Acquired Activated Protein C Resistance Associated with Anti-Protein S Antibody as a Strong Risk Factor for DVT in Non-SLE Patients

Junzo Nojima1, 2, Hirohiko Kuratsune3, Etsuji Suehisa1, Tomio Kawasaki4, Takashi Machii3, Teruo Kitani3, Yoshinori Iwatani2, Yuzuru Kanakura1, 3

1Laboratory for Clinical Investigation, Osaka University Hospital, Suita, Osaka, Japan, 2Department of Clinical Laboratory Science, School of Allied Health Sciences, Faculty of Medicine, Osaka University, Osaka, Japan, 3Department of Hematology and Oncology, Osaka University Graduate School of Medicine, Suita, Osaka, Japan, 4Division of Vascular Surgery, Department of Cardiovascular Surgery, Osaka University Graduate School of Medicine, Suita, Osaka, Japan

Correspondence to: Dr. Junzo Nojima, Laboratory for Clinical Investigation, Osaka University Hospital, 2-15 Yamadaoka, Suita, Osaka 565-0871, Japan – Tel.: 81-6-6879-6636, Fax: 81-6-6879-6635, E-mail: nojima@hp-lab.med.osaka-u.ac.jp

Keywords
Acquired activated protein C resistance, anti-phospholipid antibodies, anti-protein C antibodies, anti-protein S antibodies, deep vein thrombosis

Summary
Anti-phospholipid (aPL) antibodies (Abs) are well known to be associated with thromboembolic events in patients with systemic lupus erythematosus (SLE). However, the clinical relevance of aPL Abs in patients without SLE (non-SLE) who have venous thromboembolism remains unclear. We evaluated 143 non-SLE patients with a first episode of clinically suspected deep vein thrombosis (DVT) by using objective tests for diagnosing DVT and laboratory tests including the activated protein C resistance (APC-R) test, the factor V Leiden test, and various aPL Abs. The prevalence of acquired APC-R, in which case there was no factor V Leiden mutation, was significantly higher in patients with DVT (15/58 cases, 25.9%, p <0.001) than in those without DVT (3/80 cases, 3.7%), and confirmed that acquired APC-R was a strong risk factor for DVT (odds ratio [OR], 8.95; 95% confidence intervals [CI], 2.45-32.7; p <0.001). Multivariate logistic analysis revealed that the presence of LA, aCL, anti-β2-glycoprotein I, anti-prothrombin and anti-protein C Abs was not reliable as a risk factor for DVT in non-SLE patients, and that the presence of anti-protein S Abs was the most significant risk factor for DVT (OR, 5.88; 95% CI, 1.96-17.7; p <0.002). Furthermore, the presence of anti-protein C Abs was strongly associated with acquired APC-R (OR, 57.8; 95% CI, 8.53-391; p <0.0001). These results suggest that acquired APC-R may reflect functional interference by anti-protein S Abs of the protein C pathway, which action may represent an important mechanism for the development of DVT in non-SLE patients.

Introduction
Anti-phospholipid (aPL) antibodies (Abs) are a heterogeneous group of Abs, including anti-cardiolipin Abs (aCL) and lupus anticoagulant (LA) (1). These antibodies are frequently found in the plasma of patients with systemic lupus erythematosus (SLE) (2), and have been reported to be associated with venous thromboembolic events such as deep vein thrombosis (DVT) and pulmonary embolism (PE) in these patients (3-7). However, the precise mechanism responsible for venous thromboembolism (VTE) in patients with aPL Abs remains unclear.

A number of previous studies have suggested that the prevalence of DVT is associated with the congenital or acquired abnormalities of the protein C pathway (8, 9). Recently, it was shown that aPL Abs may inhibit phospholipid-dependent reactions of the protein C pathway, e.g., the thrombin/ thrombomodulin activation of protein C and/or the activated protein C/protein S degradation of factor Va (10). More recently, the presence of LA was reported to be associated with acquired activated protein C resistance (APC-R) in patients with SLE (11). However, the prevalence of LA in non-SLE patients with VTE much lower (about 10%) (12, 13) than that in SLE patients with VTE (about 80%) (14), and so the results of clinical studies on SLE patients cannot be extrapolated to non-SLE patients.

