

# Systemic Lupus Erythematosus Patients with Central Nervous System Involvement Show Autoantibodies to a 50-kD Neuronal Membrane Protein

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## Summary

An antibody was detected in the sera from patients with systemic lupus erythematosus (SLE) and central nervous system (CNS) involvement that reacted with a 50-kD antigen in the plasma membrane of brain synaptic terminals. The 50-kD antigen was solubilized with Triton X-100 from preparations enriched with synaptic plasma membranes, and was partially purified by molecular sieve filtration column chromatography. The sera of 19 of 20 CNS-SLE patients showed strong to moderate immunoreactivity with the 50-kD protein in Western blots. Immunoreactivity with the 50-kD protein was also detected in the cerebrospinal fluid of CNS-SLE patients. Control sera from healthy individuals did not react with the 50-kD protein. Low to background reactivity was detected in 35% of a group of SLE patients without CNS manifestations, and in 3% of patients displaying other connective tissue diseases. A total of 100 individuals were tested in this study. Purified autoantibodies to the 50-kD protein from CNS-SLE patients were used for immunofluorescent labeling of neuroblastoma cells. The immunofluorescent staining revealed a distinct macular distribution pattern on the surface of the cell membrane. Taken together, the data suggest that the 50-kD protein may be an important target for autoantibodies, preponderantly found in CNS-SLE patients, and that the antigen may play a role in the pathogenesis of some neurological manifestations in SLE.

Systemic lupus erythematosus (SLE) is an autoimmune disease characterized by multiple autoantibodies and myriad clinical manifestations. Central nervous system (CNS)<sup>1</sup> involvement is a major clinical feature of SLE recognized in 35–75% of the patients. It is most commonly manifested as organic brain syndrome, psychosis, and seizures, indicating a diffuse and reversible involvement of the CNS (1–3) with corresponding anatomical lesions (4, 5). Routine serological tests show that serum autoantibodies to nuclear proteins and DNA, and serum complement levels, are less reliable indicators for active neuropsychiatric disorders in SLE than other manifestations of SLE, such as skin eruptions or nephritis (6). Therefore, the diagnosis of CNS-SLE is difficult and remains based on exclusion.

A useful tool for identification and characterization of molecules associated with CNS-SLE are the brain-reactive au-

toantibodies detected in these patients. Serum autoantibodies crossreacting with neuronal antigens have been implicated in the pathogenesis and onset of overt neuropsychiatric disorders in CNS-SLE (3). Correlations have been found between brain crossreactive lymphocytotoxic autoantibodies in patient's serum or in cerebrospinal fluid (CSF) and neuropsychiatric disorders, as well as between neuroblastoma binding serum autoantibodies and clinical nervous system dysfunction in SLE (7–9). In neuroblastoma cells, a 97-kD membrane protein of unknown function is the target antigen for autoantibodies from a limited number of CNS-SLE patients (10). Furthermore, autoantibody reactivity with neurofilament (11) and the ribosomal P protein (12) has been associated with SLE. The latter showed good correlation with neuropsychiatric involvement in SLE; therefore, synthetic ribosomal P peptide can now be used to assay for respective autoantibodies and hopefully to identify patients with psychosis secondary to SLE (13). In the search for a target antigen that could be useful as a more general marker for CNS involvement in SLE and that could also be putatively involved in the immunopathogenic mechanisms of this disease, we uti-

<sup>1</sup> Abbreviations used in this paper: CNS, central nervous system; CSF, cerebrospinal fluid.

lized a preparation of brain synaptic membranes and Western blotting to screen sera from a large sample of SLE patients with and without CNS manifestations. The rationale for using a synaptic membrane preparation was based on the assumption that an autoantibody that has a putative pathogenic role in CNS-SLE is most likely to be directed against a functional surface molecule with a key location in the neuronal population of the brain. We report here that a neuronal membrane antigen of 50 kD in synaptic terminals is a target for autoantibodies in SLE patients with CNS involvement.

## Materials and Methods

**Study Population.** Sera were obtained from 100 individuals. Of these, 43 were diagnosed with SLE and fulfilled the American College of Rheumatology criteria. All SLE patients were antinuclear antibody (ANA) positive and most tested positive for anti-double-stranded DNA. Further serological testing of the SLE patients was not performed due to obvious disease symptoms. 20 of the SLE patients were diagnosed with CNS disorders in addition to active SLE. Of the 20 patients, 5 were male. About half of the female patients had no record of pregnancies. Clinical diagnosis of CNS activity in these patients was determined independently of the tests performed in this study. CNS manifestations included seizures, psychosis, severe protracted headaches, visual blurring, photophobia, acute organic brain syndrome, cerebrovascular accident (infarction and hemorrhage), and transverse myelitis. None of the CNS manifestations could be attributed to a coexisting disease other than SLE. The 23 SLE patients without CNS involvement had no known current or previous neurological manifestations. All SLE patients had no prior transfusion record. The non-SLE patients included one patient with rheumatoid vasculitis and acute organic brain syndrome, six patients with rheumatoid arthritis, three with polymyositis, eight with progressive systemic sclerosis, 10 with chronic schizophrenia, three with hypertensive cerebrovascular accidents, five with idiopathic seizure disorders, and one with paraneoplastic cerebellar degeneration. 20 individuals were normal. The well-defined ribonucleoprotein La-positive serum as well as the crude recombinant La antigen and bacterial control used in this study were a generous gift from Dr. K. B. Elkon (Cornell University, Ithaca, NY). Defined anti-Ro human antisera were a gift from Dr. John B. Harley (Oklahoma Medical Research Foundation). Rabbit calreticulin and rabbit anti-calreticulin antibody were kindly provided by Dr. Richard Fine (Boston University Medical School).

