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A study of immune responses to myelin and cardiolipin in patients with systemic lupus erythematosus (SLE)

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SUMMARY

Sera from 39 patients with SLE, 20 patients with cerebrovascular disease with no evidence of SLE, and 20 normal controls were tested for antibodies to cardiolipin (CL), brain total upper (UPG) and lower phase (LPG) glycolipids, myelin basic protein (MBP), myelin, and single strand DNA (ssDNA) by ELISA. Binding to the glycolipids and MBP was negative or negligible in all the groups, but significant binding was observed against CL, myelin and ssDNA in some of the SLE patients. Many sera from SLE patients with cerebral disorders and high CL binding also demonstrated high binding to myelin. These sera also labelled cell surface antigens on neonatal mouse neurons and astrocytes by immunofluorescence in tissue culture. A correlation was found to exist between anti-CL and anti-myelin antibodies in SLE patients with cerebral lesions, but not between anti-ssDNA and anti-CL antibodies. As much as 80–90% of the specific activity of these antibodies could be absorbed out by the relevant antigens but only partially by the other antigens. In the control groups binding was low and no specific absorption could be demonstrated.

Keywords SLE myelin cardiolipin autoimmunity

INTRODUCTION

Cardiolipin (CL) is diphosphatidyle glycerol, a negatively charged phospholipid. Anti-CL antibodies are associated with many clinical conditions such as venous and arterial thrombosis (Harris *et al.*, 1983; Colaco & Male, 1985) thrombocytopenia (Harris *et al.*, 1986), recurrent abortion (Lubbe *et al.*, 1983; Lockshin *et al.*, 1985), pulmonary hypertension (Asherson *et al.*, 1983) and neurological disorders (Colaco, Scadding & Lockhart, 1987) both in patients with SLE and others who fulfil the criteria for the anti-phospholipid (AP) syndrome (Harris *et al.*, 1987) (a condition which involves the presence, at high titres, of AP antibodies and two or more manifestations related to these antibodies, but no SLE).

Several investigations have shown that anti-CL antibodies cross-react both with DNA (Lafer *et al.*, 1981) and other negatively charged phospholipids, such as phosphatidic acid (PA), phosphatidyl serine (PS) and phosphatidyl inositol (PI), substances containing similar diester-linked phosphate groups (Sutjita *et al.*, 1989), but not phosphatidyl ethanolamine (PE) or choline (PC) (Gharavi *et al.*, 1987). The structure of CL is related to PS, PA and DNA. The phosphodiester groups are separated by equal numbers of carbon atoms in CL and DNA, and in CL, PS and PA have a negative charge. Koike *et al.* (1982) have reported that anti-DNA antibodies derived from SLE-prone mice cross-reacted with anti-CL, but several other studies (Harris *et al.*, 1985; Weidmann *et al.*, 1988) reported no relation between anti-CL levels and anti-DNA.

Human central nervous system (CNS) myelin consists of 70% lipids (phospholipids 30·2%, glycolipids 19·2% and cholesterol 19·4%) and 30% proteins (the major protein being myelin basic protein (MBP) 12%) (Morell, 1984). Glycolipids can be divided into brain total upper (UPG) (mainly gangliosides) and lower phase (LPG) (mainly neutral glycolipids, such as cerebrosides) and phospholipids are mainly PE, PS, PC and PI. The lipid content of the peripheral nervous system is similar to that of the CNS. In view of the evidence of cerebral disorders in SLE, especially with the involvement of anti-CL antibodies, we decided to investigate the possibility of cross-reactivity between anti-CL and myelin in SLE patients who also had neurological deficits, as well as binding of these patients' sera to neuronal cells in culture.

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MATERIALS AND METHODS

ELISA

ELISAs were used to test the sera against CL, ssDNA, UPG, LPG, myelin and MBP under coded conditions. The methods for these have been previously described (Khalili-Shirazi, 1991). DNA (calf-thymus sodium salt type-1) and CL were purchased (Sigma Chemical Co., Poole, UK). Alkaline-phosphatase-

conjugated anti-human immunoglobulin (IgG+IgM+IgA combined, from Dako, High Wycombe, UK), was used as the second antibody in the ELISAs. Myelin, UPG and LPG were prepared from mouse and rat brains as previously described (Khalili-Shirazi, 1988). The purity of myelin was examined on a 15% SDS–PAGE, and found to be about 90% pure, containing myelin proteins reported in the literature (Morell, 1984), Wolfgram proteins, proteolipid proteins, DM20 and MBP. The MBP used in this study was prepared from purified rat myelin. Briefly, myelin (3 mg protein/ml Tris-HCl buffer) was suspended in 0·2 M HCl, stirred for 1 h at 37°C and the membranes were pelleted. The supernatant was concentrated and applied to a Sephadex G50 column (Pharmacia, Milton Keynes, UK) and the proteins were monitored at absorbance at 280 nm (A_{280}). The purity of MBP containing fractions was checked by PAGE on a 15% gel.

