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## ANTI-RIBOSOMAL P ANTIBODIES ARE ASSOCIATED WITH NEPHRITIS, VASCULAR THROMBOSIS AND LYMPHOCYTOPENIA IN PATIENTS WITH SYSTEMIC LUPUS ERYTHEMATOSUS

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**Abstract** : In the present study, anti-ribosomal P antibody in sera of patients with systemic lupus erythematosus was assayed using an enzyme-linked immunosorbent assay, and its association with clinical symptoms of the patients was analyzed. The presence of anti-ribosomal P antibody was associated with increased frequency of lupus nephritis in the presence of anti-DNA antibody, and was associated with increased frequency of vascular thrombosis in the presence of anti- $\beta$ 2 glycoprotein I antibody and/or lupus anticoagulant. The level of anti-ribosomal P antibody correlated inversely with the peripheral lymphocyte counts.

**Key words** : anti-DNA antibody, anti-ribosomal P antibody, lupus nephritis, lymphocytopenia, vascular thrombosis

### INTRODUCTION

Anti-ribosomal P (anti-P) antibody is one of the autoantibodies found in patients with systemic lupus erythematosus (SLE)<sup>1-3)</sup>. The presence of anti-P antibody is reportedly associated with neuropsychiatric disorders in SLE patients<sup>4-8)</sup>. Recent findings indicate that the presence of anti-P antibody is also related to renal damage<sup>9-12)</sup> and vascular thrombosis<sup>13)</sup> in SLE patients. We investigated the correlation of anti-P antibody and anti-DNA antibody on lupus nephritis, and investigated the correlation of anti-P antibody and anti- $\beta$ 2 glycoprotein I (anti- $\beta$ 2GPI) antibody or lupus anticoagulant on antiphospholipid syndrome (APS), especially vascular thrombosis, in SLE patients. In addition, the levels of anti-P antibody

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were compared with titers of anti-DNA antibody and anti- $\beta$ 2GPI antibody or lupus anticoagulant, and were also compared with the results of laboratory tests.

#### PATIENTS AND METHOD

##### *Patients and sera*

Ninety-two patients with SLE attending or admitted to the Rheumatology or Nephrology Division of Ohta Nishinouchi Hospital were enrolled in the present study. Diagnosis of SLE was based on the American College of Rheumatology (ACR) revised criteria for SLE<sup>14</sup>). As disease controls, 36 patients were enrolled: 12 with rheumatoid arthritis (RA), 4 with systemic sclerosis (SSc), 7 with polymyositis/dermatomyositis (PM/DM), and 13 with various kinds of vasculitis. Diagnosis of RA, SSc and PM/DM was based on the respective ACR classification criteria. As normal controls, healthy staff members were enrolled. Sera were obtained from clotted blood and stored at  $-20^{\circ}\text{C}$  until used.

##### *Clinical features of SLE patients*

Clinical features of the 92 SLE patients were classified, based on the major symptoms, into the following 4 groups: lupus nephritis (LN), neuropsychiatric SLE (NPL), APS and "other symptoms". The numbers of patients with these major symptoms (in combination or single symptoms) are shown in Table 1. A total of 51 patients suffered from LN; in 40 of those patients, LN was confirmed histologically by renal biopsy. Twenty-one patients suffered from NPL (psychosis, epilepsy, meningitis, or cerebral nerve involvement). Twenty-six patients suffered from APS: 4 patients associated with spontaneous abortion; 10 patients with venous thrombosis, mostly of the deep veins in the legs; and 12 patients with arterial thrombosis in various organs including brain, lung and kidney. Fifteen patients

Table 1. Classification of patients depending on involved organs or symptoms

| Involved organs or symptoms | No. of patients | No. of patients with |     |     |                |
|-----------------------------|-----------------|----------------------|-----|-----|----------------|
|                             |                 | LN                   | NPL | APS | Other symptoms |
| LN+NPL+APS                  | 2               | 2                    | 2   | 2   | 0              |
| LN+NPL                      | 1               | 1                    | 1   | 0   | 0              |
| LN+APS                      | 14              | 14                   | 0   | 14  | 0              |
| NPL+APS                     | 2               | 0                    | 2   | 2   | 0              |
| LN                          | 34              | 34                   | 0   | 0   | 0              |
| NPL                         | 16              | 0                    | 16  | 0   | 0              |
| APS                         | 8               | 0                    | 0   | 8   | 0              |
| Other symptoms              | 15              | 0                    | 0   | 0   | 15             |
| Total                       | 92              | 51                   | 21  | 26  | 15             |

APS, antiphospholipid syndrome; LN, lupus nephritis; NPL, neuropsychiatric lupus

suffered from other symptoms including erythema, arthralgia, pleuritis and pericarditis. Two patients suffered from a combination of LN, NPL and APS. Seventeen patients suffered from 2 symptoms: LN+NPL in 1 patient, LN+APS in 14 patients, and NPL+APS in 2 patients. The numbers of patients suffering from LN, NPL or APS alone were 34, 16 and 8, respectively. Fifteen patients did not suffer from LN, NPL or APS, and were classified as having other symptoms.

