG, A.D. (1977) Hematologic aspects of systemic lupus erythematosus. Current concepts. *Ann. intern. Med.* **86,** 220.

CHURG, J. & SOBIN, L.H. ED. (1982) Renal disease. *Classification and Atlas of Glomerular Diseases.* p. 128. Igaku-Shoin, Tokyo, New York.

COHEN, P., RAPOPORT, R. & EISENBERG, R. (1985) Hidden autoantibodies against common serum proteins in murine systemic lupus erythematosus. *J. exp. Med.* **161,** 1587.

DIGHIERO, G., GUILBERT, B., FERMANT, J.P., LYMBERI, P., DANON, F. & AVRAMEAS, S. (1983) Thirty-six human monoclonal immunoglobulins (MIg) with antibody reactivity against cytoskeleton, proteins, thyroglobulin and native DNA. Immunological studies and clinical correlations. *Blood* **62,** 264.

DIGHIERO, G., LYMBERI, P., MAZIE, J.C., ROUYRE, S., BUTLER-BROWNE, G.S., WHALEN, R.G. & AVRAMEAS, S. (1983) Murine hybridomas secreting natural monoclonal antibodies reacting with self antigens. *J. Immunol.* **131,** 2267.

DIGHIERO, G., LYMBERI, P., HOMBERG, D., LUNDQUIST, I., COUTINHO, A. & AVRAMEAS, S. (1985) High frequency of natural autoantibodies in normal newborn mice. *J. Immunol.* **134,** 765.

DIXON, F.J., THEOPHILOPOULOS, A.G., IZUI, S. & MACCONAHEY, I.J. (1980) Murine systemic lupus erythematosus: aetiology and pathogenesis. In *Progress in Immunobiology* (eds M. Fougereau & J. Dausset) p. 959. Academic Press, New York.

FAABER, P., CAPEL, P.J.A., RIJKE, G.P.M., VIERMINDEN, G., VAN DE PUFTE, L.B.A. & KOENE, R.A.P. (1984) Cross-reactivity of anti-DNA antibodies with proteoglycans. *Clin. exp. Immunol.* **55,** 502.

FELTKAMP, T.E.W. & BOODE, J.H. (1970) Elution of antibodies from biopsy tissue. *J. clin. Pathol.* **23,** 629.

GUILBERT, B., DIGHIERO, G. & AVRAMEAS, S. (1982) Naturally occurring antibodies against nine common antigens in normal human sera. Detection, isolation, and characterization. *J. Immunol.* **128,** 2779.

JACOB, L., LETY, M.-A., LOUVARD, D. & BACH, J.F. (1985) Binding of a monoclonal anti-DNA autoantibody to identical protein(s) present at the surface of several human cell types involved in lupus pathogenesis. *J. clin. Invest.* **75,** 315.

JACOB, L. & TRON, F. (1982) Monoclonal anti-deoxyribonucleic antibodies. *J. Immunol.* **128,** 895.

JAFFE, B.M., EISEN, H.M., SIMMS, E.S. & POTTER, M. (1969) Myeloma proteins with anti-hapten antibody activity E-2-4 dinitrophenyl binding by the protein produced by mouse plasmocytoma MOPC 460. *J. Immunol.* **103,** 872.

KOFFLER, D., SCHUR, P.H. & KUNKEL, H.G. (1967) Immunological studies concerning the nephritis of systemic lupus erythematosus. *J. exp. Med.* **126,** 607.

KOIKE, T., MARUYAMA, N., FUNAKI, H., TOMIOKA, H. & YOSHIDA, S. (1984) Specificity of mouse hybridoma antibodies to DNA. II-Phospholipid reactivity and biological false positive serological test for syphilis. *Clin. exp. Immunol.* **57,** 345.

KUNKEL, H.G. & TAN, E.M. (1964) Autoantibodies and disease. *Adv. Immunol.* **4,** 351.

LAFER, E.M., RAUCH, J., ANDRZEJEWSKI, C., MUDD,

JR.D., FURIE, B., SCHWARTZ, R.S. & STOLLAR, B.D. (1981) Polyspecific monoclonal lupus autoantibodies reactive with both polynucleotides and phospholipids. *J. exp. Med.* **153,** 897.

LITTLE, J.R. & EISEN, H.N. (1966) Preparation and characterization of antibodies specific for the 2-4-6 trinitrophenyl group. *Biochemistry* **5,** 3385.

SCHWARTZ, R.S. & STOLLAR, B.D. (1985) Origins of anti-DNA autoantibodies. *J. clin. Invest.* **75,** 321.

SERBAN, D., RORDORF-ADAM, C., SUN, Y-Z & GORDON, J. (1985) Monoclonal and serum anti-DNA antibodies cross-reactive with 2,4,6-trinitrophenyl derivatives. *J. Immunol.* **135,** 3122.

SHELANSKY, M.L., GASKIN, F. & CANTOR, C. (1973) Microtubules assembly in the absence of added nucleotides. *Proc. natn. Acad. Sci. USA.* **70,** 765.

SHOENFELD, Y., RAUCH, J., MASSICOTTE, H., SYAMAL, K.D., ANDRE-SCHWARTZ, J., STOLLAR, B.D. & SCHWARTZ, R.S. (1983) Polyspecificity of monoclonal lupus autoantibodies produced by human hybridomas. *N. Engl. J. Med.* **24,** 414.

SIEGEL, N.J. & HAYSLETT, J.P. (1981) Renal involvement in systemic lupus erythematosus. In: *Kidney in Systemic disease* (ed. Wadi N. Suki & Garabed Eknoyan) p. 55. Wiley Medical, New York.

SPUDISH, J.A. & WATT, S. (1971) The regulation of rabbit skeletal muscle contraction. *J. biol. Chem.* **246,** 4866.

SVEC, K.H., BLAIR J.D. & KAPLAN, M.H. (1967) Immunopathologic studies of systemic lupus erythematosus (SLE). I. Tissue-bound immunoglobulins in relation to serum antinuclear immunoglobulins in systemic lupus and in chronic liver disease with LE cell factor. *J. Clin. Invest.* **46,** 558.

TAN, E.M., CHOEN, A.S., FRIES, J.F., MASI, A.T., MCSHANE, D.J., ROTHFIELD, N.F., SCHALLER, J.G. TALAZ, N. & WINCHESTER, R.J. (1982) The 1982 revised criteria for the classification of SLE. *Arthritis Rheum.* **25,** 1271.

TAN, E.M. (1982) Autoantibodies to nuclear antigens (ANAF): their immunobiology and medicine. *Adv. Immunol.* **33,** 167.

WHALEN, R.G., BUTLER-BROWNE, G.S. & GROS, F. (1978) Identification of a novel form of myosin light chain present in embryonic muscle tissue and cultured muscle cells. *J. mol. Biol.* **126,** 415.

WINFIELD, J.B., FAIFERMAN, K. & KOFFLER, D. (1977) Avidity of anti-DNA antibodies in serum and IgG glomerular eluates from patients with systemic lupus erythematosus: association of high avidity antinative DNA antibodies with glomerulonephritis. *J. clin. Invest.* **59,** 90.