Correlation of antibodies to ribosomal P protein with psychosis in patients with systemic lupus erythematosus

Y Nojima, S Minota, A Yamada, F Takaku, S Aotsuka, R Yokohari

Abstract
Ninety one Japanese patients with systemic lupus erythematosus (SLE) were studied to determine the clinical significance of antibodies to ribosomal P protein (anti-P). Anti-P was detected by western blotting in 38 of 91 patients (42%). Clinical symptoms of SLE were compared between patients with and without anti-P. The occurrence of lupus psychosis was significantly higher in patients with anti-P than in those without anti-P (9/38 v 1/53). No significant association was found between anti-P and other symptoms of SLE. These data strongly support the suggestion proposed by previous workers that anti-P is a marker autoantibody for the development of lupus psychosis.


Central nervous system disease has long been recognised as a serious complication of systemic lupus erythematosus (SLE). Multiple factors can cause neuropsychiatric manifestations in patients with SLE such as drugs, metabolic abnormalities, infections, and the disease itself (central nervous system lupus). Although numerous laboratory tests have been proposed it is often difficult to diagnose correctly central nervous system lupus, which is important for the management of patients with SLE.

Bonna and co-workers have suggested a close association between lupus psychosis and antibodies to ribosomal P protein (anti-P), which had been previously identified by Elkon et al. as an autoantibody found in patients with SLE. Their findings are considered to contribute greatly to the successful management of lupus psychosis; however, reports supporting their data from other laboratories have not been published. In this study we have attempted to determine the clinical association of anti-P among non-selected Japanese patients with SLE.

Patients and methods
PATIENTS AND SERUM SAMPLES
Serum samples used in this study were obtained from the venous blood of 91 patients with SLE (80 women, 11 men) who attended the third department of internal medicine of Tokyo University or the department of collagen diseases of the National Medical Center. All patients met the American Rheumatism Association criteria for the classification of SLE. Control serum samples were also obtained from 50 normal subjects matched to the patient group for age and sex and from 32 patients with primary psychosis, including 18 patients with schizophrenia, seven patients with depression, and seven patients with epilepsy.

Clinical symptoms were evaluated by retrospective chart review. Dermatological disease was diagnosed by the presence of a malar rash, discoid rash, photosensitivity, or mouth ulcers. Articular disease was defined by a non-erosive arthritis. Serositis included pericarditis and pleuritis. Renal disease was diagnosed by the presence of persistent proteinuria of 0.5 g/day or cellular casts seen on analysis of urine. Haematological disorders were defined by persistent haemolytic anaemia, leucocytopenia (<4×10^9/l), lymphopenia (<1.5×10^9/l), or thrombocytopenia (<100×10^9/l). Central nervous system disease was diagnosed when seizures, coma, or psychosis occurred in the absence of other known causes of central nervous system diseases, such as drugs, infections, or metabolic abnormalities. Lupus psychosis was diagnosed when a marked behavioural disturbance including depression, mania, paranoia, hallucinations, or catatonia was observed in the absence of other known factors which could affect mental status.

Ten patients in our study developed lupus psychosis during their clinical courses.

WESTERN BLOTTING FOR THE DETECTION OF ANTI-P
Antibodies to ribosomal P protein were detected by western blotting using ribosomes as antigen sources. Ribosomes were purified from rat liver according to the method described elsewhere. Polyacrylamide gel electrophoresis was performed using the discontinuous buffer system described by Laemmli in the presence of 0.1% sodium dodecyl sulphate. Western blotting was performed as described previously. In brief the nitrocellulose sheets on which ribosomal proteins were electrotransferred from the gel were cut into strips and incubated for 30 min at room temperature in blocking buffer (10 mM phosphate buffered saline containing 5% non-fat dry milk), followed by a one hour incubation at room temperature with constant agitation in 1:20 dilutions of serum from patients with SLE or normal controls. After washing three times with phosphate buffered saline containing 5% non-fat dry milk, strips were incubated for one hour with alkaline phosphatase conjugated goat antibodies to human IgG (TAGO, Burlingame, CA, USA). The strips were rewashed and then developed for colour with 5-bromo-4-chloro-3-indolyl phosphate and nitroblue tetrazolium.
Results
Autoantibodies to ribosomal P proteins were detected in 38 (42%) of 91 patients with SLE by western blotting. The figure shows the representative blotting data. Antibodies to ribosomal P proteins were defined as positive when serum samples reacted simultaneously with the three ribosomal P proteins P0, P1, or P2 (38 000, 19 000, and 18 000 daltons). Neither normal controls nor patients with primary psychosis showed any reactivity against ribosomal P proteins under our experimental conditions.

The table gives a clinical analysis of patients with SLE with and without anti-P. There was no difference in the occurrence of anti-P in male (45%) and female patients (41%). There was no difference in the mean age at the time of the study between patients positive for anti-P (mean (SD) 35·5 (15·7) years) and negative for anti-P (36·3 (16·8) years). The incidence of multisystem organ disease including dermatological, articular, cardiac, lung and haematological disorders was not significantly different between the two groups. Of 10 patients who developed lupus psychosis during their clinical courses, nine were found to have anti-P. When these frequencies were compared with those of anti-P in randomly selected patients with SLE, significant differences were observed (p<0·01, χ² test). No significant association was observed between anti-P and other central nervous system manifestations excluding psychosis.