Although the most common antigenic targets of aPL Abs are β2-glycoprotein I (β2-GP I) and prothrombin, recent studies suggest that other phospholipid-binding proteins, particularly protein C and protein S, may be important targets as well (1, 15, 16). When we examined the prevalence of IgG Abs against phospholipid-binding plasma proteins (β2-GP I, prothrombin, protein C, protein S and annexin V) in patients with SLE, we found that the prevalence of aPL Abs against protein C or protein S was significantly higher in the SLE patients with venous thrombosis than in those with arterial thrombosis or thrombocytopenia or without thrombotic complications (17).

In the present study, we evaluated 143 DVT suspected patients who had acute or chronic swelling of the lower legs by using objective tests for diagnosing DVT and laboratory tests including those to determine the levels of various aPL Abs, and APC-R phenotype and genotype.

Patients, Materials and Methods
Study population. Consecutive patients between January 1998 and November 2000 who were referred to the thromboembolism consultants at Osaka University Hospital with clinically suspected deep vein thrombosis (DVT) were potentially eligible for the study. Since the objective of this study was to elucidate the degree of association between the presence of aPL Abs and/or acquired APC-R and the prevalence of DVT in patients without SLE, all patients diagnosed with SLE were excluded from this study population. One-hundred and forty-three patients were evaluated with objective tests for...
diagnosing DVT and with laboratory tests including those to determine the levels of various aPL Abs (LA, aCL, anti-β-2-GP I Abs, anti-prothrombin Abs, anti-protein C Abs, and anti-protein S Abs), activated protein C sensitivity ratio, factor V Leiden mutation, and coagulation tests (protein C, protein S, anti-thrombin). Diagnosis of DVT was made based on clinical manifestations and findings by duplex scanning, radioisotope venography, contrast venography, and radioisotope lung scanning. As controls, we also studied 50 plasma samples from normal healthy volunteers, which samples had been previously taken from the staff of Osaka University Hospital. None of them had any history of thrombotic complications, and there was no abnormality found by the blood examinations (blood cell counts, coagulation tests, liver function tests, and examinations for autoimmunity). Blood samples were taken into vacuum tubes (5.0 mL total volume, SEKUSIU, Japan) containing 0.5 mL of 3.13% trisodium citrate (Na₂C₃H₅O₇·2H₂O), and platelet-poor plasma was prepared by double centrifugation at 2800 g for 15 min at 15°C. Informed consent was obtained from all patients and control subjects.

Detection of anticardiolipin Abs (aCL) and lupus anti-coagulant (LA). The aCL levels were measured by a standard ELISA system as previously described (18, 19). The cut-off level for aCL Abs (398 mAbsorbance) was taken from a previous report (17). The LA activity was detected by use of both the diluted Russell Viper Venom time (dRVVT) and STACLOT LA test. The dRVVT (Gradipore Ltd, Sydney, Australia) and STACLOT LA test (Diagnostica Stago, Asnieres-sur-Seine, France) were performed by using commercially available screening and confirmatory tests as previously reported (14).