**Antigen Preparation.** Approximately 400 g of bovine brain cortex were used. After removal of the meninges the gray matter was separated by suction, and synaptic plasma membranes were prepared from the gray matter using the methanol of Jones and Matus (14). All buffers contained 1  $\mu$ M PMSF and aprotinin (both from Sigma Chemical Co., St. Louis, MO) as proteolytic inhibitors. The gray matter was diluted 1:1 with 10% sucrose, homogenized in a blender (Waring, New Hartford, CT) and centrifuged in a GSA rotor at 2,500 rpm (DuPont Co., Wilmington, DE) for 20 min at 4°C. The supernatant was centrifuged in an SS-34 rotor at 7,500 rpm for 20 min at 4°C, the resulting pellet diluted in 10 mM Tris buffer, pH 7.4, and made to 34% sucrose using a 48% sucrose stock solution. This material was overlaid with 28.5% sucrose and centrifuged for 2 h at 22,000 rpm in an SW 27 rotor (Beckman Instruments, Inc., Fullerton, CA). The interface was collected and washed with 10 mM Tris buffer by centrifugation in a Ti 45 rotor at 33,000 rpm for 60 min at 4°C. The yield was 3.2 mg/g of tissue.

Electron microscopical evaluation of the synaptosomal pellet revealed some large mitochondria, however, the subsequent osmotic shock and gradient centrifugation eliminated most of this contamination. In addition the mitochondrial fraction tested negative for the 50-kD antigen. The pellets were treated with 0.5% Triton X-100 to solubilize the membranes. Extracted proteins were separated on a Sephadex G-100 column (1  $\times$  96 cm) using 10 mM Tris buffer, pH 7.4, with 0.5% Triton X-100 and 1 mM EDTA. Fractions of 2 ml were collected and assayed by SDS-PAGE. Protein-containing fractions were assayed by immunoblot with serum from CNS-SLE patients diluted 1:200.

**Electrophoresis and Immunoblotting.** SDS-PAGE was performed according to the method of Laemmli (15). Gradient gels (5–15%) were used and stained with coomassie blue where indicated. Electrophoretic transfer of proteins to nitrocellulose was performed using a transfer apparatus (Bio-Rad Laboratories, Richmond, CA) according to manufacturer's procedure. Nitrocellulose filters (Bio-Rad Laboratories) were fixed in isopropanol and saturated with 0.1% BSA and 0.05% Tween-20 in Tris-buffered saline (TBS), pH 7.5. Filters were incubated at 4°C overnight with human serum diluted 1:200 in TBS containing 0.1% BSA and 0.05% Tween-20, or with CSF diluted 1:2. Control sera were used at dilutions of 1:100. After three washes with TBS containing 0.05% Tween-20, the filters were incubated with peroxidase-conjugated goat anti-human antibody (Boehringer Mannheim Biochemicals, Indianapolis, IN) for 1 h at room temperature diluted with TBS containing 0.1% BSA and 0.05% Tween-20. After three washes with TBS containing 0.05% Tween-20 and one wash with 20 mM Tris and 0.5 M NaCl, pH 7.5 bands were visualized with 4-chloro-1-naphthol (Bio-Rad Laboratories) and 0.015% hydrogen peroxide.

**Serum Fractionation.** Serum from a strongly positive CNS-SLE patient was precipitated with ammonium sulfate at 40% saturation, washed two times, dissolved, and dialyzed overnight against 100 mM Tris buffer, pH 7.8. Ig fractionation was performed by fast performance liquid chromatography (Pharmacia Fine Chemicals, Piscataway, NJ). The dialyzed Ig was loaded on a Mono-Q ion exchange column (Pharmacia Fine Chemicals) equilibrated in the same buffer as above. Fractions were eluted using 15–50% of 0.5 M NaCl in 100 mM Tris buffer, pH 7.8, linear gradient, and dialyzed against PBS immediately.

**Immunofluorescence.** B104 rat neuroblastoma cells (16) were grown on coverslips in RPMI supplemented with 5% FCS and 5% horse serum. Coverslips were coated with poly-D-lysine and fibronectin (Sigma Chemical Co.) for cell adhesion. Confluent B104 cells were rinsed in PBS and fixed in 4% paraformaldehyde for 30 min. Fixed and unfixed cells were washed in PBS/0.1% BSA and incubated with FPLC-purified Ig diluted 1:200 in PBS/0.1% BSA for 2 h at room temperature or with affinity-purified antiserum at XXX. The cells were washed three times with PBS and incubated for 45 min at room temperature with goat anti-human Ig conjugated to fluorescein. After three washes in PBS/0.1% BSA, the cells were rinsed once in PBS and mounted in fluoromount (Lipshaw). Slides were examined under an Axiomat microscope (Carl Zeiss) and photographed on a Kodak TMAX 400 film.

