Lack of Clinical Association with Antibodies to Ribosomal P Proteins in Indian Patients with Systemic Lupus Erythematosus

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ABSTRACT. ***Objective.*** We examined the prevalence and clinical association of the antiribosomal antibodies in our cohort of patients with systemic lupus erythematosus (SLE).

Methods. IgG antiribosomal P protein (anti-P) antibodies were detected in 202 consecutive patients with SLE and 212 age and sex matched healthy subjects by an in-house ELISA, using the 22-mer C-terminal peptide. In 13 patients, IgG anti-P antibodies were also tested in paired cerebrospinal fluid (CSF) and sera samples. Clinical variables were compared in the antibody-positive and negative groups using appropriate statistical tests.

Results. Of the 202 patients, 15 were male. Their median age was 30 years and the median disease duration was 36 months. Thirty-one patients (15.35%) were positive for IgG anti-P antibodies, of which 24 were also positive by Western blot. No association with SLE Disease Activity Index, nervous system disease, nephritis, hepatitis, skin disease, arthritis, and juvenile onset disease could be demonstrated. Levels of IgG anti-P antibodies in CSF were 100-fold less compared to levels in serum, and correlated well with the latter ($r = 0.86$; $p < 0.01$).

Conclusion. The prevalence of IgG anti-P antibodies is similar in Indian and Caucasian patients with SLE. No association with specific organ involvement or age at onset could be demonstrated. (J Rheumatol 2006;33:1987–9)

Key Indexing Terms:
CONNECTIVE TISSUE DISEASE
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Antibodies to ribosomal P proteins (anti-P), directed against P_0, P_1, and P_2 proteins located on the 60S subunit of eukaryotic ribosomes, occur specifically in systemic lupus erythematosus (SLE)[1]. A common epitope of 22 amino acid residues is located at the carboxy-terminal end of all the 3 P proteins[2]. The prevalence of these antibodies in SLE varies between 6% and 46% in various ethnic groups[3,4]. Clinical association with neuropsychiatric disease, especially psychosis[3,5,6], nephritis[7,8], hepatitis[7,9], skin[10], and age at onset of disease[11] is described in some but not in all studies[10,12]. We investigated the prevalence of these antibodies and its relation with clinical manifestations in our cohort of patients with lupus.

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MATERIALS AND METHODS

All consecutive patients with SLE [American College of Rheumatology (ACR) 1982 criteria[13]] attending our clinic between January 2000 and August 2002 were included in the study. Disease activity was measured at the time of sample collection using the SLE Disease Activity Index (SLEDAI). Cumulative clinical manifestations up to the time of assessment were included. Active neuropsychiatric lupus was diagnosed according to ACR guidelines[14]. Nephritis was diagnosed if the patient had urinary sediment abnormalities and proteinuria > 500 mg in 24 hours. Hepatitis was diagnosed if the levels of serum bilirubin and serum alanine and/or aspartate aminotransferases were abnormal after excluding drug and viral etiologies. Sera from 212 age and sex matched healthy individuals were collected as controls. Paired cerebrospinal fluid (CSF) and serum samples were collected from 13 patients with neuropsychiatric lupus during CSF examination. All samples were stored at –40°C until analysis.

ELISA for IgG anti-P antibodies. ELISA was done using the C-terminal 22-mer peptide of ribosomal P proteins, Lys-Lys-Glu-Glu-Lys-Lys-Glu-Glu-Ser-Glu-Glu-Glu-Asp-Glu-Asp-Met-Gly-Phe-Gly-Leu-Phe-Asp. A quantity of 50 μl/well of peptide (5 μg/ml) was coated and incubated at 37°C for 1 h, and then at 4°C overnight. The following morning, the plates were washed with phosphate buffered saline (PBS) and blocked with 200 μl PBS containing 3% bovine serum albumin (PBS-BSA) for 2 h at 37°C. Then 100 μl/well of 1:100 diluted test and positive control (1:400–1:25,600) sera in 0.1% PBS-BSA was added in duplicate and incubated 2 h at 37°C. Bound antibodies were developed with rabbit anti-human IgG-horseradish peroxidase (Dako, Glostrup, Denmark) and OPD in substrate buffer and read at 492 nm with an ELISA reader (Tecan, Spectra, Salzburg, Austria). The value of the test sera was expressed as arbitrary units and read against the standard. Sera with values above mean + 5 SD (14.7 au/ml) of 212 healthy controls were taken as posi-