Anti-CL quantitative standards used for standardizing the ELISA were the standardized positive sera 19, 62, 61 and negative sera (gift of Dr G. Hughes, Lupus Research, Rayne Institute, St Thomas's Hospital, London, UK). We quantified these sera in IgG and IgM ELISA units. The order of activity of these sera (at 1:50) is: sera 19 (high-acting), IgG = 268, IgM = 44; sera 62 (medium-acting), IgG = 28, IgM = 24; sera 61 (low-acting), IgG = 7·4, IgM = 8·2 and negative sera = 0. The optical densities (OD) of these sera at 1/50 dilution against CL in our ELISA were found 19 = 1·185, 62 = 0·555, 61 = 0·355 and negative control = 0·1. Therefore, in our following studies the OD readings < 0·1 were treated as negative, 0·1–0·35 as low, 0·36–0·55 as medium and 0·56–1·18 as high acting CL antibodies. In order to validate our binding to CL, an ELISA plate was half coated with CL in ethanol and the other half with ethanol only and tested against three positive sera in triplicate at 1/50. The mean (s.d.) values of nine replicates obtained were 1·118 (0·418) for CL in ethanol and 0·128 (0·022) for ethanol only.

Patients

All of the sera were tested blind and only when the initial screening was completed were they divided into Groups I to III.

Group I Sera from 39 patients with SLE were provided from the out-patients clinic of Tygerberg Hospital, covering multiracial patients from the Western Cape. The sera were sent coded and the study was carried out blind. Later, the patients were divided into SLE patients with or without cerebral complaints. These were called 'cerebral SLE' and 'non-cerebral SLE' patients; 'SLE patients' refers to the undifferentiated original group.

Group II Twenty patients from Groote Schuur Hospital Out-patient clinics, Cape Town, were studied. These patients had a variety of non-immune vascular complaints including strokes, large vessel occlusion and myocardial infarction. None of them had any autoimmune disease.

Group III Twenty normal blood-donors were used. The sera were provided from the two out-patient clinics serving multiracial communities. The ethnic origin (South African Government's obsolete Race Classification) of patients was later found to be a mixture of Cape-coloured and White patients, and no Black patients. This is because SLE is very rare amongst the Black population, and the controls had accordingly been kept compatible.

Absorption studies

Serum samples were diluted at 1/50 in phosphate-buffered saline (PBS) buffer containing 5% fetal calf serum and divided into four parts. One part (0·8 ml) was absorbed with myelin (250 μg protein/ml), one with CL (10 μg/ml), and one with ssDNA (20 μg/ml), for 4, 24, or 48 h at 4°C. One part remained unabsorbed. After these periods of absorption the sera were tested against myelin, CL and ssDNA.

Western blotting

This was carried out by the method of Towbin, Staebelin & Gordon (1979). SDS–PAGE (15%) was run, using 26 μg of delipidated myelin per lane and blotted on to Immobilon (Millipore, Harrow, UK). Sera from three of the cerebral-SLE patients at 1/50 and a pool of 10 normal controls (from group III) also at 1/50 were incubated with the myelin blots. These blots had been previously blocked for 12 h with 3% milk powder (Marvel) in PBS. Anti-human immunoglobulin (G+A+M) antibody conjugated to alkaline phosphatase (Dako) was used as the second antibody and the blots were then developed. The gel itself was stained with amido black or Coomasie blue. This experiment was repeated twice with the same sera.

Immunofluorescence

This was carried out on neonate mouse brain cell cultures, adapted for neuronal and astrocyte growth. The method for this cell culture and for the immunofluorescent technique have previously been described (Khalili-Shirazi, 1988). An endothelial cell line (BAE8) (gift from Department of Biochemistry, UCT Medical School, Cape Town) was also used.

Statistical analysis

An unpaired *t*-test, Student's *t*-test and correlation of coefficient analysis were carried out using a Nanostat computer program (Alpha Bridge, London, UK).

RESULTS

All sera were initially screened against myelin, CL and ssDNA by ELISA (Fig. 1). The SLE group showed a greater range of anti-myelin and anti-CL binding than the other two groups. Binding of the SLE patients to both myelin and CL was significantly higher than those of the control groups, with the *P* values for anti-myelin (group II) $P = 0{\cdot}0002$ and for group III $P < 0{\cdot}0001$. For anti-CL binding the corresponding values were $P = 0{\cdot}004$ and $P = 0{\cdot}0082$. Six of the high CL-binding sera were titrated by doubling dilutions (in 1% BSA/PBS) from 1/35 to 1/1120. Binding curves were sigmoidal with the extreme ODs being $1{\cdot}03 \pm 0{\cdot}09$ to $0{\cdot}67 \pm 0{\cdot}09$ for myelin; $0{\cdot}6 \pm 0{\cdot}1$ to $0{\cdot}15 \pm 0{\cdot}006$ for CL; and $1{\cdot}4 \pm 0{\cdot}13$ to $0{\cdot}68 \pm 0{\cdot}24$ for ssDNA.