Discussion
The presence of autoantibodies reactive with cytoplasmic ribosomes in serum samples from some patients with autoimmune diseases have been known for more than 20 years. Although they have been described to be relatively specific for SLE their precise antigenic specificities and clinical significance have long been controversial. Elkon et al. have shown that the target molecules immunoreactive with SLE autoantibodies were the three large subunit ribosomal P proteins P0 (38 000 dalton), P1 (19 000), and P2 (17 000). Antibodies to ribosomal P protein react with a shared epitope common to all P proteins that is evolutionarily conserved in eukaryotes. Most importantly they showed a striking association between anti-P and the development of lupus psychosis. This is an important finding as differentiation between lupus psychosis and other neuropsychiatric disturbances caused by drugs, infections, or metabolic abnormalities is often difficult. Our study has strongly supported their findings.

We were able to find anti-P in 42% (38/91) of patients who fulfilled the American Rheumatism Association criteria for the classification of SLE. This represents a greater proportion than the 12% frequency of anti-P among patients with SLE described by Elkon and coworkers. They used western blotting and radioimmunoassay with a synthetic peptide as antigen in the detection of anti-P and found no difference in the frequencies of anti-P determined by these two methods. The incidence of autoantibodies can be affected by multiple factors including the specificity and sensitivity of the assays used or differences in the patient population studied. As we adopted essentially the same technique to detect anti-P as described by Elkon and coworkers, it is unlikely that the sensitivity of the assay is the main cause of this discordant incidence. Specificity of anti-P in our study was confirmed by the following findings, as described fully elsewhere.

(a) Serum samples from patients reacted simultaneously with three polypeptides with molecular weights of 38 000, 19 000, and 17 000, all of which were derived from a large subunit ribosomal protein. (b) Two dimensional electrophoresis followed by immunoblotting showed that these proteins were acidic. (c) On indirect immunofluorescence some of these serum samples showed cytoplasmic and nucleolar staining that was a typical pattern of anti-P. (d) Using antibodies to P0 and P1/P2 affinity purified independently from the immobilised P0 and P1/P2, respectively, on nitrocellulose sheets, we was found that serum

Clinical findings in patients with systemic lupus erythematosus (SLE) with and those without antibodies to ribosomal P protein (anti-P)

<table>
<thead>
<tr>
<th>Clinical findings</th>
<th>No (%) of patients with SLE</th>
<th>p Value</th>
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<tbody>
<tr>
<td></td>
<td>Anti-P positive</td>
<td>Anti-P negative</td>
</tr>
<tr>
<td></td>
<td>(n=38)</td>
<td>(n=53)</td>
</tr>
<tr>
<td>Dermatological disease</td>
<td>32 (84)</td>
<td>37 (51)</td>
</tr>
<tr>
<td>Articular disease</td>
<td>22 (58)</td>
<td>32 (60)</td>
</tr>
<tr>
<td>Serositis</td>
<td>6 (16)</td>
<td>12 (23)</td>
</tr>
<tr>
<td>Renal disease</td>
<td>22 (58)</td>
<td>31 (58)</td>
</tr>
<tr>
<td>CNS disease without psychosis†</td>
<td>3 (8)</td>
<td>6 (11)</td>
</tr>
<tr>
<td>Psychosis</td>
<td>9 (24)</td>
<td>1 (2)</td>
</tr>
<tr>
<td>Haematological disease</td>
<td>29 (76)</td>
<td>34 (64)</td>
</tr>
</tbody>
</table>

*NS=not significant.
†CNS=central nervous system.
samples from our patients reacted with a shared epitope common to all P proteins. All of these data indicate that we detected bona fide anti-P with the same specificity as that described by Elkon and coworkers.\cite{1,2,3,4,5} We studied a non-selected group of patients with SLE. The higher prevalence of anti-P in our study group than in that of Elkon and coworkers\cite{1,2,3,4,5} might be partly due to racial differences, although this possibility cannot be addressed until inter-laboratory exchanges of serum samples are feasible. We could not find a serum sample with anti-P specificities in 50 normal controls and in 32 patients with primary psychosis. Therefore the appearance of anti-P is actually related to the disease processes of SLE in Japanese patients.

Regardless of the discrepancy just described, the potential clinical significance of anti-P was also shown by our study of Japanese patients with SLE. It should be stressed, however, that more than half of the patients with anti-P had no neuropsychiatric signs during their clinical courses. Additional factors might be needed for the full development of lupus psychosis. Anti-P may be an epiphenomenon with little or no pathogenic importance. This seems unlikely, however, considering the strong clinical association, the selective increase of anti-P during the active psychosis,\cite{6,7,8,9,10,11,12,13,14} and the enrichment of anti-P in cerebrospinal fluid in a psychotic patient.\cite{14} The mechanism of action of anti-P is not yet known, and why more than half the patients with anti-P do not have psychiatric episodes remains to be determined. It is necessary to solve these problems to understand precisely the relation between anti-P and lupus psychosis and the possible protection of patients from this serious complication of SLE.

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