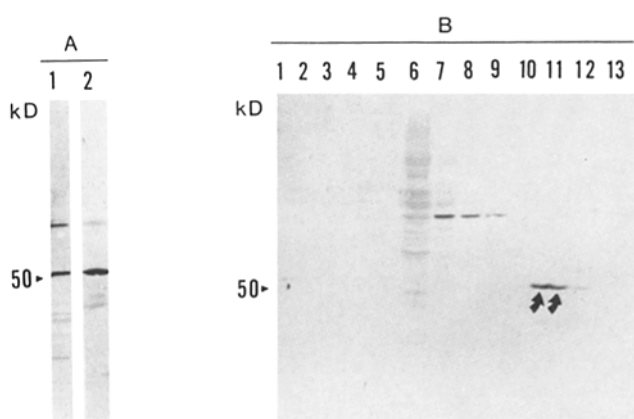
**Absorption of Patients Sera.** Tissues (5 g) from bovine brain, kidney, spleen, heart, and lung were finely diced and then homogenized in a Dounce glass homogenizer (Wheaton Scientific, Millville, NY). Homogenates from each tissue were washed three times with 20 ml of PBS containing 1  $\mu$ M PMSF and aprotinin (both from Sigma Chemical Co.) by centrifugation at 2,000 g at 4°C for 20 min. Each homogenate pellet was resuspended in equal volume of serum for 2 h at 4°C. After centrifugation at 2,000 g for 20 min at 4°C, the resulting supernatant was used as absorbed

serum in Western blot experiments. Up to three consecutive absorptions were performed. For some immunofluorescence experiments, a partially purified 50-kD protein fraction was conjugated to CNBR Sepharose 4B beads. The conjugated protein fraction was combined with an equal volume of FPLC-purified serum, incubated as above and centrifuged at 400 *g* for 5 min. The supernatant was used as controls for immunofluorescence experiments.

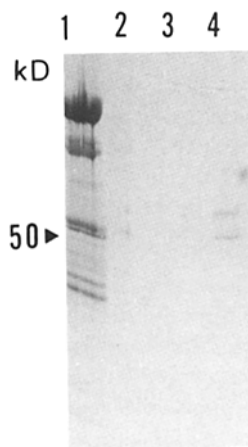
**Affinity Purification of Human Antiserum.** Partially purified preparation of the 50-kD protein was subjected to SDS-PAGE and electrophoretic transfer onto nitrocellulose membrane. The membranes were fixed, washed, and blocked as described above and incubated overnight at 4°C with the serum from a CNS-SLE patient. The membranes were washed and stored. The first lane of each membrane was treated with alkaline phosphatase-conjugated anti-human antibody. This reaction was visualized with a solution of 100 mM Tris, pH 9, 100 mM NaCl, 5 mM MgCl<sub>2</sub>, 0.3 mg/ml nitro blue tetrazolium, and 0.15 mg/ml bromo 4-chloro-3-indoyl phosphate (Pharmacia Fine Chemicals). This reaction was used to demark the 50-kD area on the remaining nitrocellulose membrane. The appropriate area on the remaining nitrocellulose membrane was excised and the bound antibody was eluted by incubation with a small volume of 0.1 M glycine buffer, pH 2.3. The eluted antibody was neutralized with 0.5 M Tris-HCl buffer, pH 8.0, and dialyzed against PBS. Purified antibody was stored in PBS/1% BSA.

## Results

The 50-kD neuronal membrane protein was detected by Western blotting experiments using an extract of bovine brain tissue and sera from SLE patients with CNS abnormalities (Fig. 1 *A*, lane 1). Synaptic plasma membranes showed an enrichment in the 50-kD protein content using the same sera in Western blotting experiments (Fig. 1 *A*, lane 2). The protein appears to be integral to the synaptic plasma membrane, since it could only be solubilized with detergent. Chromatography of a Triton X-100-solubilized synaptic plasma membrane preparation on a G-100 column yields fractions enriched with the 50-kD protein as shown on the Western blot of



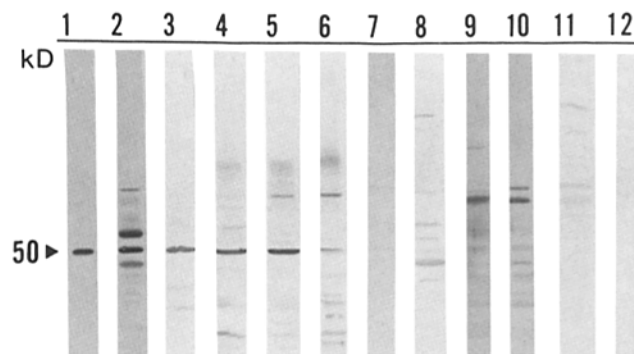
**Figure 1.** (*A*) Immunoblot of brain bovine homogenate (lane 1) and synaptosomal membranes (lane 2) using serum from a CNS-SLE patient. (*B*) Immunoblot of fractions from G-100 column chromatography of solubilized bovine brain synaptic plasma membranes using the serum of the same patient as in *A*. Lanes 1–13 represent the immunoblot of fractions 10–23 of the G-100 column. The 50-kD molecule eluted in fractions 20–22, shown in lanes 10–12.



**Figure 2.** SDS-PAGE and coomassie blue staining of the hydroxylapatite chromatography of fractions 20–22 of the G-100 column show coelution of the 50-kD protein with another protein of 65 kD at 75 mM phosphate concentration (lane 4). Lane 1 has bovine brain-coated vesicles used as protein standard; lanes 2 and 3 represent fractions eluting just before the 50-kD protein.

the SDS-PAGE of these fractions (Fig. 1 *B*, lanes 10–12). These fractions were pooled, and the detergent was removed by repeated concentration and dilution using Centricon filtration system (Amicon Corp., Danvers, MA). After dialysis against 5 mM potassium phosphate buffer, the pooled fractions were chromatographed on a small hydroxylapatite column using a 20–200-mM linear potassium phosphate gradient. The 50-kD protein eluted at 70 mM potassium phosphate concentration. The protein coeluted with a 65-kD protein (Fig. 2, lane 4), and no other proteins were detected in this fraction by coomassie blue stain.