ELISA for anti-β-2-GP I, anti-prothrombin, anti-protein C, and anti-protein S Abs. Recent studies have indicated that aPL Abs do not recognize the native forms of β-2-GP I and prothrombin on plain polystyrene ELISA plates, but do bind to the conformationally changed structures of β-2-GP I and prothrombin coated on γ-irradiated polystyrene ELISA plates (3, 20, 21). In this study, we used a specific ELISA system for detecting the IgG aPL Abs specific for various phospholipid-binding plasma proteins, in which human plasma proteins (β-2-GP I, prothrombin, protein C, and protein S) were directly immobilized on γ-irradiated polystyrene plates. The ELISA assays were performed in duplicate. Such plates (Nunc-Immunoplate, Maxi-Sorp, Kastrup, Roskilde, Denmark) were coated overnight at 4°C with 50 μL per well of human β-2-glycoprotein I, prothrombin, protein C, or protein S (Diagnostica Stago), each suspended at a concentration of 10 μg/mL in Tris-buffer saline (TBS, 50 mM Tris-HCl, 0.1 M NaCl, pH 7.4). The wells were blocked for 60 min at room temperature with 50 μL of TBS containing 1.0% bovine serum albumin (BSA, Sigma, St. Louis, MO, USA), and then washed 3 times with TBS containing 0.1% Tween 20 (TBS Tween). Thereafter, 50 μL of plasma sample (diluted 101 times with 1.0% BSA-TBS, 0.1% Tween 20) was added to each well. Following 60 min of incubation at room temperature, the wells were washed with TBS-Tween. Horseradish peroxidase-conjugated goat anti-human IgG (γ-chain specific; A-2290) F(ab')2 fragment of affinity isolated Ab (Sigma) was used; and the color was developed by use of tetramethylbenzidine (TMB) solution (Moss, Inc, MD, USA). The absorbance was measured at 450 nm. Also, we determine the levels of anti-β2-GP I, anti-prothrombin, anti-protein C, and anti-protein S Abs in 50 healthy control subjects. The level of each Ab in healthy control subjects was as follows: anti-β2-GP I Ab, 222 ± 4.4 (mean ± SD); anti-prothrombin Ab, 210 ± 98.8; anti-protein C Ab, 229 ± 90.6; and anti-protein S Ab, 237 ± 8.6. Each of the antibody levels detected by ELISA in 50 normal control subjects were log transformed to approximate normality using the Stat Flex program before performing statistical analysis. The mean + 3 SD of each Ab in normal controls was decided as the normal cut-off point. The cut-off values for anti-β2-GP I, anti-prothrombin, anti-protein C, and anti-protein S Abs were 415, 506, 501, and 503, milliabsorbance units, respectively. A result was regarded as positive when the log-transformed absorbance exceeded each cut-off value. All assays were performed in duplicate using the Biomek 2000 Laboratory Automation Workstation (Beckman Instruments, Inc. USA). To assess ELISA assay performance both intra-assay and interassay coefficient of variations (CV) were calculated. The intra-assay CV was established from 24 measurements of a control sample within an assay run and was calculated to be 5.4%, 4.2%, 6.4%, and 5.8% for anti-prothrombin, anti-β2-GP I, anti-protein C, and anti-protein S Abs respectively. Interassay CV was calculated from measurements of a control sample assayed on 10 different assay runs and was 9.6%, 8.8%, 9.8%, and 8.2% for anti-prothrombin, anti-β2-GP I, anti-protein C, and anti-protein S Abs respectively.

APC resistance assay and factor V Leiden test. APC assay was performed in duplicate by use of an activated partial thromboplastin time (APTT)-based assay on the KC-10 coagulometer (Amelung, Germany). APTT was measured in the presence and absence of APC in the Coatest Activated Protein C Resistance Kit (Coatest, Chromogenix, Molndal, Sweden). The test was performed with undiluted patient plasma. The results were expressed as the ratio of APTT in the presence and absence of APC (APTT with APC/APTT without APC). The APC sensitivity ratio in 50 healthy controls was 2.72 ± 0.27 (mean ± SD). The APC sensitivity ratio of less than 2.18, which was the mean-2SD in healthy controls, was decided as the normal cut-off value in the APC-R study. Factor V Leiden status was determined by extracting genomic DNA from the plasma of the patients, as previously reported (22).

The mixing experiment with APC-R assay. Briefly, 50 μL of patient’s plasma was incubated with 50 μL of normal pooled plasma form 20 healthy donors. After 5 min of incubation at 37°C, the APC-R assay was performed as above.

Measurement of protein C, protein S, and anti-thrombin. Protein C activity was measured by use of a chromogenic substrate assay kit employing PGPA-MNA (Berichrom, Behring-Werke, Marburg, Germany) as previously reported (23). Protein C antigen was measured by using a homogeneous enzyme immunoassay as previously reported (24). Protein S activity was determined by a clotting assay (Staclot Protein S, Diagnostica Stago), as previously reported (25). Plasma levels of total and free protein S antigens were measured by using enzyme-linked immunosorbent assay kits (Asserachrom Total Protein S and Asserachrom Free Protein S; Diagnostica Stago). Antithrombin III activity was measured with a chromogenic substrate (S-2238, Kabi Vitrum, Stockholm, Sweden) used in an assay kit (Testzym, Daiichi Pure Chemicals, Tokyo, Japan), as previously reported (25). Antithrombin antigen was assayed by single radial immunodiffusion using M-Partigen plates (Behring-Werke).