When SLE (group I) was divided into cerebral ($n = 17$) and non-cerebral ($n = 22$) SLEs and all the data were pooled, the final mean OD values ± s.e.m. for anti-myelin and CL activities were calculated. The cerebral SLE had ODs of $0{\cdot}411 \pm 0{\cdot}022$ for CL, and $0{\cdot}505 \pm 0{\cdot}021$ for myelin. The corresponding values for non-cerebral SLE were $0{\cdot}243 \pm 0{\cdot}014$ for CL and $0{\cdot}464 \pm 0{\cdot}021$ for myelin binding.

The results from testing the SLE sera in duplicates against CL, myelin and ssDNA (Fig. 1) were divided into cerebral and non-cerebral SLEs and compared. A significant correlation

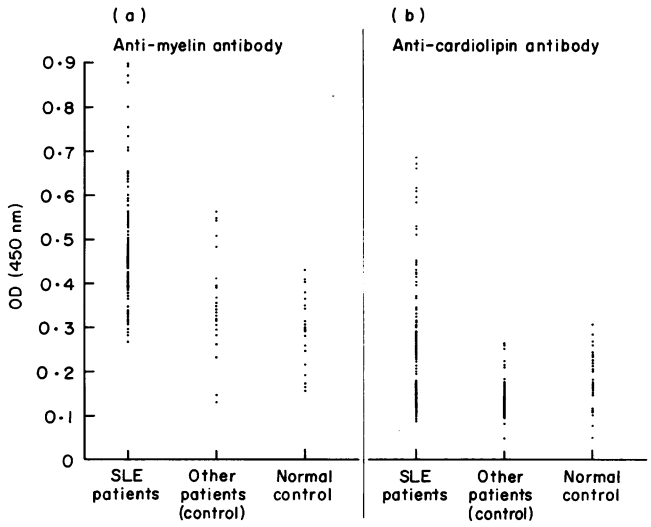

Fig. 1. The initial antibody binding of sera (1:50 dilution) from SLE patients (group I), control patients (group II) and normal control (group III) to (a) myelin and (b) CL in an ELISA (repeated twice). Antibody responses are presented as distribution of the direct ODs at 450 nm of each group against the two antigens. The mean OD and s.d. for these groups to antigens (a) and (b) is as follows: Group I (a) 0·505 ± 0·079, (b) 0·333 ± 0·124, group II (a) 0·368 ± 0·029, (b) 0·172 ± 0·029 and group III (a) 0·304 ± 0·015, (b) 0·205 ± 0·028.

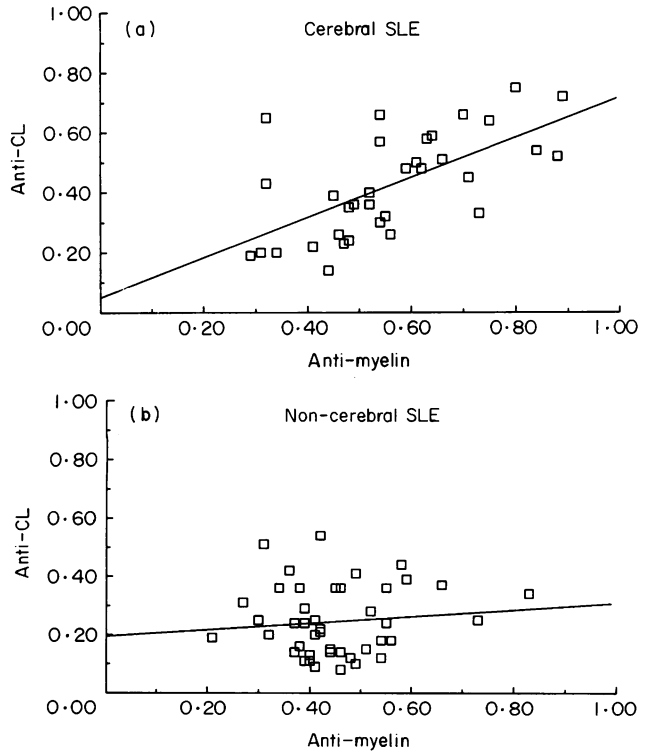

Fig. 2. Correlation between anti-CL and anti-myelin are shown for (a) cerebral (17) and (b) non-cerebral (22) SLE patients. The data are directly taken from Fig. 1 (study repeated twice, therefore 34 values for cerebral and 44 values for non-cerebral SLE patients are presented). The mean anti-CL and anti-myelin activity was found to be 0·421 ± 0·172 and 0·562 ± 0·165 for cerebral SLE and 0·246 ± 0·165 and 0·449 ± 0·115 for non-cerebral SLE patients.