Fractions from the G-100 column chromatography enriched in 50-kD protein content as well as purified synaptic plasma membranes and sera of patients with CNS-SLE, SLE, normal individuals, and patients with unrelated diseases were used in Western blotting experiments to screen for serum binding (representative reactivities are shown on Fig. 3, lanes 1–12) to establish whether the presence of the autoantibody to the 50-kD protein is restricted to patients with CNS-SLE. The



**Figure 3.** Representative immunoblots from the screen of CNS-SLE, SLE patients, patients with unrelated diseases, and normal individuals using bovine brain synaptic plasma membranes. Lanes 1–5 represent immunoblots with serum from subjects with: CNS-SLE with monospecificity to the 50-kD protein (lane 1) and CNS-SLE (lane 2–5). Lane 6 shows a CNS-SLE patient with very low reactivity. Lanes 7, 8, and 10 show representative reactivities of sera from the group of SLE patients without CNS disorders found reactive with the 50-kD protein. Lane 9 shows a patient with rheumatoid arthritis. Lane 11 shows nonreactive sera from patients with SLE, and lane 12 is a normal human serum control.

total study population comprised 100 individuals. Of those, 20 were patients with CNS-SLE, 23 with SLE without known neurological disease, 20 normal individuals, six were patients with rheumatoid arthritis, three with polymyositis, one with rheumatoid vasculitis, and the remaining were patients with unrelated diseases such as progressive systemic sclerosis, schizophrenia, stroke, idiopathic seizure disorders, and paraneoplastic cerebellar degeneration. The 20 patients with CNS-SLE displayed a variety of CNS manifestations ranging from seizures, psychosis, and severe protracted headaches to acute organic brain syndrome, visual blurring, and sensorimotor polyneuropathy (Table 1). In addition to the CNS abnormalities, these patients had active SLE symptoms including skin manifestations, nephritis, pleuropericarditis, and polyarthritis. At the time of sampling of the sera for reactivity with the 50-kD protein, 19 patients had active CNS disease.

**Table 1.** Summary of CNS-SLE Patients Tested Positive for Autoantibody Reactivity with the 50-kD Protein

CNS-SLE Patients		Anti-50-kD reactivity	CNS manifestations
Age	Sex		
yr			
25	F	++	Headache, visual blurring, photophobia
15	F	++	Organic brain syndrome
32	F	++	Seizures, vertigo, headaches
24	M	++	Seizures, nausea, photophobia
29	F	++	Transverse myelitis, intracerebral hemorrhage
67	F	++	Polyneuropathy, acute organic brain syndrome
38	M	++	Psychosis
31	F	+	Seizures, psychosis
30	F	+	Psychosis
39	F	-	h/o* psychosis, depression
18	F	+	Psychosis
24	M	++	Seizures, psychosis
16	F	++	Transverse myelitis
28	F	++	Transient ischemic event, headaches
14	F	+	Transient ischemic event
19	F	++	Pseudotumor cerebri
30	F	++	Psychosis
27	F	+	Cerebrovascular accident
35	F	++	Seizures
24	F	+	Psychosis

Range: strong positive, ++; moderately positive, +; negative, -.  
\* History of.

We found strong autoantibody reactivity in all but one of the patients with active CNS-SLE (Table 2). This patient had a history of CNS-SLE with depression and psychosis, but did not have active disease at the time of blood sampling. None of the normal individuals displayed reactivity with the 50-kD molecule. A low level of reactivity was detected in 35% of the SLE patients without CNS involvement, while the remaining SLE patients did not react with the 50-kD protein. We could not ascertain any particular clinical or serological correlations present in the 35% of the reactive SLE patients. The incidence of positivity in patients with unrelated diseases was <3% and involved a patient with rheumatoid vasculitis and organic brain syndrome. Moreover, in this case the reactivity was very weak.

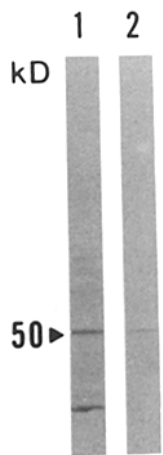
Western blots were also performed using CSF samples from patients with CNS-SLE, SLE, or normal individuals and purified synaptic plasma membranes. CSF samples of five CNS-SLE patients were assayed and showed reactivity with the 50-kD protein. A representative reactivity is shown in Fig. 4, lane 2. Autoantibody reactivity to the 50-kD protein was absent in CSF of normal patients. Two patients with SLE were also tested and displayed no reactivity (not shown).

To determine whether the 50-kD molecule is brain specific, we screened several bovine tissue extracts for the presence of this protein. The serum from a patient with strong reactivity with the 50-kD protein was used in Western blotting with homogenates from brain, spleen, lung, skeletal muscle, heart muscle, liver, and kidney. As shown in Fig. 5 B, the patient's serum reacts strongly with a protein of the same molecular mass in liver (lane 7), kidney (lane 6), spleen (lane 2), and brain (lane 1). Weak reactivity was present in skeletal muscle (lane 4) and lung (lane 3), and no reactivity was found in heart muscle (lane 5). To characterize further the tissue specificity and molecular homogeneity of the 50-kD protein, serum samples from CNS-SLE patients were absorbed with various tissue homogenates before Western blotting on synaptic membrane preparations. Preincubation of serum with brain homogenate was most efficient in removing the serum

**Table 2.** Presence or Absence of Anti-50-kD Reactivity by Diagnosis

Diagnosis	Neuronal 50-kD positivity
CNS-SLE	19/20
SLE without CNS	8/23
Rheumatoid vasculitis with OBS*	1/1
Rheumatoid arthritis	0/6
Polymyositis	0/3
Progressive systemic sclerosis	0/8
Chronic schizophrenia	0/10
Hypertensive cerebrovascular accident	0/3
Idiopathic seizure disorders	0/5
Paraneoplastic cerebellar degeneration	0/1

\* Organic brain syndrome.

