Antiribosomal P Protein Antibodies in Cerebrospinal Fluid Are Associated with Neuropsychiatric Systemic Lupus Erythematosus

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ABSTRACT. ***Objective.*** To investigate whether antiribosomal P protein antibodies (anti-P) are present in the cerebrospinal fluid (CSF) of patients with systemic lupus erythematosus (SLE), and if presence of anti-P in CSF is more strongly related to the appearance of neuropsychiatric SLE (NPSLE) than anti-P in serum.

Methods. CSF and serum samples from 70 patients with SLE were used. Patients were divided into 4 groups: 21 patients with neurologic syndromes of the central nervous system (CNS); 19 patients with diffuse psychiatric/neuropsychological syndromes; 10 patients with complex presentations (neurologic syndromes of the CNS plus diffuse psychiatric/neuropsychological syndromes); and 20 patients without NPSLE based on diagnostic criteria for 19 NPSLE syndromes proposed by the American College of Rheumatology. IgG anti-P in CSF and serum samples were detected by Western blotting using rat liver ribosomes. Inhibition assay was performed using 5 anti-P-positive CSF samples preincubated with synthetic ribosomal P peptide. Western blotting results were compared with those from ELISA with synthetic ribosomal P peptide as antigen. The association of CSF and serum anti-P with NPSLE was analyzed.

Results. CSF and serum IgG anti-P by Western blotting were detected, respectively, in 20 (28.6%) and 32 (45.7%) of 70 patients. The presence of IgG anti-P by Western blotting in the CSF was supported by positive results in the inhibition assay and significant association with CSF IgG anti-P titers by ELISA. The frequency of CSF anti-P by Western blotting in SLE patients with serum anti-P was significantly higher than in SLE patients without serum anti-P (18/32 vs 2/38; $p < 0.001$). The frequency of CSF anti-P by Western blotting in patients with NPSLE was significantly higher than in patients without NPSLE (19/50 vs 1/20; $p < 0.01$). The frequency of CSF anti-P by Western blotting in the group with complex presentations (10/10) was significantly higher than in the other 3 groups [neurologic syndromes of CNS (5/21); diffuse psychiatric/neuropsychological syndromes (4/19); and patients without NPSLE (1/20)] ($p < 0.001$). The frequency of serum anti-P by Western blotting in patients with NPSLE was not significantly higher than in patients without NPSLE (25/50 vs 7/20; $p = 0.192$).

Conclusion. These results suggest that the presence of IgG anti-P in CSF of SLE patients may be involved in the appearance of NPSLE, especially in complex presentations. Measurement of IgG anti-P in CSF by Western blotting may be more useful for diagnosis of NPSLE than measurements in serum. (J Rheumatol 2005;32:34–9)

Key Indexing Terms:
ANTIRIBOSOMAL P PROTEIN ANTIBODIES
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CEREBROSPINAL FLUID
NEUROPSYCHIATRIC SYSTEMIC LUPUS ERYTHEMATOSUS
NEUROLOGIC SYNDROMES OF THE CENTRAL NERVOUS SYSTEM
DIFFUSE PSYCHIATRIC/NEUROPSYCHOLOGICAL SYNDROMES

In 1987, Bonfa, *et al* found that serum antiribosomal P protein antibodies (anti-P) were highly specific for lupus psychosis[1]. However, 7 subsequent studies of serum anti-P have yielded conflicting results[2-8]. It has not yet been elucidated whether serum anti-P contribute to the appearance and development of neuropsychiatric systemic lupus erythematosus (NPSLE) including psychiatric disease.

In addition, an association of anti-P in the cerebrospinal fluid (CSF) with NPSLE remains uncertain. Golombek, *et al* detected anti-P in the CSF of all 4 of their patients with lupus psychosis[9]. However, 5 other independent studies found no significant presence of anti-P in CSF[1,2,7,10,11], although a few patients with NPSLE had CSF anti-P[10,11]. We measured anti-P in the CSF from patients with SLE by Western blotting using rat liver ribosomes as a source of antigen, and analyzed whether the presence of anti-P in CSF could be more strongly associated with NPSLE than anti-P in the serum.