tive. CSF samples were tested undiluted. An inhibition assay was done to confirm specificity by preincubating the peptide for 2 h with some positive sera (1:100) at 37°C, then overnight at 4°C, and testing for anti-P activity. The percentage inhibition was calculated using the following formula: percentage inhibition = (1 – OD inhibited/OD blank inhibition) × 100. Increasing concentrations of the peptide (0.25, 0.49, 0.98, 1.95, 3.91, 7.81, 15.62, 31.25, and 62.5 μg/ml) inhibited binding by 35%, 43%, 53%, 59%, 82%, 91%, 95%, 96%, and 97%, respectively, in a dose-dependent manner. The maximum inhibition obtained was 97%, confirming the specificity of the assay.

Western blot for IgG anti-P. In all the sera positive by ELISA, antibodies to ribosomal P were confirmed by Western blotting, using an enriched fraction of ribosomal proteins prepared from rat liver by density-gradient ultracentrifugation[15]. Sera with the presence of any or all of the 3 bands were taken as positive.

Statistical analysis. Statistical analysis was performed using chi-squares with Yates' correction or Fisher's exact test and Pearson's correlation coefficient.

RESULTS

There were 202 patients (15 males), whose median age was 30 (range 10–64) years; median duration of disease was 36 (range 1–360) months. The median age of 212 controls (28 males) was 28 (range 19–55) years. Thirty-one (15.35%) patients with SLE had IgG anti-P by ELISA (Figure 1), out of which 24 (77.4%) were positive by immunoblotting. No control tested positive. We did correlation coefficient analysis of the antibody level in those who were positive with the age at presentation, but failed to find any significance ($r = 0.117$, $p > 0.05$).

Patients' clinical features were arthritis (161), rash (133), nephritis (91), nervous system (69), childhood onset (53), and hepatitis (3). Fifty-six had diffuse central nervous system disease including generalized seizures (30), acute confusional state (2), psychosis (12), depression (7), and mood disturbances (2). None of these manifestations were associated with the presence of IgG anti-P by ELISA (Table 1). SLEDAI data were available for 105 patients. The median score was 4 (range 0–41) and had no relation with levels of anti-P antibodies ($r = 0.04$, p nonsignificant).

Thirteen paired CSF and sera samples were collected from patients with nervous system involvement (seizures in 5, acute confusional state in 4, psychosis in 2, cranial neuropathy and autonomic neuropathy in 1 each). The level of IgG anti-P in CSF was 100-fold less than that in serum samples, and correlated well with the serum levels ($r = 0.86$; Figure 2).

Table 1. Association of clinical features with IgG anti-P antibodies in 202 patients with SLE. No associations were significant (chi-square test/Fisher's exact test).

Clinical features (n)	Anti-P-Positive, n = 31 (%)	Anti-P-Negative, n = 171 (%)
Juvenile (53)	8 (25.8)	45 (26.3)
Arthritis (161)	23 (74.2)	138 (80.7)
Rash (133)	23 (74.2)	110 (64.3)
Nephritis (91)	13 (41.9)	78 (45.6)
Hepatitis (3)	1 (3.2)	2 (1.1)
Nervous system (69)	9 (29)	60 (35.1)
Diffuse NPSLE (56)	8 (25.8)	48 (28.1)
Psychosis (12)	1 (3.2)	11 (6.4)
Depression (7)	0	7 (4.1)

NPSLE: neuropychiatric SLE.