Statistical analysis. The non-parametric Mann-Whitney test was used to compare the levels of aPL Abs between DVT-positive group and DVT-negative group. Fisher’s exact probability test was used to compare the prevalence of aPL Abs or APC-R between DVT-positive group and DVT-negative group. The Kruskal-Wallis test and the Dunn multiple comparison test were used to compare the values of the APC ratio among groups. As an approximation of the relative risk, the odds ratios (OR) and 95% confidence intervals (CI) were calculated for several putative risk factors by using multivariate logistic regression analysis with the statistical program Stat Flex. An OR was considered statistically significant when the lower limit of the 95% confidence intervals (CI) was >1.0 or if the upper limit was <1.0. The variable that achieved statistical significance in the first analysis was tested in a second analysis by multivariate logistic regression analysis. In the multivariate logistic regression analysis, a value of p <0.05 was considered to be statistically significant to indicate a risk factor.

Results

Patients groups with or without DVT, and normal control group. One-hundred and forty-three patients who had acute or chronic swelling of the lower legs, indicating a possibility of DVT, were evaluated with objective tests for diagnosing DVT. Sixty-three of them were assigned to the DVT-positive group. This is the first episode in 51 of 63 patients with DVT, and 12 had the past history of thrombotic events. All of 63 patients with DVT did not have any kinds of oral anticoagulant treatment at the time of presentation in this study. The other 80 subjects, who had no abnormality in the objective tests for diagnosing DVT, were considered as the DVT-negative group. Table 1 shows the main characteristics of the DVT-positive group, DVT-negative group, and the control group. There were no significant difference in age and sex amongst these 3 groups.
It has been established that patients who were deficient in protein C, protein S, or antithrombin had a markedly increased risk for DVT (8, 9). Therefore, we studied the concentrations of protein C, protein S, and antithrombin in our 63 patients with DVT, and found that 5 of them had a deficiency in protein C or protein S (3 for protein C and 2 for protein S). The remaining 58 patients with DVT had no congenital abnormalities in protein C, protein S or antithrombin.

**Association between acquired APC-R and DVT.** APC-R was present in 18 (13.0%) of 138 patients (58 with DVT and 80 without DVT). All 18 patients with APC-R tested negative for the factor V Leiden mutation. Furthermore, the APC-sensitivity ratios of these 18 patients were not increased significantly after the mixing experiment in which pooled plasma from 20 healthy donors was mixed 1:1 with each of patient plasma. Therefore, the remaining 18 patients were considered to have acquired APC-R. The prevalence of acquired APC-R was significantly higher in patients with DVT (15/58 cases, 25.9%, p < 0.0001) than in those without DVT (3/80 cases, 3.7%). The results revealed that the presence of acquired APC-R was a strong risk factor for the prevalence of DVT (OR, 8.95; 95% CI, 2.45-32.7; p < 0.001, Table 2).

**Association between various aPL Abs and DVT.** The prevalence of LA, anti-protein C Abs, and anti-protein S Abs was significantly higher in patients with DVT (LA, 6 cases, 10.3%, p < 0.05; anti-protein C, 16 cases, 27.6%, p < 0.001; anti-protein S, 25 cases, 43.1%, p < 0.0001; Fig. 1) than in those without DVT (1 case, 1.3%; 3 cases, 3.8%; 6 cases, 7.5%; respectively). On the contrary, there were no statistical differences in the prevalence of aCL, anti-β2-GP I Abs, and anti-anti-thrombin Abs between the patients with and without DVT (aCL, 3.4% vs. 1.3%, respectively; anti-β2-GP I Abs, 6.9% vs. 2.5%; anti-anti-thrombin Abs, 29.3% vs. 15.0%; Fig. 1). Multivariate logistic analysis revealed that only the presence of anti-protein S Abs was associated with the prevalence of DVT (OR, 5.88; 95% CI, 1.96-17.7, p < 0.002, Table 3).