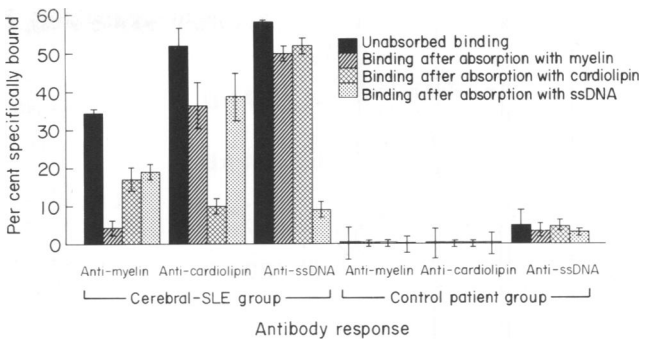

Fig. 3. A normalized representation of absorption of group I (with cerebral complaints) and group II patients by myelin, CL and ssDNA is shown. Each histogram represents the mean ± s.d. of three experiments, and each experiment was carried out with samples in duplicates. The specificity of anti-myelin, anti-CL and anti-ssDNA in the SLE sera was tested by a series of absorption studies and the results were normalized as follows. In each study the OD of each unabsorbed sample for each antigen was taken as 100%. The OD values of the corresponding absorbed samples were calculated as a percentage of that and the mean and the s.d. calculated for each group. The specific binding to each antigen was calculated in order to distinguish the level of patients' binding above that of the non-specific normal control (group III). The mean OD value for the unabsorbed binding of the normal control sera to each antigen was calculated as a percentage of the corresponding binding by the test patients' sera (groups I and II). This value was then deducted from the binding (100%) of that group. The remaining value represents the percentage of specific binding of that group. The same was carried out for bindings to each antigen, before and after a 24 h absorption. The abscissa therefore represents the normal control binding level. In the cerebral SLE patients, myelin absorbed out nearly all of the specific anti-myelin activity, but it only partially absorbed the anti-CL or the anti-ssDNA activities. The specific binding of group II patients was low. The pattern of absorption after 48 h of incubation did not change, only the overall levels of binding decreased. The OD range for binding in cerebral SLE patients was 1·532 and 0·7, whereas those of the control patients (group II) and normal controls (group III) were 0·39 to 0·2 and 0·78 to 0·16 respectively. The data are presented as a percentage of anti-myelin, CL and ssDNA specific binding before and after absorption by each antigen.

between the anti-CL and anti-myelin response was found in the cerebral SLE patients (with the correlation coefficient of 0·644 and $P = 0·0001$), but despite the high anti-myelin activity in some of the non-cerebral SLE patients, the correlation between these two antibodies was not significant (0·096 and $P = 0·538$) (Fig. 2). No correlation between these two antibodies in groups II and III was found (results not shown). Also, there was no significant correlation between binding activity of CL and ssDNA.

Adsorption of both anti-myelin and anti-CL antibody binding of sera from SLE patients was significant when performed by the test antigen ($P < 0·001$) (Fig. 3). The extent of the absorption ($P = 0·02$) by the other antigens varied from patient to patient, indicating heterogeneity of antibody specificity in different patients. Binding to ssDNA showed only very limited absorption by myelin or CL ($0·1 > P > 0·02$). In every case absorption by the test antigen was significantly higher than that of the other two antigens ($P < 0·001$), reducing the level of binding close to that observed by normal sera. However, the difference between the absorption by the other two antigens was not significant ($P > 0·1$). Only CL, and not myelin or ssDNA,

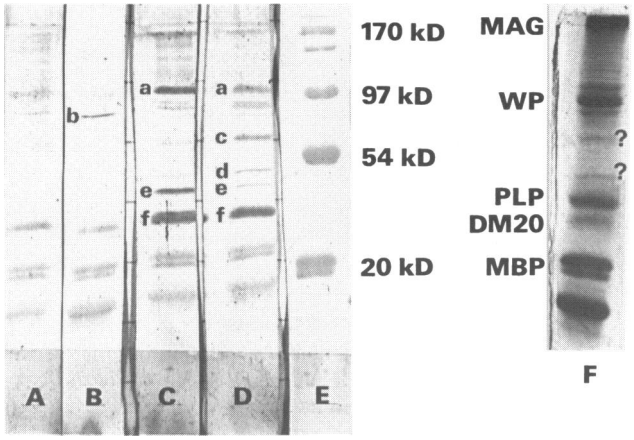

Fig. 4. A profile of a dried gel from an SDS–PAGE of CNS myelin and the blots from Western blotting of a pool of normal control sera (A), three cerebral-SLE patients (B, C, D) and molecular weight markers (E). The gel profile (F) of myelin demonstrates the main myelin proteins: myelin associated glycoprotein (MAG) 90–100 kD molecular weight, Wolfgram proteins (WP) consisting of 45–60 kD proteins, proteolipid proteins (PLP and DM20) 31 kD and 25 kD, and MBP consisting of 21·5 kD, 18·5 kD, 17 kD and 14 kD proteins. The bands in the patients' blots which either had a higher intensity of binding, or a different molecular weight from those of the normal control are marked a–f, the molecular weights for which are: a = 54 kD, b = 50 kD, c = 43·5 kD, d = 36 kD, e = 31 kD and f = 29 kD. This experiment was repeated twice with the same result. The cerebral-SLE patients were selected from the most severe cases.