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MATERIALS AND METHODS

Patients. Seventy patients with SLE (63 women, 7 men; mean ± SD age 32.3 ± 13.7 yrs, range 13–71 yrs) who were hospitalized in Jichi Medical School Hospital between March 1989 and August 2001 and from whom samples of both CSF and serum were obtained almost simultaneously entered this study. All patients fulfilled the American College of Rheumatology (ACR) 1982 revised criteria for the classification of SLE[12]. The diagnoses of NPSLE were made by neurologists and psychologists based on the diagnostic criteria for 19 NPSLE syndromes proposed by the ACR[13]. Seventy patients with SLE were divided into 4 groups as shown in Table 1. In this study neurologic syndromes of the peripheral nervous system were not included in NPSLE. A complex presentation (the third group) was defined as having both neurologic syndromes of the central nervous system (CNS) and diffuse psychiatric/neuropsychological syndromes occurring concurrently in a patient. The fourth group comprised 20 patients without evidence of NPSLE, including one with polyneuropathy, one with bacterial meningitis, one with mumps, one with postherpetic neuralgia, one with Lance-Adams syndrome, one with psychiatric disease due to thrombotic thrombocytopenic purpura, one with suspected metastatic meningitis, 2 with hysteria, and 11 without abnormality.

Western blotting for detection of anti-P. IgG anti-P were detected by Western blotting using ribosomes as antigen sources. Ribosomes were purified from rat liver as described[1]. Polyacrylamide gel electrophoresis was performed using the discontinuous buffer system described by Laemmli[14] in the presence of 0.1% sodium dodecyl sulfate. Western blotting was performed as described[15]. Undiluted CSF and 1:20 diluted serum samples in phosphate buffered saline (PBS) were applied to strips, and anti-P were defined as positive when at least one band of 3 ribosomal P proteins P0 (38 kDa), P1 (19 kDa), or P2 (17 kDa) was detected.

Synthetic peptide corresponding to the carboxyl-terminal 22 amino acids of the human ribosomal P0 protein (ribosomal P peptide). Ribosomal P peptide was synthesized using a solid-phase method (Peptide Institute Inc., Minoh, Japan) as described[16]. The purity of the peptide was confirmed as 93.7% by analytic high performance liquid chromatography, amino acid analysis, and microsequencing. The synthetic ribosomal P peptide was conjugated to bovine serum albumin (BSA; Sigma, St. Louis, MO, USA) at a 11:1 molar ratio using glutaraldehyde as described[17].

Inhibition assay. CSF samples were mixed with equal volume of the ribosomal P peptide solution (1 mg/ml) or PBS alone and incubated at 4°C overnight. CSF samples were finally diluted 1:2 with this mixture. Western blotting was performed after incubation using these mixed CSF samples.

ELISA for detection of anti-P. IgG anti-P titers were measured by ELISA using ribosomal P peptide-BSA conjugate as antigen. Wells of 96-well microtiter plates (Nunc immuno module F8 maxisorp; Nunc, Roskilde, Denmark) were coated with 100 µl of ribosomal P peptide-BSA conjugates at 5 µg/ml of ribosomal P peptide in PBS at 4°C overnight. The wells were blocked with Block Ace (Dainippon Pharmaceutical, Osaka, Japan) diluted 1:4 with distilled water for 2 h at room temperature. Before being added to the ribosomal P peptide-BSA conjugate-coated wells, CSF and serum samples were diluted 1:2.5 and 1:200, respectively, in PBS containing 1% BSA. The following procedure was performed as described[8]. The values for these serum and CSF samples were determined from a standard curve using the high-titer positive serum as described[8] and expressed as arbitrary units/ml and arbitrary CSF units/ml, respectively.

Statistical analysis. Proportions of positive tests in different groups were compared by Fisher's exact test. Differences between groups were compared by the nonparametric Mann-Whitney U test.

RESULTS

Frequency of IgG anti-P in serum and CSF samples. CSF and serum anti-P were detected, respectively, in 20 (28.6%) and 32 (45.7%) of the 70 patients. Figure 1 shows the representative Western blotting data using CSF and serum samples from 9 patients with SLE. Both serum and CSF samples from 4 patients with SLE (lanes 1–4) showed the positive bands. Serum samples but not CSF samples from 2 patients with SLE (lanes 5, 6) showed positive bands. Neither serum nor CSF from one patient with SLE (lane 7) showed positive bands. CSF samples from 2 patients with SLE (lanes 8, 9) showed positive bands, but serum samples did not. All anti-P-positive

Table 1. NPSLE manifestations of 70 SLE patients and frequency of serum and CSF IgG anti-P by Western blotting (WB) in 4 different patient groups. Of 20 patients positive for CSF IgG anti-P by Western blotting, 18 had serum IgG anti-P by Western blotting (one had seizure disorders + acute confusional state and one had transverse myelopathy + acute confusional state).