Furthermore, the levels of each Ab were examined in patients with or without DVT. As shown in Fig. 2, the levels of anti-protein C Abs and anti-protein S Abs were significantly higher in the patients with DVT (419.6 ± 30.6 for anti-protein C, mean ± SE, p < 0.0001; 528.3 ± 40.9 for anti-protein S, p < 0.0001) than in those without DVT (anti-protein C, 262.3 ± 12.6; anti-protein S, 291.6 ± 14.9). On the contrary, there were no statistical differences in the levels of anti-β2-GP I Abs and anti-anti-thrombin Abs between patients with or without DVT (anti-β2-GP I, 212.7 ± 30.9 vs. 168.7 ± 12.9; anti-anti-thrombin, 412.2 ± 45.6 vs. 309.2 ± 29.2).

**Association between various aPL Abs and acquired APC-R.** The results of multivariate logistic analysis are shown in Table 4. A significant association was observed between the prevalence of acquired APC-R and the presence of anti-protein S Abs (OR, 57.8; 95% CI, 8.53-391; p < 0.0001), and 15 (83.3%) of the 18 patients with acquired APC-R had anti-protein S Abs. There was no association between acquired APC-R and the presence of LA, aCL, anti-β2-GP I Abs, anti-antithrombin Abs, or anti-protein C Abs. The prevalence of anti-anti-thrombin Abs and anti-protein C Abs was also high in patients with acquired APC-R (anti-anti-thrombin Abs, 11/18, 61.1%; anti-protein C Abs, 10/18, 55.6%). However, multivariate logistic regression analysis revealed that the presence of anti-protein S Abs was the only significant risk factor for acquired APC-R. Furthermore, the mean value of APC sensitivity ratios was significantly lower in patients with anti-protein S Abs (DVT-positive group, mean ± SD, 2.25 ± 0.29, p < 0.0001; DVT-negative group, 2.11 ± 0.21; p < 0.002) than in those without anti-protein S Abs (DVT-positive group, 2.73 ± 0.55; DVT-negative group, 2.76 ± 0.65, Table 5).

---

**Table 1** Characteristics of the patients groups with or without DVT and control group

<table>
<thead>
<tr>
<th></th>
<th>DVT-positive</th>
<th>DVT-negative</th>
<th>Normal control</th>
</tr>
</thead>
<tbody>
<tr>
<td>number of cases</td>
<td>63 cases*</td>
<td>80 cases</td>
<td>50 cases</td>
</tr>
<tr>
<td>female/male (n)</td>
<td>39/24</td>
<td>50/30</td>
<td>28/22</td>
</tr>
<tr>
<td>mean age</td>
<td>56.0</td>
<td>51.1</td>
<td>49.2</td>
</tr>
<tr>
<td>age range</td>
<td>21-82</td>
<td>23-85</td>
<td>25-63</td>
</tr>
</tbody>
</table>

DVT: deep vein thrombosis.
There were no significant differences in age and sex amongst these three groups.
*Five congenital cases were included.

**Table 2** Association between acquired APC-R and DVT

<table>
<thead>
<tr>
<th>APC-R</th>
<th>DVT-positive (58 cases)</th>
<th>DVT-negative (80 cases)</th>
<th>Multivariate logistic analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive cases</td>
<td>15 (25.9%)</td>
<td>3 (3.7%)</td>
<td><strong>8.95</strong></td>
</tr>
<tr>
<td>Negative cases</td>
<td>43 (74.1%)</td>
<td>77 (96.3%)</td>
<td><strong>2.45-32.7</strong></td>
</tr>
</tbody>
</table>

APC-R: activated protein C resistance. DVT: deep vein thrombosis.
Statistical analysis was performed using logistic analysis.
OR: odds ratio. CI: confidence interval.
An OR was considered statistically significant when the lower limit of the 95% CI was > 1.0.
A value of p=0.05 was considered to be statistically significant to indicate a risk factor.
*Results shown to be statistically significant.
Discussion

Venous thromboembolic events, such as DVT and PE, are one of the common manifestations in the anti-phospholipid syndrome (APS) (12, 14, 16, 26). However, the precise mechanism responsible for VTE in patients with aPL Abs remains unclear. The present study is the first to show that the presence of anti-protein S Abs is associated with the prevalence of acquired APC-R in non-SLE patients and that anti-protein S Abs is a strong risk factor for DVT.