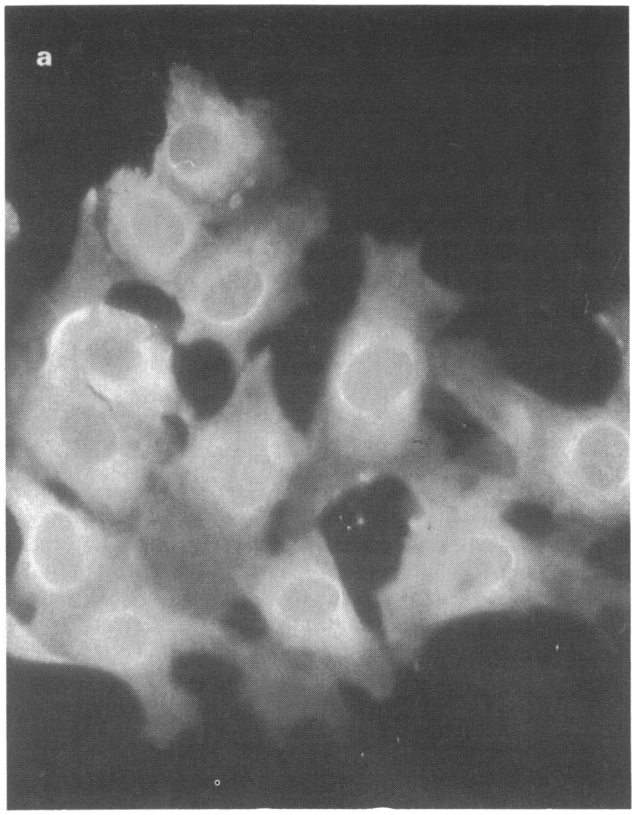

Fig. 5. Immunofluorescent binding of a pool of normal sera (1/35) and that of a cerebral-SLE patient (1/35) are presented. Six other cerebral-SLE patients were also tested and showed similar results. (a) Binding of cerebral SLE sera to fixed endothelial cells; (b) binding of normal sera to fixed endothelial cells; (c) binding of cerebral-SLE sera to fixed brain cells; (d) binding of normal sera to fixed brain cells; (e) binding of cerebral-SLE sera to unfixed brain cells; (f) binding of normal sera to unfixed brain cells. (Magnification = ×310).

could significantly absorb the anti-CL binding; similarly, only myelin or only ssDNA could absorb the anti-myelin or the anti-ssDNA binding (Fig. 3). In the normal controls and the control patient group, no absorption by any test antigen could be detected. In this study the binding of cerebral SLE sera to myelin, CL and ssDNA was 35%, 53% and 58% above those of the normal controls. All sera gave negative results in ELISA with MBP. The sera showed varying degrees of binding to the brain glycolipids, but this was considered non-specific since it was not reduced by absorption.

In order to examine further the antigen binding of the cerebral SLE sera to myelin, the three highest-binding sera from this group and a pool of 10 normal control sera were tested against myelin, by Western blotting. This experiment was repeated twice. Normal sera showed some background binding, but each of the patient sera tested had extra bands binding to myelin proteins in addition to those seen by the normal sera (Fig. 4). Serum B only reacted with a myelin protein of molecular weight 50 kD, serum C reacted with 54 kD and 31 kD, whilst serum D reacted with 54 kD, 43·5 kD, 36 kD and 31 kD proteins. The myelin protein bands of 29 kD and 31 kD are within proteolipid proteins' estimated molecular weights (Morell, 1984). The 36 kD and 43·5 kD bands also appear weakly on the stained myelin gel, but their identity is not known (not all the myelin protein bands are as yet identified). The 50-kD band is probably a Wolfgram protein. In addition, sera C and D bind to a 29-kD band to which serum B and the normal control also bind. However, the density of binding is much stronger with sera C and D. These findings of binding of different myelin proteins by different sera further support the idea of heterogeneity in the anti-myelin antibodies.

Immunoglobulin binding from all three groups was tested against fixed (for intracytoplasmic antigens) and unfixed (for cell-surface antigens) endothelial cell line and primary brain cell cultures. In this study five cerebral SLE sera, four non-cerebral SLE sera (one with high myelin binding), two from control group II and a pool of 10 normal control (group I) sera were examined. All of the SLE sera bound similarly to the fixed endothelial cells, but at various intensities. The binding was densely cytoplasmic and nuclear (Fig. 5a). After absorption with ssDNA there was no change in the cytoplasmic staining, but the intensity of nuclear staining of some of the sera had decreased. There was no staining when using normal control sera with fixed (Fig. 5b) or unfixed endothelial cells. Four out of the five cerebral SLE sera also labelled unfixed endothelial cells. This labelling was only seen on the cell surface, with no intracellular staining. However, this surface staining disappeared when the sera were absorbed either with myelin or with CL.