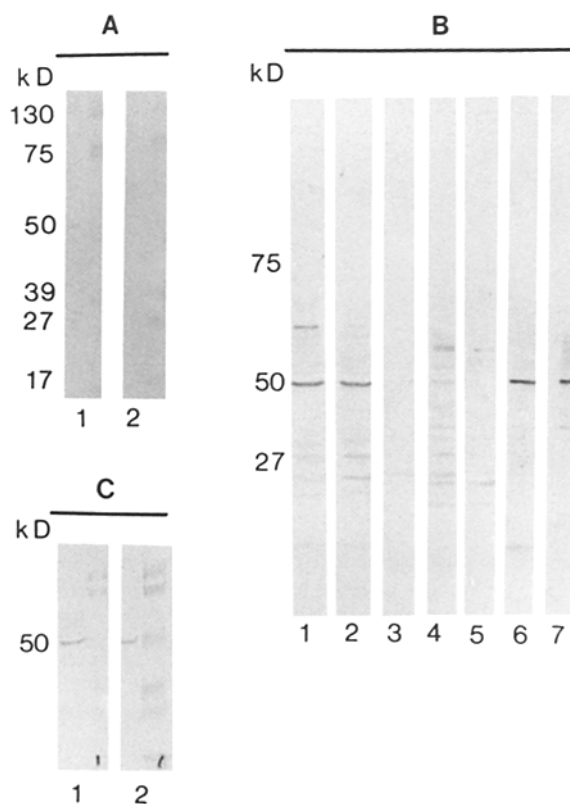


**Figure 4.** Autoantibody reactivity with the 50-kD protein can be detected in the CSF of CNS-SLE patients by immunoblot. Lane 1 represents the immunoblot of bovine brain synaptic membrane preparation with the serum of a CNS-SLE patient. Lane 2 represents the immunoblot of synaptic membrane fractions with the CSF from the same patient.

reactivity in Western blots (Fig. 5 A, lane 2). Serum adsorption with kidney (Fig 5 A, lane 1), liver, and spleen homogenates diminished serum reactivity with brain but could not remove this reactivity completely even after long preincubation times and multiple absorptions.

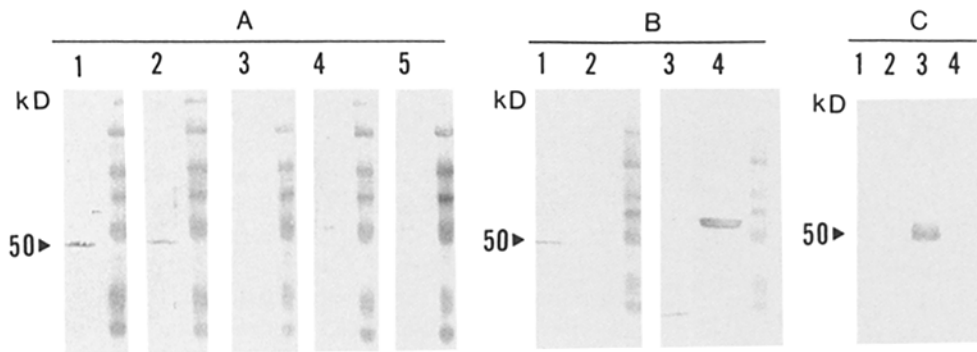
To ascertain that the 50-kD antigen described here is different from other known 50-kD antigens associated with SLE, we studied the crossreactivity of purified antibodies to the 50-kD antigen with the ribonuclear protein La(SS-B) and calreticulin, a protein of identical amino acid sequence with the human Ro(SS-A) antigen. Further, we looked at the crossreactivity of rabbit antisera to calreticulin and well-defined autoantibodies to the Ro and La antigens with our 50-kD antigen preparations. As shown in Fig. 6 A, lanes 3–5, human anti-Ro antisera did not crossreact with our 50-kD antigen preparation, nor did defined anti-La antisera (Fig. 6 C, lanes 1 and 2). In addition, purified anti-50-kD antiserum did not crossreact with calreticulin (Fig. 6 B, lane 3) and anticallreticulin antisera showed no reactivity with our 50-kD antigen preparation.

We examined the surface distribution of the 50-kD molecule using B104 rat neuroblastoma cells and serum from a CNS-SLE patient with strong reactivity to this protein. The Ig fraction from this serum was precipitated with ammo-



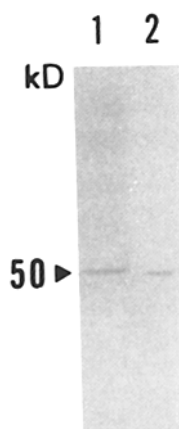
**Figure 5.** (A) Western blot of bovine brain synaptic plasma membrane preparation with serum from a CNS-SLE patient with anti-50-kD reactivity and preabsorbed with kidney (lane 1) and brain (lane 2). Both reactivities are shown adjacent to low molecular mass markers. (B) Western blot of bovine tissue homogenates using serum from a CNS-SLE patient with high antibody titer to the 50-kD protein. Lane 1, brain; lane 2, spleen; lane 3, lung; lane 4, skeletal muscle; lane 5, heart muscle; lane 6, liver; lane 7, kidney. (C) Western blot of bovine brain homogenate using serum from a CNS-SLE patient fractionated by FPLC ion exchange chromatography. The peak fraction containing the 50-kD reactivity is shown in lane 2 adjacent to low molecular mass markers. Lane 1 shows the reactivity of the whole serum adjacent to low molecular mass markers.