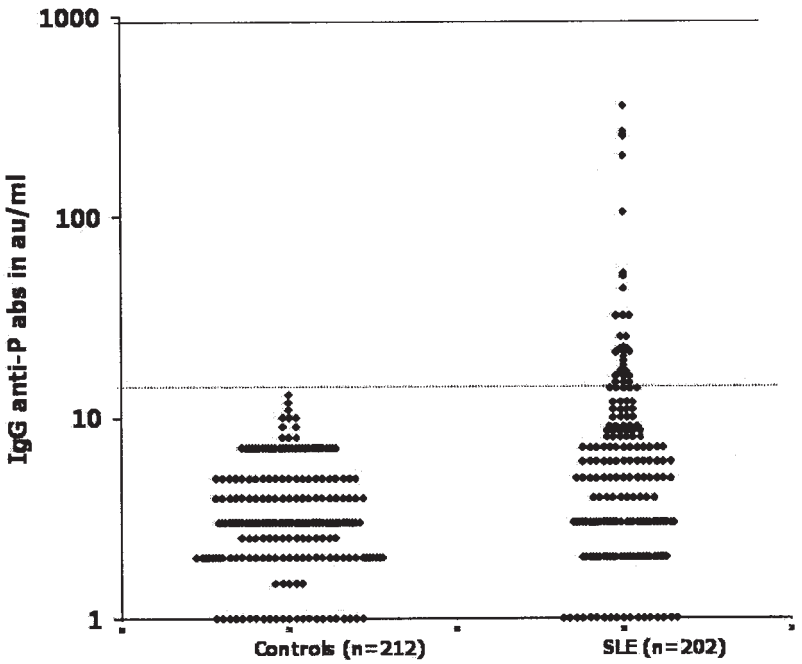

Figure 1. Values of IgG anti-P antibodies by ELISA in 212 controls and 202 patients with SLE. The cutoff value of positivity was taken as the mean + 5 standard deviations of the control sera.

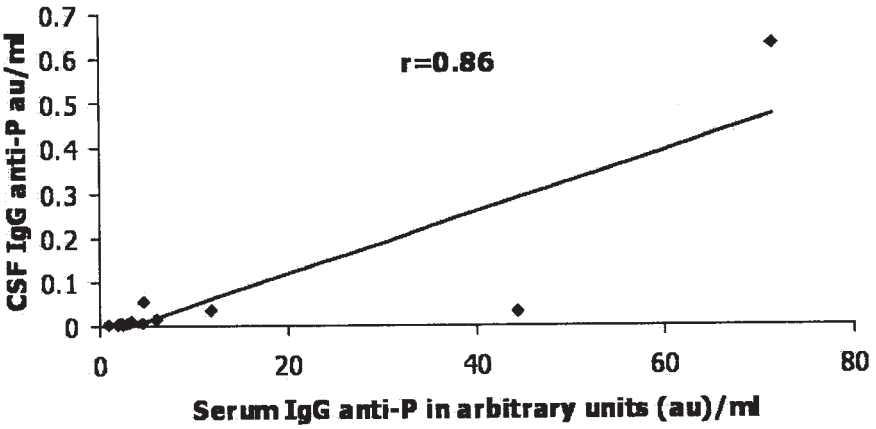

Figure 2. Correlation between serum and CSF IgG anti-P antibodies in 13 patients who had CSF examination. The coefficient of correlation was statistically significant ($r = 0.86$; $p < 0.01$).

DISCUSSION

Our study revealed that 15% of patients with SLE had IgG anti-P antibodies by a sensitive and specific assay, but there was no association between presence of anti-P and the clinical disease variables. The presence of antibodies in the CSF correlated with the presence of antibodies in serum. The prevalence is comparable to that described in recent reports[4], but less than the figures of 36%–38% reported from China[16]. This may be due to ethnic variations, considering the linkage to HLA-DQ-B1*0602[4,16]. The genetic background of Asian North Indians is closer to that of Caucasians, and our anti-P prevalence data are comparable to the 13%–20% reported from Greece[4].

ELISA was more sensitive than Western blotting, which is more specific. We confirmed that serum levels had a good correlation with CSF levels, as reported previously[5]. Obtaining CSF samples is difficult and we were able to study only 13 samples, of which only 3 were positive, limiting the significance of our findings. Despite using a sensitive and specific peptide based assay and a large sample size, we failed to find any association between presence of anti-P antibodies and clinical features of lupus as described by others[10,12]. The variability from studies reporting an association could be related to differences in the genetic background of our population. Thus IgG antibodies to ribosomal proteins have little clinical significance.

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