Hereditary APC-R caused by the factor V Leiden mutation is clearly associated with an increased risk of DVT (27, 28). Acquired APC-R, a phenotypic APC-R that occurs in the absence of the factor V

<table>
<thead>
<tr>
<th>Antibodies</th>
<th>DVT-positive (58 cases)</th>
<th>DVT-negative (80 cases)</th>
<th>Multivariate logistic analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>LA</td>
<td>6 / 58 (10.3%)</td>
<td>1 / 80 (1.3%)</td>
<td>5.78 0.53-63.3 0.16</td>
</tr>
<tr>
<td>aCL</td>
<td>2 (3.4%)</td>
<td>1 (1.3%)</td>
<td>1.51 0.01-407 0.89</td>
</tr>
<tr>
<td>anti-β 2GP I</td>
<td>4 (6.9%)</td>
<td>2 (2.5%)</td>
<td>2.55 0.14-45.1 0.53</td>
</tr>
<tr>
<td>anti-PT</td>
<td>17 (29.3%)</td>
<td>12 (15.0%)</td>
<td>0.07† 0.01-0.62 0.02</td>
</tr>
<tr>
<td>anti-PC</td>
<td>16 (27.6%)</td>
<td>3 (3.8%)</td>
<td>3.24 0.73-14.3 0.13</td>
</tr>
<tr>
<td>anti-PS</td>
<td>25 (43.1%)</td>
<td>6 (7.5%)</td>
<td><strong>5.88†</strong> 1.96-17.7 &lt;0.002</td>
</tr>
</tbody>
</table>


Statistical analysis was performed using univariate and multivariate logistic analysis.

OR: odds ratio. CI: confidence interval.

An OR was considered statistically significant when the lower limit of the 95% CI was > 1.0.

A value of p<0.05 was considered to be statistically significant to indicate a risk factor.

*Results shown to be statistically significant.

†Results shown to be statistically significant negative factor.
Fig. 2 Levels of anti-β2-GP I, anti-prothrombin, anti-protein C, and anti-protein S Abs in the non-SLE patients with or without deep vein thrombosis (DVT). ●: patients with DVT (58 cases). ○: patients without DVT (80 cases). Statistical analysis was performed by using the non-parametric Mann-Whitney tests.

### Table 4 Association between the presence of each aPL Ab and acquired APC-R

<table>
<thead>
<tr>
<th>Antibodies</th>
<th>APC-R-positive (18 cases)</th>
<th>APC-R-negative (120 cases)</th>
<th>Multivariate logistic analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>LA</td>
<td>2 / 18 (11.1%)</td>
<td>5 / 120 (4.2%)</td>
<td>0.26 0.01-4.80 0.37</td>
</tr>
<tr>
<td>aCL</td>
<td>2 (11.1%)</td>
<td>1 (0.8%)</td>
<td>56.5 0.35-9076 0.12</td>
</tr>
<tr>
<td>anti-β2-GP I</td>
<td>3 (16.8%)</td>
<td>3 (2.5%)</td>
<td>4.28 0.09-207 0.47</td>
</tr>
<tr>
<td>anti-PT</td>
<td>11 (61.1%)</td>
<td>18 (15.0%)</td>
<td>0.20 0.03-1.42 0.11</td>
</tr>
<tr>
<td>anti-PC</td>
<td>10 (55.6%)</td>
<td>9 (7.5%)</td>
<td>5.75 0.82-40.4 0.08</td>
</tr>
<tr>
<td>anti-PS</td>
<td>15 (83.3%)</td>
<td>16 (13.3%)</td>
<td>57.8* 8.53-391 &lt;0.0001</td>
</tr>
</tbody>
</table>

Statistical analysis was performed using univariate and multivariate logistic analysis.
OR: odds ratio. CI: confidence interval.
An OR was considered statistically significant when the lower limit of the 95% CI was > 1.0.
A value of p<0.05 was considered to be statistically significant to indicate a risk factor.
*Results shown to be statistically significant.