All five of the cerebral SLE sera strongly stained fixed brain cells (Fig. 5c). The pattern of staining was similar to that of the fixed endothelial cells, but the degree of nuclear staining varied. After absorption with ssDNA, there was no change in the cytoplasmic staining, but the nuclear staining had decreased in three of the samples. Absorbtion with myelin or CL was not

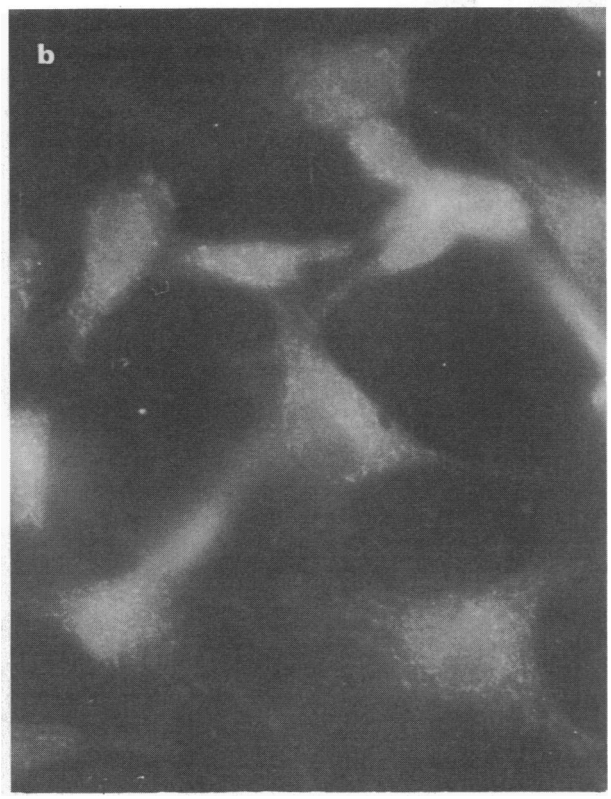

Fig. 5(b).

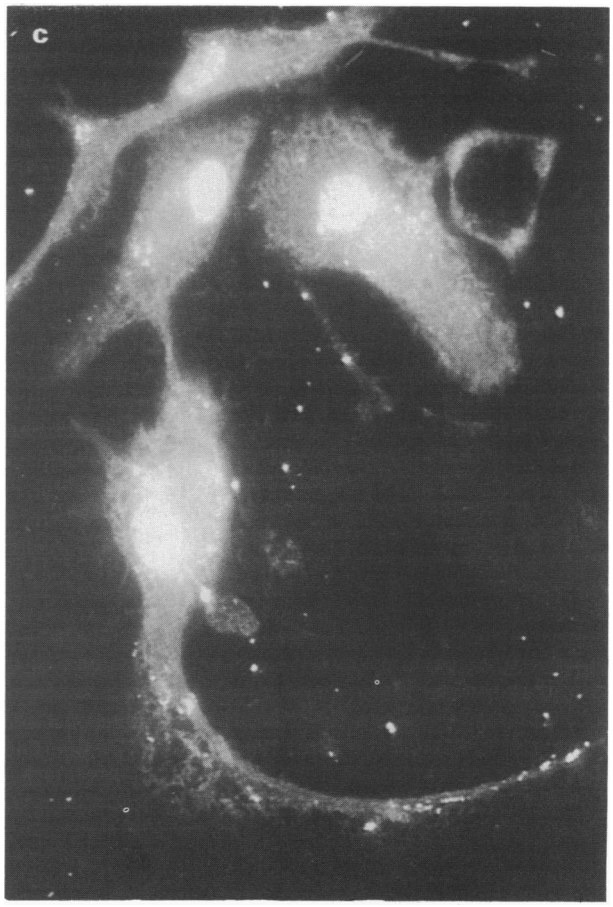

Fig. 5(c).

carried out, since loss of surface staining after such absorbtions had already been observed with the unfixed endothelial cells. The non-cerebral SLE sample with the highest myelin binding was also tested on fixed brain cells, and the results were the same as the above, but the staining with the pooled normal sera was negative (Fig. 5d). All of the five cerebral SLE sera bound to the unfixed brain cells (Fig. 5e). None of the four non-cerebral SLE, or the two control sera group II, or the pooled normal control sera (Fig. 5f) bound to unfixed brain cells. The binding of the cerebral SLE sera was totally surface labelling with no intracytoplasmic staining. This pattern did not alter after absorption with ssDNA, and the lack of staining of all the other groups also did not change after they were absorbed with ssDNA.

DISCUSSION

SLE is a multi-organ autoimmune disease with a variety of circulating autoantibodies, the origin of which remains complex. Anti-DNA antibodies are thought to cause tissue damage by forming immune complexes in the circulation, with subsequent deposition in kidneys and possibly other organs. A smaller proportion of patients produce antibodies to intracellular ribonucleoprotein particles such as Ro, La, Sm, U1-RNP or ribosomes, with 25% of patients having autoantibodies that bind nuclear proteins (Tan, 1982), and 12% which react against ribosomal P protein located in the nucleus of mammalian cells (Lerner & Steitz, 1979; Bonfa & Elkon, 1986). There appear to be antibody populations present in most lupus sera that can react with cellular polypeptides, Sm and U1-RNP cellular antigens (Francoeur, 1989). Anti-Sm and P antibodies are associated with SLE and are also present in MRL-lpr mice and it is suggested that they are regulated at the antibody induction or regulation level (Elkon *et al.*, 1989). The presence of Sm and U1-RNP antigen in structurally related RNP (Lerner & Steitz, 1979) was thought to explain the anti-Sm/anti-U1RNP association, but the failure to cross-inhibit anti-Sm or anti-P activity excluded the possibility that their association may be due to a cross-reactive epitope (Elkon *et al.*, 1989). Anti-P peptide antibody has been associated with lupus psychosis (Bonfa *et al.*, 1987). Neurological abnormalities are common in SLE patients (Fulford *et al.*, 1972; Hughes, 1980) and the two immunopathological mechanisms most widely suggested in SLE are the immune-complex mediated vasculitis and the action of autoantibodies to cell membrane molecules. The anatomical location of the vasculopathy often differs from the neurologic site responsible for clinical findings. Bluestein (1987) suggested that the changes in the vessels of the nervous system can not be sufficient by themselves to account for most of the neuropsychiatric manifestations of SLE. Antibodies reacting with neurons in brain sections have frequently been found (Bresnihan *et al.*, 1979). Cerebrospinal fluid of 20 out of 27 SLE patients with CNS disease, but only two out of 18 SLE patients without CNS involvement, had increased antineuronal IgG antibodies (Bluestein, Williams & Steinberg, 1981). In addition, sera from 45% of neuropsychiatric SLE patients, but only 14% of SLE patients with no cerebral involvement and 12% of the controls, demonstrated binding activity to a neuroblastoma cell line (How *et al.*, 1985). Thus, the most diffuse neuropsychiatric