#### *Autoantibodies*

Anti-ribosomal P (anti-P) antibody was measured using the Ribosomal P ELISA kit (Medical and Biological Laboratory, Nagoya, Japan). Briefly, 100  $\mu$ l of 1:100-diluted serum was added to each well of a 96-well micro-plate. The wells were coated with the 22 C-terminal amino acids of recombinant ribosomal P0 protein. The plates were incubated for 1 hour at room temperature. After washing 4 times with phosphate-buffered saline (PBS; pH 7.4), 100  $\mu$ l of peroxidase-labeled goat polyclonal antibody against human immunoglobulins was added. After washing 4 times with PBS, 100  $\mu$ l of tetramethylbentidine and H<sub>2</sub>O<sub>2</sub> substrate solution was added to each well, and the optical density (OD) at 450 nm was measured using a spectrophotometer (Bio-Rad, Hercules, CA, USA). The level (index) of anti-P antibody was determined using the following formula:  $\text{index} = (\text{OD intensity of sample} - \text{OD intensity of negative control}) / (\text{OD intensity of positive control} - \text{OD intensity of negative control}) \times 100$ . Antinuclear antibody (ANA) was measured using an immunofluorescence test at the Laboratory Unit of Ohta Nishinouchi Hospital. Anti-double stranded DNA (anti-DNA) and anti- $\beta$ 2GPI antibodies were measured using a standard enzyme-linked immunosorbent assay (ELISA) at the Laboratory Unit of Ohta Nishinouchi Hospital. Lupus anti-coagulant was measured using the silica clotting time method at Mitsubishi Kagaku Bio-Chemical Laboratory, Inc (Tokyo, Japan).

#### *Laboratory testing of blood samples*

Peripheral blood cell counts and complement levels (CH50) were measured using routine methods at the Laboratory Unit of Ohta Nishinouchi Hospital.

#### *Statistical analysis*

Statistical analysis was performed using the J-STAT software package for Windows. Differences between two groups were evaluated using the chi square test. Correlation between two values was assessed using Spearman's rank correlation coefficient test. P values less than 0.05 were considered to indicate significance.

## RESULTS

*Anti-ribosomal P antibody in sera of patients with SLE and various connective tissue-vascular diseases*

Serum levels of anti-P antibody (mean $\pm$ SD) were as follows: SLE sera, 23.5 $\pm$ 35.1 indices; SSc/PM/DM patients, 18.4 $\pm$ 15.3 indices; vasculitis patients, 21.5 $\pm$ 18.4 indices; healthy subjects, 3.54 $\pm$ 2.27 indices. Levels of anti-P antibody greater than 10.35 indices (mean $\pm$ 3SD) were considered positive for anti-P antibody. The frequencies of positivity for anti-P antibody were as follows: SLE, 43.5% (40/92); RA, 0% (0/12); SSc/PM/DM, 9.1% (1/11); vasculitis patients, 30.8% (4/13) (data not shown).

*Levels and frequency of anti-ribosomal P antibody in subgroups of SLE patients*

Levels and frequency of positivity for anti-P antibody in subgroups were as follows: LN, 25.3 $\pm$ 39.7 indices, 46.2% (24/51); NPL, 21.6 $\pm$ 28.0 indices, 47.6% (10/21); APS, 25.9 $\pm$ 33.7 indices, 73.1% (19/26); other symptom group, 8.9 $\pm$ 13.2 indices, 12.5% (2/15) (Table 2). There were 3 SLE patients who were negative for anti-nuclear antibody but positive for anti-P antibody; they exhibited elevated levels of anti-P antibody (41.3 $\pm$ 17.8 index). Two of those 3 patients suffered from nephritis; 1 of these 2 patients also suffered from APS, and the other suffered from NPL syndrome only.