### Table 5 Comparison of the values of APC-sensitivity ratio among patients with or without anti-protein S Abs and/or DVT

<table>
<thead>
<tr>
<th>Groups</th>
<th>anti-PS</th>
<th>DVT</th>
<th>Number of cases</th>
<th>APC-sensitivity ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>+</td>
<td>+</td>
<td>25</td>
<td>2.25 ± 0.29*</td>
</tr>
<tr>
<td>B</td>
<td>+</td>
<td>−</td>
<td>6</td>
<td>2.11 ± 0.21*</td>
</tr>
<tr>
<td>C</td>
<td>−</td>
<td>+</td>
<td>33</td>
<td>2.76 ± 0.65</td>
</tr>
<tr>
<td>D</td>
<td>−</td>
<td>−</td>
<td>74</td>
<td>2.73 ± 0.55</td>
</tr>
</tbody>
</table>

Statistical analysis was performed using the Kruskal-Wallis test and the Dunn multiple comparison test.
*P<0.001 vs. group C or D. There were no significant differences in the mean values of APC-sensitivity ratio between group A and B or group C and D.
Leiden mutation, has been reported in patients with APS (11, 29, 30), in patients with VTE (31, 32), in pregnancy (33), and in individuals using oral contraceptives (34, 35). However, the mechanism by which acquired APC-R is generated in these conditions has not been elucidated. More importantly, the association of acquired APC-R with VTE and/or aPL Abs has not been clearly established.

The present study provides unique evidence for an association between acquired APC-R and objectively documented DVT in non-SLE patients. In this study, APC-R was present in 13.0% (18/138 cases) of the non-SLE patient (58 with DVT and 80 without DVT), none of whom had factor V Leiden mutation. The prevalence of acquired APC-R was significantly higher in patients with DVT (15/58 cases, 25.9%, p <0.001) than in those without DVT (3/80 cases, 3.7%). Non-SLE patients with acquired APC-R had an 8.95 (OR) increased risk of DVT compared to non-SLE patients without acquired APC-R (95% CI, 2.45-32.7; p <0.001).

Recently, the presence of anti-β2-GP I Abs and/or LA was reported to be associated with acquired APC-R in patients with SLE (11, 36, 37), and acquired APC-R induced by these Abs has been hypothesized to be a possible mechanism of aPL Ab-associated thromboembolic complications in patients with SLE (38). Since the prevalence of anti-β2-GP I Abs and/or LA in patients with VTE who do not have SLE (non-SLE) is much lower than that in SLE patients with VTE, the etiology of VTE in the vast majority of the non-SLE patients has not been clear.

It has been suggested that the complexes of phospholipid and plasma proteins such as protein C and protein S are also recognized by aPL Abs and that these Abs are also involved in thrombotic complications (1, 15, 16). Recently, by means of specific ELISA systems we examined the prevalence of aPL Abs to various phospholipid-binding plasma proteins (β2-GP I, prothrombin, protein C, protein S, and annexin V) in SLE patients with arterial thrombosis, venous thrombosis, thrombocytopenia, fetal loss, and without complications; and we found that the prevalence of anti-protein C and anti-protein S Abs was significantly higher in the SLE patients with venous thrombosis than in those with arterial thrombosis, thrombocytopenia or without these complications, whereas the prevalence of anti-β2-GP I Abs was significantly higher in patients with arterial thrombosis than in those with venous thrombosis (17). These findings suggest that anti-protein C/anti-protein S Abs and anti-β2-GP I Abs may play a differential role in the occurrence of thrombotic complications, with anti-protein C and/or anti-protein S Abs being primarily associated with venous thrombosis and anti-β2-GP I Abs, primarily with arterial thrombosis. To clarify the association between the various types of aPL Abs and DVT and/or acquired APC-R in non-SLE patients, we examined the prevalence of the various aPL Abs (LA, aCL, anti-β2-GP I Abs, anti-prothrombin Abs, anti-protein C Abs, and anti-protein S Abs) and acquired APC-R in non-SLE patients (58 with DVT and 80 without DVT). The prevalence of LA, anti-protein C Abs, and anti-protein S Abs was significantly higher in patients with DVT than in those without it. On the contrary, there were no statistical differences in the prevalence of aCL, anti-β2-GP I Abs, and anti-prothrombin Abs between the 2 groups. Multivariate logistic regression analysis confirmed that the presence of anti-protein S Abs was only associated with prevalence of DVT. Multivariate logistic analysis also suggested that the presence of anti-prothrombin Abs was a significant negative factor for DVT (OR, 0.07; 95%CI, 0.01-0.62; p <0.02; Table 3), because there were a lot of DVT-negative patients who had anti-prothrombin Abs without anti-protein C Abs and anti-protein S Abs. However, this observation seems to be due to a phenomenon on statistics processing. It is not likely that the presence of anti-prothrombin Abs prevents the DVT.