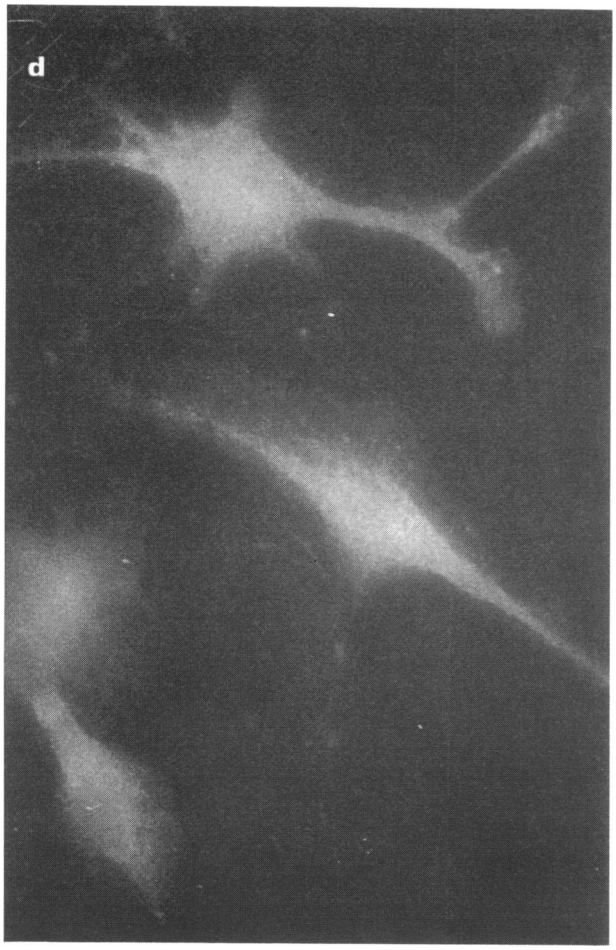

Fig. 5(d).

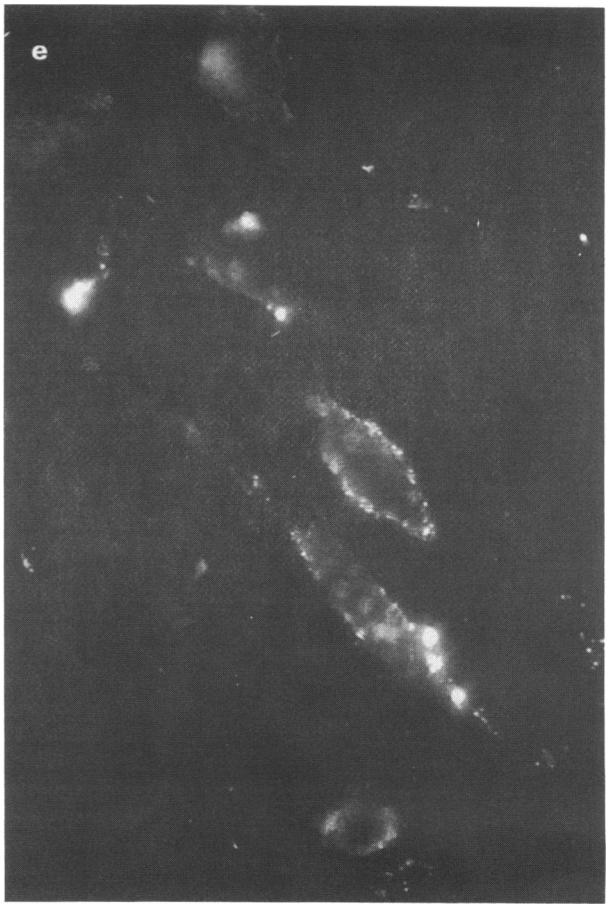

Fig. 5(e).

manifestation of SLE may result from binding of autoantibodies to molecules on neuronal membranes, interfering with a cell's ability to respond to or propagate neuronal signals (Bluestein, 1989). The advantage of this theory over anti-P antibodies is the fact that these antibodies are membranous, whereas ribosomal P protein is intracellular and would require the antibody to enter the cell (Bluestein, 1987). The involvement of anti-CL antibodies with cerebral disorders has been reported (Winfield *et al.*, 1983; Asherson & Harris, 1986; Asherson *et al.*, 1987). AP antibodies have also been reported to be involved with other neurological disorders (Hull *et al.*, 1984; Colaco *et al.*, 1987; Alarcon-Segovia, 1988; Harris, Asherson & Hughes, 1988; Mackworth-Young, Loizou & Walport, 1989). In our study, high anti-CL antibodies were found (especially in SLE patients with neurological disorders).