onium sulfate and subsequently fractionated by ion exchange chromatography. We obtained an Ig fraction that showed reactivity specific for the 50-kD protein in Western blots of whole bovine brain homogenates (Fig. 5 C, lane 2) as well



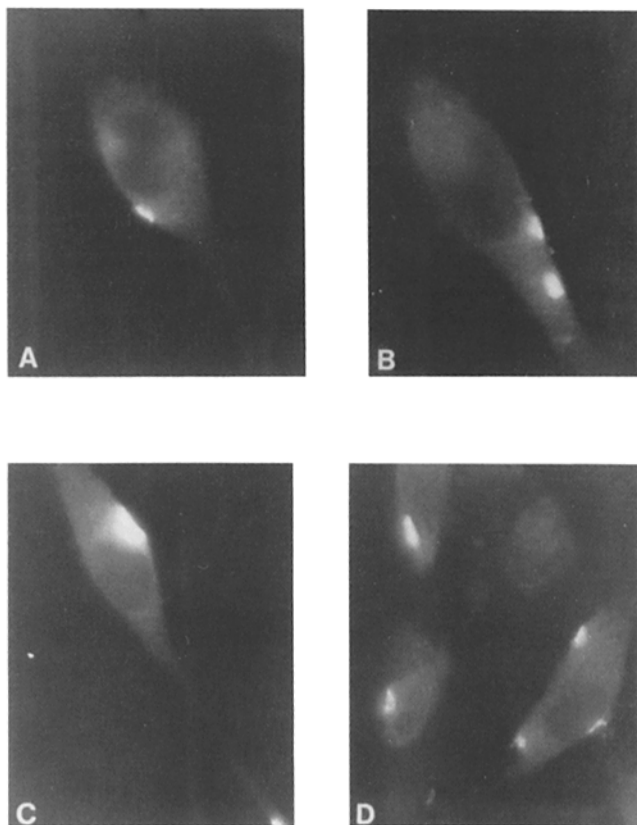
**Figure 6.** (A) Immunoblot of partially purified 50-kD protein from bovine brain synaptic plasma membranes using purified serum from two CNS-SLE patients with positive reactivity (lanes 1 and 2, respectively) and well-defined human antisera to the Ro antigen from three patients with SLE (lanes 3, 4 and 5, respectively). (B) Immunoblot of partially purified 50-kD protein from bovine brain synaptic plasma membranes (lanes 1 and 3) and calreticulin (lanes 2 and 4) using rabbit antiserum to calretic-

ulin (lanes 1 and 2) and purified anti-50-kD antiserum from a patient with CNS-SLE. (C) Immunoblot with a well-defined La antigen-positive serum on partially purified 50-kD protein from bovine brain synaptic plasma membranes (lane 1 and 2), recombinant La antigen (lane 3), and a control bacterial extract (lane 4).



**Figure 7.** Immunoblot of human postmortem fetal brain homogenate (lane 1) and B104 whole cell homogenate (lane 2) using purified serum from a CNS-SLE patient.

as in Western blots of human postmortem fetal brain homogenate (Fig. 7, lane 1) and B104 whole cell homogenate (Fig. 7, lane 2). When prefixed but not permeabilized B104 cells were labeled with this fraction, a distinct macular surface fluorescence was observed consistently (Fig. 8, A-D). Fluorescent patches were seen on the cell body and dendritic projections. Two to three large patches were seen per cell in most preparations. Labeling of unfixed cells gave the same



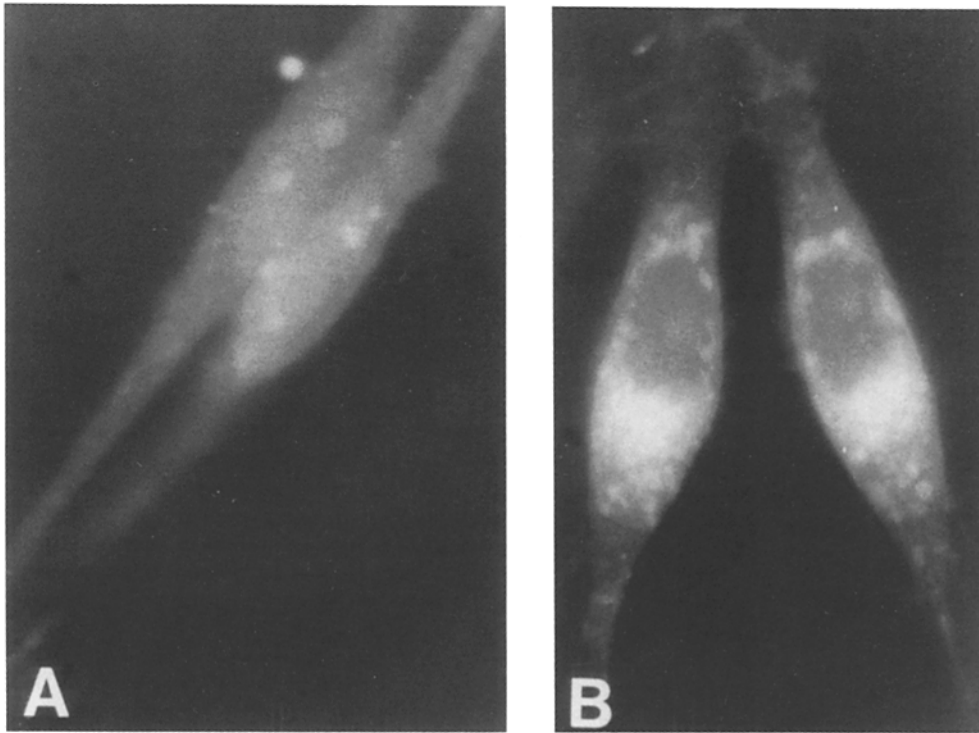
**Figure 8.** Immunofluorescent labeling of B104 neuroblastoma cells with purified anti-50-kD autoantibody from a CNS-SLE patient. One to three patches of intense fluorescence per cell are usually observed.