Patient Group	No. (%) of Patients Positive for Serum IgG Anti-P by WB	No. (%) of Patients Positive for CSF IgG Anti-P by WB
1. Neurologic syndromes of the CNS, n = 21	9 (42.9)	5 (23.8)
Aseptic meningitis, n = 6	3 (50.0)	3 (50.0)
Cerebrovascular diseaes, n = 10	4 (40.0)	0 (0)
Headache, n = 2	0 (0)	0 (0)
Seizure disorders, n = 3	2 (66.7)	2 (66.7)
2. Diffuse psychiatric/neuropsychological syndromes, n = 19	8 (42.1)	4 (21.1)
Acute confusional state, n = 1	0 (0)	0 (0)
Anxiety disorder, n = 7	3 (42.9)	2 (28.6)
Mood disorder, n = 4	2 (50.0)	0 (0)
Psychosis, n = 7	3 (42.9)	2 (28.6)
3. Complex presentations, n = 10	8 (80.0)	10 (100)
Seizure disorders + acute confusional state, n = 6	5 (83.3)	6 (100)
Seizure disorders + psychosis, n =1	1 (100)	1 (100)
Transverse myelopathy + acute confusional state, n = 2	1 (50.0)	2 (100)
Transverse myelopathy + anxiety disorder, n = 1	1 (100)	1 (100)
4. Patients without NPSLE, n = 20	7 (35.0)	1 (5.0)

NPSLE: neuropsychiatric systemic lupus erythematosus; anti-P: antiribosomal P protein antibodies.

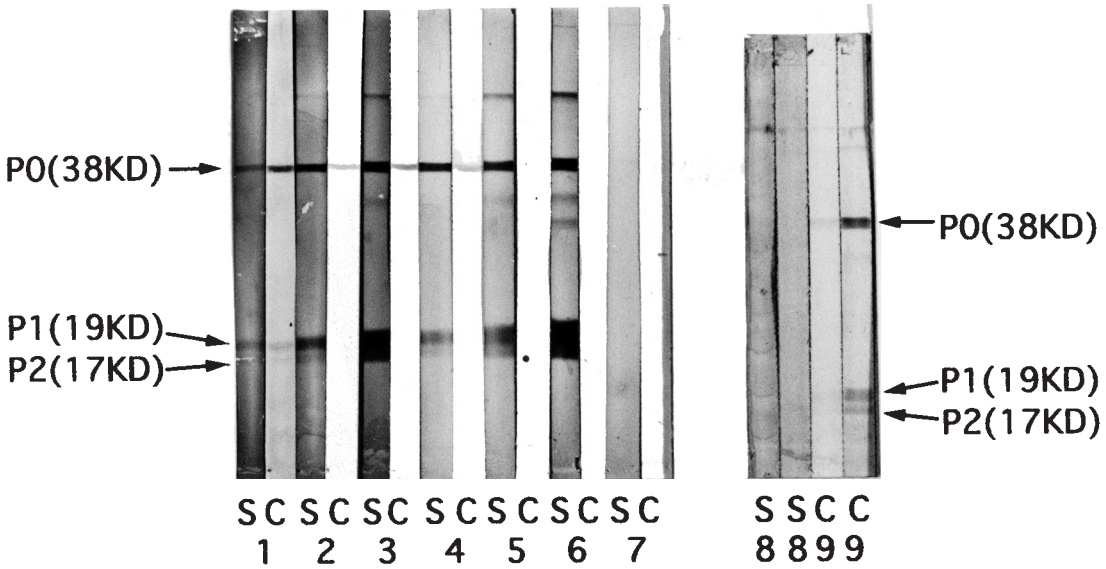

Figure 1. Western blotting analysis for serum and CSF IgG antiribosomal P protein antibodies in 9 patients with SLE (lanes 1–9). P0: ribosomal P0 protein; P1: ribosomal P1 protein; P2: ribosomal P2 protein; S: serum; C: cerebrospinal fluid.

serum and CSF samples clearly showed bands corresponding to ribosomal P protein P0 (38 kDa). Of 32 patients positive for serum anti-P, 18 (56.3%) had CSF anti-P. Of 38 patients negative for serum anti-P, 2 (5.26%) had CSF anti-P (Figure 1). These 2 patients showed complex presentations (one with seizure disorders and acute confusional state, the other with transverse myelopathy and acute confusional state) of NPSLE (Table 1). The frequency of CSF anti-P by Western blotting in patients positive for serum anti-P was significantly higher than that in patients who were negative for serum anti-P ($p < 0.001$; sensitivity of 56.3% and specificity 94.7%; κ coefficient 0.49).