*Association of the presence of anti-P antibody and anti-DNA antibody with lupus nephritis*

The 92 SLE patients were divided into 4 groups depending on presence or absence of anti-DNA antibody and anti-P antibody: group 1, positive for both anti-DNA antibody and anti-P antibody; group 2, positive for anti-DNA antibody and negative for anti-P antibody; group 3, negative for anti-DNA antibody and positive for anti-P antibody; group 4, negative for both anti-DNA and anti-P antibody. Numbers of patients and frequency of positivity for lupus nephritis in these 4 groups

Table 2. Prevalence and mean levels of anti-P antibody in SLE patients classified into 4 subgroups

|   | subgroups       |                 |                 |                |
|---|-----------------|-----------------|-----------------|----------------|
|   | LN              | NPL             | APS             | Other symptoms |
| No. of patients                                   | 51              | 21              | 26              | 15             |
| No. of patients positive for anti-P antibody (%)  | 24<br>(46.2%)   | 10<br>(47.6%)   | 19<br>(73.1%)   | 2<br>(12.5%)   |
| Levels (index) of anti-P antibody (mean $\pm$ SD) | 25.3 $\pm$ 39.7 | 21.0 $\pm$ 28.0 | 25.9 $\pm$ 33.7 | 8.9 $\pm$ 13.2 |

APS, antiphospholipid syndrome; LN, lupus nephritis; NPL, neuropsychiatric lupus

are shown in Table 3. Frequency of lupus nephritis was significantly higher for group 1 (81.3% ; 26/32) than for group 2 (51.5% ; 17/33) ( $p < 0.05$ ). There was no significant difference in frequency of lupus nephritis between group 3 and group 4.

*Association of the presence of anti-P antibody and anti- $\beta$ 2GPI antibody and/or lupus anticoagulant with antiphospholipid syndrome*

The 92 SLE patients were divided into 4 groups depending on presence or absence of anti-P antibody and anti- $\beta$ 2GPI antibody and/or lupus anticoagulant : group 1, positive for either anti- $\beta$ 2GPI antibody or lupus anticoagulant and positive for anti-P antibody ; group 2, positive for either anti- $\beta$ 2GPI antibody or lupus anticoagulant and negative for anti-P antibody ; group 3, negative for both anti- $\beta$ 2GPI antibody and lupus anticoagulant and positive for anti-P antibody ; group 4, negative for both anti- $\beta$ 2GPI antibody and lupus anticoagulant and negative for anti-P antibody. Numbers of patients and frequency of positivity for APS in these

Table 3. Number of patients with lupus nephritis in 4 groups classified by presence or absence of anti-DNA antibody and anti-P antibody

| anti-DNA | anti-P | No. of patients<br>Lupus nephritis |          | total |
|----------|--------|------------------------------------|----------|-------|
|          |        | positive                           | negative |       |
| +        | +      | 26 (81.3%)                         | 6        | 32    |
| +        | -      | 17 (51.5%)                         | 16       | 33    |
| -        | +      | 4 (33.3%)                          | 8        | 12    |
| -        | -      | 4 (26.7%)                          | 11       | 15    |
| Total    |        | 51 (55.4%)                         | 41       | 92    |

anti-DNA, anti-double stranded DNA antibody ; anti-P, anti-ribosomal P antibody ; \*,  $p < 0.05$  ; ns, not significant

Table 4. Numbers of patients with antiphospholipid syndrome in 4 groups classified by presence or absence of anti-P antibody and anti- $\beta$ 2GPI antibody and/or lupus anticoagulant (LAC)

| anti- $\beta$ 2GPI<br>and/or LAC | anti-P | No. of patients<br>Antiphospholipid syndrome |          | total |
|----------------------------------|--------|--|----------|-------|
|                                  |        | positive                                     | negative |       |
| +                                | +      | 19 (61.3%)                                   | 12       | 31    |
| +                                | -      | 7 (25.0%)                                    | 21       | 28    |
| -                                | +      | 0 (0%)                                       | 9        | 9     |
| -                                | -      | 0 (0%)                                       | 24       | 24    |
|                                  |        | 26   | 65       | 92    |

anti- $\beta$ 2GPI, anti- $\beta$ 2 glycoprotein I antibody ; anti-P, anti-ribosomal P antibody ; LAC, lupus anticoagulant ; \*\*,  $p < 0.01$  ; ns, not significant