The presence of LA was reported to be strongly associated with DVT or PE in non-SLE patients, as it was relatively high (9-14%) in such non-SLE patients (12, 13). In our study, the prevalence of LA was also high in patients with DVT (6/58 cases, 10.3%) as compared with that in patients without DVT (1/80 cases, 1.3%). However, when we performed the multivariate logistic regression analysis, it became clear that the presence of LA was not a risk factor for DVT. It is of great importance to note that 43.1% of patients with DVT had anti-protein S Abs, the prevalence being much higher than that of LA (10.1%).

It has been established that patients who have congenital abnormalities of protein C, protein S, or antithrombin have a markedly increased risk for DVT (8, 9, 23, 39). Therefore, we determined the concentrations of protein C, protein S, and antithrombin in our 63 patients with DVT, and found that only 5 of them had a protein C or protein S deficiency (3 with protein C deficiency and 2 with protein S deficiency). On these 5 patients, all of them had no Abs to protein C and/or protein S. These 5 DVT cases were considered to be associated with congenital abnormalities of protein C or protein S. Furthermore, there were no protein C or protein S deficiency in patients with anti-protein C and/or anti-protein S Abs. It is important to notice that the presence of aPL Abs against protein C and/or protein S was not associated with decrease in their protein concentration deficiency.

Concerning the relationship between aPL Abs and acquired APC-R, the presence of LA has recently been reported to be associated with acquired APC-R in patients with SLE (16). However, the prevalence of LA was detected in only 11.1% (2/18 cases) of the non-SLE patients with acquired APC-R in our study, suggesting that LA may not play a major role in acquired APC-R in our non-SLE patients. On the other hand, 61.1% (11/18 cases), 55.6% (10/18 cases), and 83.3% (15/18 cases) of the non-SLE patients with acquired APC-R had anti-prothrombin, anti-protein C, and anti-protein S Abs, respectively. However, multivariate logistic regression analysis revealed that only the presence of anti-protein S Abs was a significant risk factor for acquired APC-R. These results suggest that acquired APC-R may reflect functional interference by anti-protein S Abs of the protein C pathway, which action may represent an important mechanism responsible for the development of DVT.

Protein S serves as cofactor of activated protein C in the inactivation of factor Va and VIIIa, by facilitating the formation of enzyme-substrate complexes on the surface of phospholipid/platelet membranes (9). It was reported that protein S itself also has anticoagulant activity, by inhibiting the activity of both tenase and Prothrombinase (40, 41). Also, Oosting et al. demonstrated that aPL Abs against phospholipid-bound protein C or protein S inhibited the protein C/platelet S-mediated inactivation of factor Va (10). It is thus reasonable to speculate that the inhibition of the anticoagulant activity of the protein C pathway by anti-protein S Abs may increase the risk of DVT. Further studies are currently in progress to elucidate the mechanisms by which anti-protein S Abs inhibit the anticoagulant activity of the protein C pathway.

References


Received March 12, 2002 Accepted after revision July 22, 2002