Inhibition assay (Figure 2). To determine whether bands corresponding to 38, 19, or 17 kDa were due to binding of anti-P in the CSF, an inhibition assay was performed using anti-P-positive CSF samples from 5 patients with SLE by Western blotting. Preincubation of all 5 CSF samples with PBS alone showed bands corresponding to 38, 19, or 17 kDa in Western blotting of rat liver ribosomes as shown in "negative" lanes (denoted "–"). But the bands corresponding to 38, 19, or 17 kDa were completely abrogated, as shown in the positive lanes ("+"), when these 5 CSF samples were preincubated with synthetic ribosomal P peptide. These results indicate that the bands corresponding to 38, 19, or 17 kDa in Western blotting of rat liver ribosomes are due to the binding of anti-P in the CSF.

Relationship between IgG anti-P by Western blotting and antibody titers by ELISA (Figure 3). The mean titer of CSF IgG anti-P in the controls (n = 17) from whom CSF samples did not show bands corresponding to 38, 19, or 17 kDa in Western blotting was 1.46 ± 0.81 CSF units/ml. A titer > 3 SD above the mean (3.89 CSF units/ml) was considered a positive result. The mean titer of serum IgG anti-P in the controls (n = 28) from whom serum samples did not show bands corresponding to 38, 19, or 17 kDa in Western blotting was 5.60 ± 3.14 units/ml. A titer > 3 SD above the mean (15.0 units/ml) was considered a positive result.

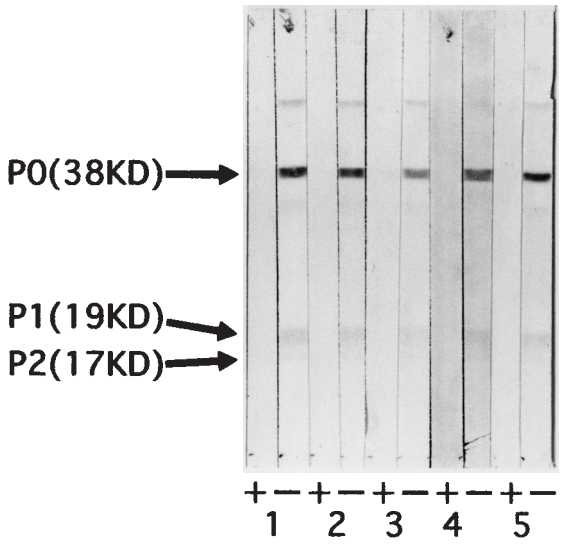

Figure 2. The reactivity of CSF IgG antiribosomal P protein antibodies from 5 patients with SLE (lanes 1–5) after incubation with PBS alone (lanes –) and the synthetic ribosomal P peptide (lanes +). Before Western blotting CSF samples were incubated with PBS alone or the synthetic ribosome P peptide (final concentration 500 µg/ml) at 4°C overnight. P0: ribosomal P0 protein; P1: ribosomal P1 protein; P2: ribosomal P2 protein.

The mean titer of CSF IgG anti-P by ELISA (23.2 ± 31.5 CSF units/ml) in 20 patients positive for CSF anti-P by Western blotting was significantly higher than that (1.99 ± 1.69 units/ml) in 50 patients negative for CSF anti-P by Western blotting ($p < 0.001$).