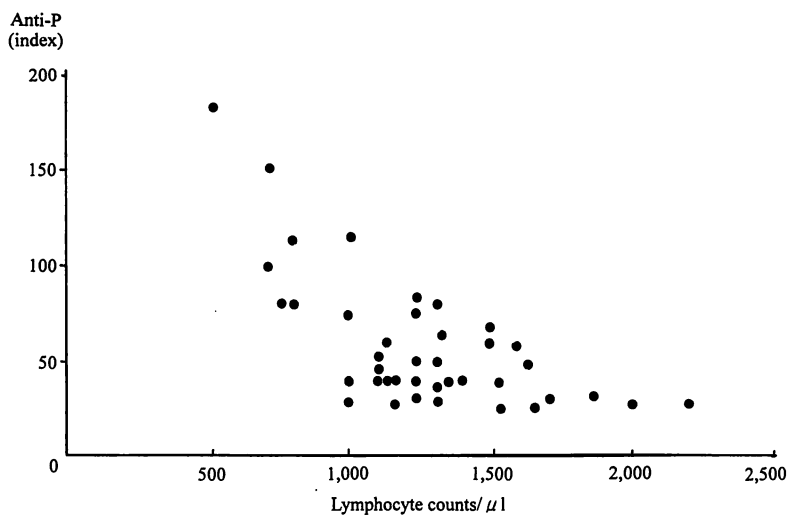


Figure 1. Correlation between the levels of anti-ribosomal P antibody (anti-P) and cell counts of peripheral blood lymphocytes, in the 40 patients with systemic lupus erythematosus who were positive for anti-P antibody.

$$Y = -0.064x + 139.42, r = -0.66178, p < 0.0001$$

4 groups are shown in Table 4. Frequency of APS was significantly higher for group 1 (61.3%; 19/31) than for group 2 (25.0%; 7/28) ( $p < 0.01$ ). APS was not observed in group 3 or group 4.

#### *Correlation between levels of anti-P antibody and results of other laboratory tests*

The levels of anti-P antibody were compared with titers of anti-DNA antibody, anti- $\beta$ 2GPI antibody and LAC, levels of serum complement, and counts of red blood cells, white blood cells, lymphocytes and platelets. In the 40 anti-P-positive SLE patients, the level of anti-P antibody correlated inversely with the lymphocyte count ( $r = -0.661$ ,  $p < 0.0001$ ) (Figure 1). There was no significant correlation between the levels of anti-P antibody and results of other laboratory tests (data not shown).

#### DISCUSSION

Reported frequencies of anti-P antibody in SLE patients range from 5 to 42%<sup>1-3,6</sup>. In the present study, anti-P antibody was found in 44% of SLE patients. This high frequency may be due to the sensitivity of the present ELISA kit and the active phase of the present patients. The present finding of a higher prevalence of anti-P antibody in patients with NPL and lupus nephritis is similar to previous findings<sup>2-6,10,11</sup>. The present finding that the presence of both anti-P antibody and anti-DNA antibody in SLE patients is associated with increased frequency of nephritis is consistent with a report by Reichlin *et al.*<sup>11</sup>, who demonstrated that anti-P and anti-dsDNA antibody were strongly associated with nephritis in juvenile-

onset SLE. The exact mechanism of the effect of anti-P antibody on nephritis is unclear. Sun et al. observed a cross reaction of anti-dsDNA antibody with ribosomal P protein on glomerular mesangial cells<sup>15)</sup>, which may explain the exacerbation of nephritis by anti-P antibody. It is also interesting that the presence of both anti-P antibody and anti- $\beta$ 2GPI antibody and/or LAC was associated with increased frequency of antiphospholipid syndrome, especially vascular thrombosis, in the present SLE patients. Yoshio et al.<sup>16)</sup> reported that anti-P antibody exhibited anti-endothelial cell activity, which may explain the thrombus formation observed in vessels in the present SLE patients. The present finding that levels of anti-P antibody correlated inversely with numbers of lymphocytes suggests that anti-P antibody has anti-lymphocyte activity. This is consistent with findings of Stafford et al.<sup>17)</sup>, who observed expression of ribosomal P protein on the cell surface of lymphocytes in both healthy volunteers and SLE patients.

There has been a few paper published which described a correlation between liver damage in SLE patients and anti-P antibody<sup>18,19)</sup>. However, we did not find a significant correlation between liver dysfunction in SLE patients and anti-P antibody in the present study.

In conclusion, the present results indicate that in SLE patients, presence of anti-P antibody and anti-DNA antibody is associated with increased frequency of lupus nephritis, and presence of anti-P antibody and anti- $\beta$ 2GPI antibody and/or LAC is associated with increased frequency of vascular thrombosis. The levels of anti-P antibody in SLE patients correlated inversely with peripheral lymphocyte counts.

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