localization pattern. Absorption of the Ig fraction with purified 50-kD protein before incubation with the B104 cells abolished the macular pattern of surface immunofluorescence (Fig. 9 A). The residual immunofluorescence seen in cells treated with absorbed autoantibody is nonspecific as it occurs in cells treated with normal human serum as well. Cells labeled with serum from SLE patients without CNS disease exhibited (not shown) nondescript diffuse surface fluorescence. To examine the intracellular distribution of this protein, B104 cells were stained with purified Ig from a CNS-SLE patient after cell membrane permeabilization with Triton X-100. The observed immunofluorescence was in the perinuclear area and confined to granular-vesicular organelles (Fig. 9 B). Cell nucleus and nucleoli were not stained. Immunofluorescent localization of the 50-kD antigen was also performed with affinity-purified anti-50-kD autoantibody from a CNS-SLE patient. Affinity-purified antibody was obtained by elution from the 50-kD band in Western blots as described in Materials and Methods. The reactivity of the affinity-purified antibody in Western blot is shown in Fig. 10, lane 3. The immunofluorescent localization pattern of this antibody (Fig. 11, A and B) is essentially the same as stated above. Absorption of affinity-purified anti-50-kD antibody with partially purified 50-kD protein preparation removed the macular surface immunofluorescence (Fig. 11 C).

### Discussion

We have found that an antibody to a 50-kD neuronal cell surface antigen appears in patients with active CNS disease secondary to SLE. In addition, we showed that purified Ig from the sera of these CNS-SLE patients react with the 50-kD antigen derived from fetal human brain. This lends support to the postulate that the reactive antibody in the sera from CNS-SLE patients is an autoantibody by ruling out the possibility of a heteroantibody interaction. Further support of this postulate is the improbability of alloantibody generation in most patients with CNS-SLE in our study, as this group of patients did not include transfused subjects and the majority of the patients were males or females without prior pregnancy record. The strongest indication of autoantibody reactivity against the 50-kD antigen, however, is the selective patient population in which it occurs. The incidence of positive immunoreactivity in 95% of the CNS-SLE patients studied is as high as the one made by Bonfa and Elkon (12) for the association of autoantibodies to the P protein with lupus psychosis. The finding that 35% of unselected SLE patients showed low levels of serum reactivity with the 50-kD antigen could conceivably identify a group of SLE patients with subclinical CNS disease. Omdal et al. (17) reported that 25 of 30 randomly selected SLE patients (83%), studied retrospectively, showed mild neuropsychiatric manifestations such as migraine and protracted headache, and concluded that clinical evaluation often failed to identify such disorders.

In the course of our work we observed that the reactivity of autoantibodies to the 50-kD protein is stronger in patients with severe neurological disorders such as seizures. The level of autoantibodies to the 50-kD antigen may become an indi-



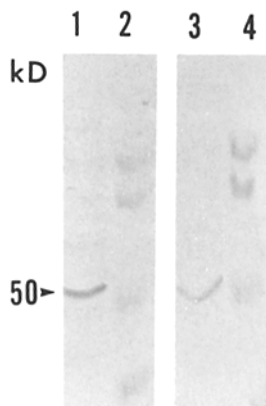
**Figure 9.** (A) Immunofluorescent labeling of B104 neuroblastoma cells with anti-50-kD autoantibody preabsorbed with partially purified 50-kD protein. (B) Immunofluorescent labeling of permeabilized B104 neuroblastoma cells with purified anti-50-kD autoantibody.

cator for the severity of the CNS disease since the only CNS-SLE patient that showed no reactivity to the antigen was in remission at the time of blood sampling. Future long-term longitudinal studies will address this possibility.

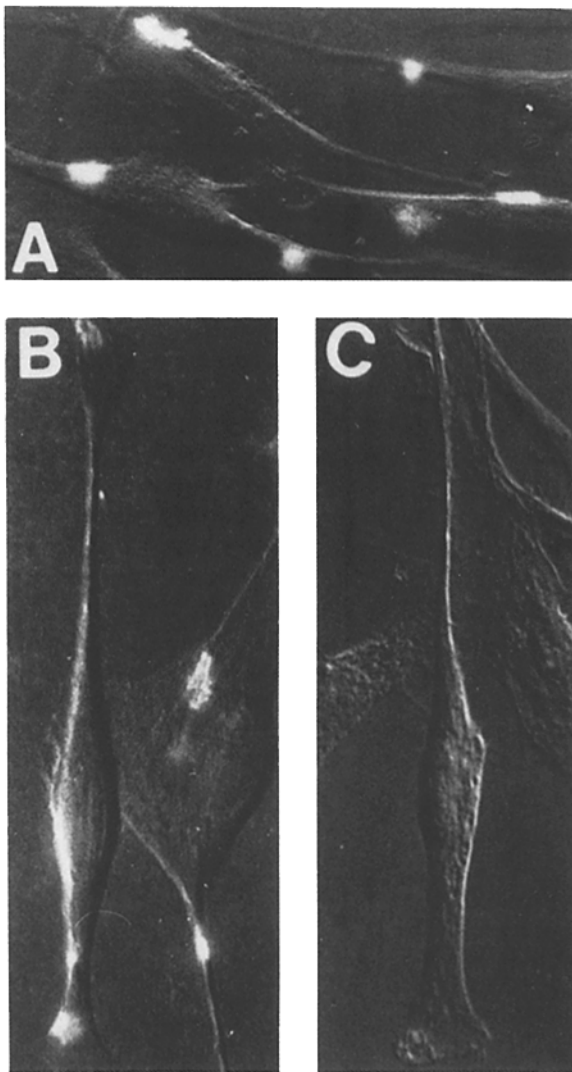
The 50-kD protein is an autoantigen present on the surface of neuronal cells. Although the identity of the antigen is unknown at the present, it appears to be distinct from previously described intracellular antigens, including Ro, Sm-RNP, and the ribosomal protein (12, 18). The molecular mass of 50-kD coincides with that of the 50-kD protein part of the La (SS-B) small ribonucleoprotein found to complex with RNA polymerase III transcripts (19). However, the immunofluorescent localization pattern of the 50-kD antigen described here differs from that of the La antigen insofar that the latter is exclusively a nuclear antigen whereas the former shows cell surface membrane localization and it is absent from