The mean titer of serum IgG anti-P by ELISA (51.7 ± 35.9

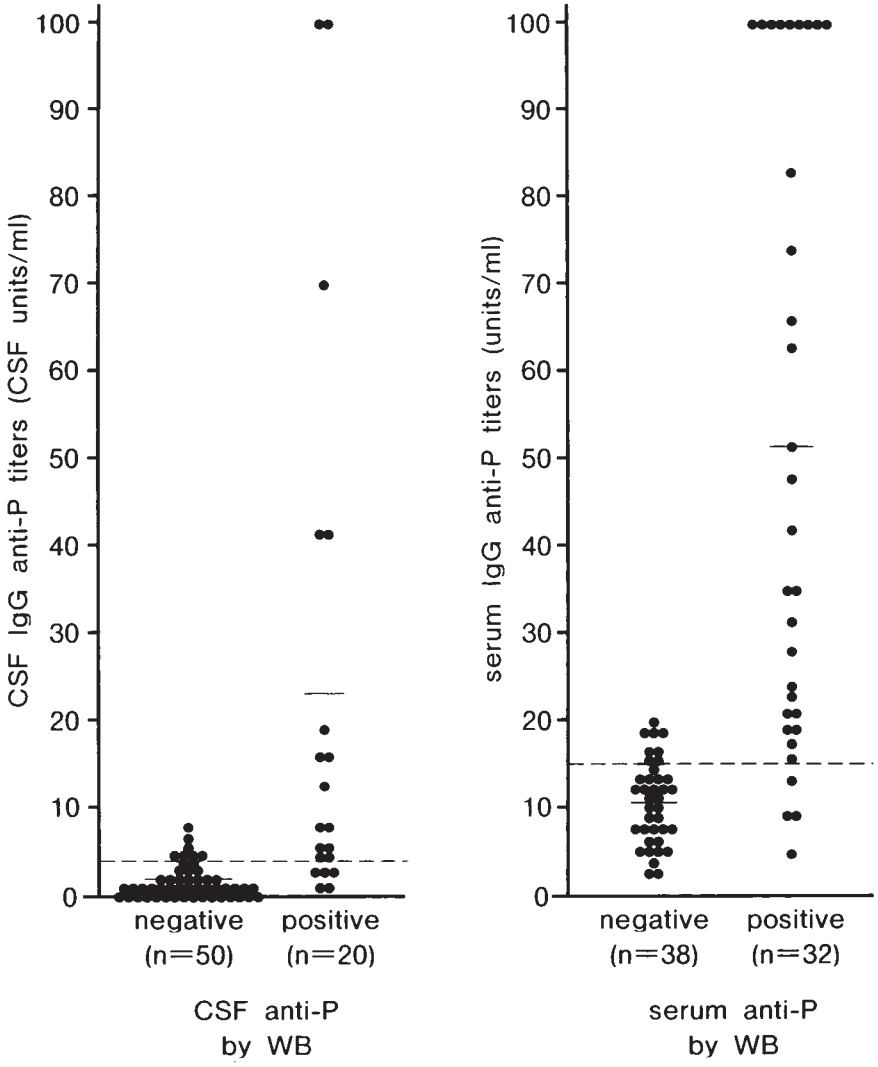

Figure 3. CSF and serum IgG antiribosomal P protein antibody titers by ELISA using synthetic ribosomal P peptide in 70 patients with SLE. Left panel shows CSF IgG anti-P titers by ELISA in 20 patients positive and 50 patients negative for CSF anti-P protein antibodies by Western blotting (WB). Right panel shows serum IgG anti-P titers by ELISA in 32 patients positive and 38 patients negative for serum anti-P by Western blotting. Horizontal bars represent the mean titers in each group. Horizontal broken lines represent the mean titers + 3 SD of CSF or serum anti-P in controls.

units/ml) in 32 patients positive for serum anti-P by Western blotting was significantly higher than that (10.6 ± 4.71 units/ml) in 38 patients negative for serum anti-P by Western blotting ($p < 0.001$).

These results suggest that the binding of IgG anti-P to ribosomal P protein in Western blotting is due to the quantification of IgG anti-P.

Relationship between IgG anti-P detected by Western blotting in serum and CSF samples and NPSLE (Table 1). Of 50 patients with NPSLE, 25 patients (50.0%; 9 with neurologic syndromes of the CNS, 8 with diffuse psychiatric/neuropsychological syndromes, and 8 with complex presentations) had serum anti-P by Western blotting. Of 20 patients without NPSLE, 7 (35.0%; one with postherpetic neuralgia and 6 with no abnormality) had serum anti-P by Western blotting. The frequency of serum anti-P by Western blotting in patients with NPSLE was not significantly higher than that in patients without NPSLE ($p = 0.192$; sensitivity of 50.0% and specificity 70.0%; κ coefficient 0.134).

Of 50 patients with NPSLE, 19 (38.0%; 5 with neurologic syndromes of the CNS, 4 with diffuse psychiatric/neuropsychological syndromes, 10 with complex presentations) had CSF anti-P by Western blotting. Of 20 patients without NPSLE, one with no abnormality (5.0%) had CSF anti-P by Western blotting. The frequency of CSF anti-P by Western blotting in patients with NPSLE was significantly higher than that in patients without NPSLE ($p < 0.01$; sensitivity of 38.0% and specificity 95.0%; κ coefficient 0.313). All of 10 patients

with complex presentations had CSF anti-P by Western blotting and the frequency of CSF anti-P by Western blotting in this group was significantly higher than in the other 3 groups ($p < 0.001$; sensitivity 100% and specificity 83.3%; κ coefficient 0.542). These results suggest the possibility that the presence of anti-P in the CSF may be associated with the appearance of NPSLE, especially in patients with complex presentations.