Production of antibodies (independent of anti-CL and DNA antibodies) reactive to endothelial cells in SLE patients (Rosenbaum *et al.*, 1988), as well as the presence of neurotoxic antibodies (Bluestein, 1978) have been reported. In a preliminary study we found complement-dependent cytotoxic activity in a cerebral SLE patient's serum toward endothelial cells (results not shown), as well as the binding to the endothelial cell line shown in Fig. 5a. Binding of cerebral SLE patients' sera to both intracellular and cell surface antigens of brain cells in culture has been demonstrated in this study. If sera from the patients who bind to endothelial cells also happen to be cytotoxic to them, then these antibodies could damage the blood-brain barrier and gain access to the brain. In this study we have shown a reaction of cerebral SLE patients' sera with myelin, and in a neonate mouse brain cell culture with neuronal and astrocytic cell-surface antigens (Fig. 5e).

The correlation between the anti-myelin and anti-CL antibodies observed in these patients could be due either to two related but not cross-reactive antibodies, or to the partial cross-reactivities of the same antibody. Myelin is a complex antigen, made up of many components. We have shown (by ELISA and Western blotting) that although CL-positive SLE sera can also be myelin positive, there is no specific activity against the gylcolipids (UPG and LPG), MBP or a particular protein of myelin. It is possible that the anti-CL antibodies in these patients may be reacting with the phospholipids in myelin, either as a result of an anti-myelin phospholipid antibody, or an antibody cross-reacting between PS in myelin and CL. Cross-reaction between anti-CL antibodies and PS has already been reported (Sutjita *et al.*, 1989). The absorption studies here (Fig. 3), however, indicate these antibodies to be separate. Therefore, it is more likely for them to be two separate antibodies possibly related, than the same antibody. It is interesting that in the SLE with no neurological complications there is no correlation between these two antibodies. The

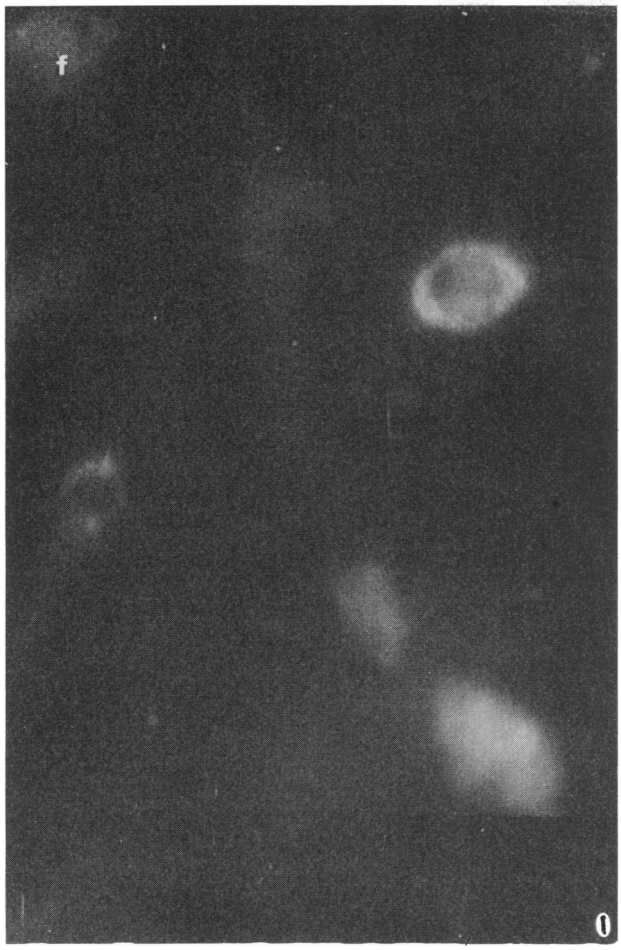

Fig. 5(f).

binding of some of the cerebral SLE sera to various myelin proteins found by Western blotting (Fig. 4) may be a secondary phenomenon, and we have no evidence as to the origin of these antibodies. There does not appear to be a relationship nor a cross-absorption between anti-ssDNA antibody and anti-myelin and anti-CL antibodies.

In the presence of the antibodies to brain cell-surface antigens, should they gain access to the brain, a humoral-mediated damage to the brain cannot be ruled out. Also, since the activity of these antibodies can be inhibited by absorption with their test antigen and not by the other antigens used, they cannot be non-specific.

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