the nucleus of the tested cell types. Anti-La antibody-positive patients have been reported to have a later age of disease onset, low frequency of lupus nephritis (20), and no associations of anti-La reactivity with CNS disease (2). Furthermore, we have shown that well-defined La positive serum does not react with the 50-kD synaptic membrane protein. The anti-50-kD antibodies do not crossreact with rabbit calreticulin, a protein of identical amino acid sequence, as the human Ro/SS-A antigen (21), which is often a target for autoantibodies in SLE patients. Alternatively, well-defined human antisera to the Ro antigen did not crossreact with the neuronal 50-kD antigen. Reactivities of rabbit antisera raised against whole brain homogenates to surface molecules of similar size (53 kD) have been reported in the A<sub>2</sub> neuroblastoma cell line (22). Absorption studies show that the 53-kD A<sub>2</sub> neuroblastoma antigen is a brain-specific antigen. Although liver, spleen, and kidney were inefficient in absorbing the 50-kD reactivity from CNS-SLE sera in the present study, the clear reactivity of these sera with the same tissues by Western blots excludes the possibility of neuronal specificity for the 50-kD protein. The 50-kD antigen appears, therefore, different from the 53-kD brain antigen identified in A<sub>2</sub> neuroblastoma cells.

The 50-kD antigen is a distinct neuronal membrane protein common for brain and neuroblastoma cells. Neuroblastoma cell lines have commonly been used to identify brain-reactive antibodies in SLE in attempts to aid diagnosis and to explain the pathogenesis in CNS-SLE (23). However, no clear associations have been shown so far. How et al. (24) demonstrated that 43% of CNS-SLE patients show reactivity with unidentified target antigens in neuroblastoma cell lines compared with 14% of unselected SLE patients. A later study of Hanly et al. (10) identified the first target antigen in neu-



**Figure 10.** Immunoreactivity of serum from a patient with CNS-SLE in a Western blot of brain homogenate (lane 1). The immunoreactivity of the same serum after affinity purification is shown in lane 3. Lanes 2 and 4 are low molecular mass markers.



**Figure 11.** (A and B) Immunofluorescent labeling of B104 neuroblastoma cells with affinity-purified anti-50-kD autoantibody from a CNS-SLE patient. Immunofluorescence image is combined with differential interference microscopy image for better visualization of the immunofluorescent localization pattern. (C) Immunofluorescent labeling of B104 neuroblastoma cells with affinity-purified anti-50-kD autoantibody preabsorbed with partially purified 50-kD protein.

roblastoma cells as a 97-kD protein of surface origin that can be precipitated by select SLE sera. In their study, two of the three patients whose sera precipitated the 97-kD antigen had overt neuropsychiatric manifestations. Although their findings suggest that the antigen may be associated with CNS-SLE, the study population was too small and a clear clinical correlation was not established. In the course of our studies we found that some CNS-SLE sera also reacted with an antigen of  $\sim 100$  kD in Western blots on synaptic plasma membranes, but the reactivity was inconsistent (data not shown). At present there is no published evidence that the 97-kD target antigen in neuroblastoma cells is also present in normal brain cells, therefore, its relevance to the mechanism of CNS-SLE remains unknown.

The 50-kD target protein described here could possibly be a clinically relevant antigen for two reasons: (a) the presence of anti-50-kD autoantibody correlates with CNS manifestations in SLE patients; and (b) the antigen is expressed on the membrane of synaptic endings, a neuronal compartment vital for normal brain function. The presence of this antigen in nonneuronal tissues offers the possibility that it is a shared antigen and may reflect similar properties and gene expression in these tissues. In addition, the presence of the 50-kD antigen in kidney, liver, and spleen may be indicative of a pattern of sensitization that occurs initially in the non-neuronal environment and causes systemic accumulation of autoantibodies. As previously proposed, the onset of CNS disease may be coupled with brain barrier dysfunction, which may lead to the ingress of the systemically accumulated autoantibody into the CSF (23). Subsequently the binding of the autoantibodies to nerve endings could impose impairment of neuronal function. It has been postulated that immune response to brain autoantigens can also be initiated intrathecally, and in fact, increased polyclonal B cell activation has been observed within the CNS in patients with CNS-SLE (25). Based on our Western blot experiments using CSF, we suggest that autoantibodies to the 50-kD antigen may gain access to the CNS. The high serum autoantibody titer of the CNS-SLE patients tested in this study, however, favors the possibility that the initial immune response resides in the systemic circulation. Future studies will address the presence of autoantibodies to the 50-kD antigen in the CSF of CNS-SLE patients and their respective titers.

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