DISCUSSION

In the study by Golombek, *et al* all 4 patients with lupus psychosis had increased CSF anti-P[9]. When paired serum and CSF samples were diluted to the same IgG concentrations and used for Western blotting, there was a selective enrichment of IgG anti-P in the CSF in only one patient[9]. Further, one patient with seizures had CSF anti-P by Western blotting[9]. In the study by Schneebaum, *et al*, CSF samples were obtained from 8 patients with raised serum IgG anti-P levels as well as active NPSLE (2 with psychosis, 4 with depression, 2 unspecified), but there was no increase in levels of IgG anti-P in CSF in any of the 8 patients[2]. Thus the authors suggested that the absence of detectable anti-P in the CSF of these patients was due to the binding of the antibody to nerve tissues[2]. In the study by Teh, *et al* IgG anti-P were not detectable in the single sample of CSF, despite a considerable elevation within the serum level (177 units/ml) in the reference-positive patient[7]. Teh also suggested the same possibility that Schneebaum, *et al* did[18]. In these 3 studies[2,7,9] a small number of the patients in whom CSF IgG anti-P were measured might be due to the low frequencies of CSF IgG anti-P. As well, the concentrations of anti-P per milligram of IgG in the CSF may be less than concentrations in sera from patients with SLE. However, in a study by Isshi and Hirohata, CSF IgG anti-P could be detected in only 3 of the 41 patients with NPSLE by ELISA with synthetic peptide corresponding to the carboxyl-terminal 22 amino acids of the *Artemia salina* ribosomal P2 protein as antigen using undiluted CSF[10]. More recently CSF IgG anti-P were detected in only 5 of the 39 patients by ELISA with 3 ribosomal P proteins (P0, P1, and P2) purified from bovine and rabbit thymus using undiluted CSF[19]. The reasons for the discrepant frequency of CSF IgG anti-P between our study and these other studies[10,19] are unclear.

It is also unclear whether anti-P are produced only outside the CNS and enter the CSF as a result of damage to the blood-brain barrier or are produced locally. Our study showed that 2 patients with complex presentations without serum anti-P had CSF anti-P by Western blotting, supporting the possibility that anti-P may be produced locally in the CSF. However, ribosomal P antigens have recently been described on the surface of endothelial cells[19]. In addition, we have confirmed that purified IgG anti-P derived from patients with SLE activate human umbilical vein endothelial cells, resulting in the increase of interleukin 6 (IL-6) production (unpublished observation). In our study the frequency of CSF anti-P by Western blotting in patients positive for serum anti-P was significantly higher than that in SLE patients negative for serum anti-P. These results raise the possibility that serum anti-P may bind to ribosomal P antigens on the endothelial surface in the brain and activate endothelial cells, leading to entry of serum anti-P into the CSF by disruption of the blood-brain barrier and the activation of intrathecal B lymphocytes by IL-6 production. Furthermore these B lymphocytes may produce anti-P in the CSF.

Koren, *et al* have observed the presence of ribosomal P antigens on the surface of neuronal cells, and suggested that the binding of anti-P to neuronal cells might participate in the development of lupus psychosis by altering neuronal function directly[20]. In our study anti-P was present not only in the CSF of patients with lupus psychosis but also those with different NPSLE manifestations. In particular, anti-P were present in the CSF of all 10 patients who had both neurologic syndromes of the CNS and diffuse psychiatric/neuropsychological syndromes occurring concurrently as complex presentations. Thus, anti-P alone may not be sufficient to cause NPSLE. Other factors such as immune complexes, complements, cytokines (IL-6 and IL-8), and autoantibodies to neuronal cells may be important. Whether anti-P in the CSF bind directly to neuronal cells and how anti-P participate in the neuronal damage and dysfunction leading to NPSLE are issues that need to be elucidated.

IgG anti-P were present in the CSF of patients with SLE, and the presence of anti-P in CSF was associated with the appearance of NPSLE, especially complex presentations. The presence of IgG anti-P in the serum did not show a significant relation. The measurement of IgG anti-P in CSF samples by Western blotting may be clinically more useful in the diagnosis of NPSLE than measurement in serum samples.

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