Antibodies against phospholipid antigens (APA) have been demonstrated in idiopathic thrombocytopenic purpura (ITP), but their clinical and pathogenetic significance has remained elusive. In this study we analyzed the prevalence and clinical features of ITP patients with elevated APA. In addition, we prospectively evaluated APA levels after treatment with corticosteroids and compared them with platelet-associated immunoglobulin (PAIgG) titers. We studied 149 patients with newly diagnosed ITP. Of these, 78 had a platelet count less than \(50 \times 10^9/L\) and received an initial treatment with oral prednisone (PDN). In 71 asymptomatic cases with platelet counts between \(50 \times 10^9/L\) and \(120 \times 10^9/L\), no therapy was scheduled. However, in five of these, the platelet count fell below \(50 \times 10^9/L\) after more than 12 months; these patients were treated with PDN. Tests for APA included the measurement of anticardiolipin antibodies (ACA) with a solid-phase immunoassay and the detection of the lupus-like anticoagulant (LA) activity with coagulation tests that included kaolin-clotting time, dilute Russell’s Viper venom time, activated partial thromboplastin time (aPTT), and dilute aPTT. Controls consisted of 174 apparently healthy subjects. Either LA or elevated ACA was seen in 69 patients (46.3%) at diagnosis. LA and ACA were both elevated in 24 cases (16.1% of the overall patient population and 34.8% of patients with high APA concentrations). No correlation was found between LA ratio values and ACA-IgG or -IgM titers, or between ACA-IgG and ACA-IgM levels. The presence of these antibodies was not associated with sex, age, platelet count, or the severity of hemorrhages. PAIgG was detected in 106 of 127 cases (83.9%). Again, no relationship was observed with clinical parameters or with APA levels. However, all cases with elevated APA also had increased PAIgG. With regard to the clinical course, we were not able to detect any significant difference between patients with normal and elevated APA. An initial complete response to prednisone treatment was observed in 43 of 83 cases (51.8%), with 13 (15.7%) achieving a prolonged complete remission. APA levels were not significantly modified after PDN therapy and on relapse. We conclude that APA positivity is a common finding in patients with ITP and does not select a category with different clinical features. APA levels are not influenced by immunosuppressive therapy with steroids and are not related to the activity of the disease. Therefore, we do not support a role for APA in the pathogenesis of ITP.

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### MATERIALS AND METHODS

#### Patients

We studied 149 patients with ITP. The diagnosis of ITP was one of exclusion. Disorders known to cause shortened survival or decreased production of platelets were ruled out, including multisystem autoimmune diseases, lymphoproliferative disorders, drug-induced thrombocytopenia, myelodysplastic syndromes, human immunodeficiency virus (HIV), and other viral as well as bacterial infections. Bone marrow aspirate was performed in all patients at presentation. The examination of a marrow specimen was considered to be consistent with the diagnosis of ITP if the cellularity was normal, the number of megakaryocytes was normal or increased (our range for normals was 0.01%–0.1% of total nucleated cells), myelodysplastic features were absent, and no tumor cells or other abnormal findings were observed. Evaluation of the cellularity was qualitative, assuming as reference standard the marrow aspirate of normal persons (normocellular). In 11 patients in whom the aspirate was hypocellular for technical reasons (excessive volume of diluting blood and/or imperfect slide preparation), a bone marrow biopsy was hypocellular and defined a separate subgroup of antibodies.

Although there has been much debate regarding the relevance and prognostic significance of APA in ITP, the pathogenetic role of these antibodies, as well as their identity with PAIg, is unclear, and clinical data are still lacking. In this study we reinvestigated this problem in a large cohort of patients uniformly treated. We addressed the extent of elevated APA titers, as defined by positivity for either LA or ACA, in patients with newly diagnosed ITP and whether such cases presented distinctive clinical manifestations or a different response to therapy. In addition, we prospectively measured APA levels in patients after treatment with prednisone and compared them with the respective PAIgG concentrations.

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was also performed to confirm diagnosis. The main clinical and laboratory features of the patient population are summarized in Table 1. No patient reported a history of thromboembolisms, autoimmune disorders, obstetric complications, drug ingestion, or recent infections. Apart from those patients with mucosal bleeding who required platelet transfusions as emergency treatment, the initial therapy for all patients with less than \(50 \times 10^9/L\) platelets consisted of oral prednisone (PDN) at a standard dose of 1 mg/kg/d for 1 month. In patients who achieved a normal count (defined as greater than \(120 \times 10^9/L\)), PDN therapy was tapered over 6 months. If steroids did not result in a sustained response and in refractory and relapsed cases, different forms of therapy were administered, including splenectomy, intravenous immunoglobulins, anti-D, and interferon. This variability reflected the diversity of the patient population, as well as its different compliance to treatment (eg, refusal of splenectomy or of anti-D treatment). Patients who had a stable disease with a platelet count between \(50 \times 10^9/L\) and \(120 \times 10^9/L\) received no treatment.

Control Group

Controls were 174 apparently healthy subjects without a history of thrombosis or autoimmune disorders (92 women and 82 men; median age, 41 years; range, 14-65 years). Three persons (1.7%) in this group were found to have either LA activity or elevated ACA levels, while increased concentrations of PAIg were detected in 4 of 96.

Laboratory Investigations

All patients with newly diagnosed ITP routinely underwent a panel of laboratory tests including anti-nuclear (ANA), anti-mitochondrial (AMA), and anti-smooth muscle (ASMA) antibodies, antibodies to viruses of the TORCH group (toxoplasmongondii; others; rubella virus; cytomegalovirus; herpes simplex virus) and screening for HIV, viruses of the TORCH group (toxoplasma gondii; others; rubella virus; cytomegalovirus; herpes simplex virus) and screening for HIV, hepatitis B, and hepatitis C infection. PAIg were detected at the time of diagnosis and after PDN therapy. LA activity and ACA levels were found to be elevated in 54/95 (52.3%) of patients who had a stable disease with a platelet count greater than \(120 \times 10^9/L\) and 149/149 (99.3%) of those patients with less than \(50 \times 10^9/L\) platelets. These results were statistically significant (\(P < 0.01\)). The proportion of positive tests in each group was determined using Student’s \(t\) test or the chi-square test, and correlation analysis with Spearman’s rank correlation test. A \(P\) value of 0.05 or less was designated as statistically significant.

Statistical Analysis

Statistical analysis was performed with the WinSTAT 2.0 (Kalma Co, Cambridge, MA) software package on an IBM computer (IBM, Boca Raton, FL). Comparisons between the groups were performed with Student’s \(t\) test or the chi-square test, and correlation analysis with Spearman’s rank correlation test. A \(P\) value of 0.05 or less was designated as statistically significant.

RESULTS

LA activity and/or increased ACA at diagnosis were found in 69 patients (46.3%; Table 2). No correlation was found between LA ratio values and ACA-IgG or ACA-IgM titres.
or between ACA-IgG and ACA-IgM levels. LA activity was present in 54 cases, ACA-IgG in 30, and ACA-IgM in 18. LA and abnormally raised ACA occurred concurrently in 24 cases (16.1% of the overall patient population and 34.8% of patients with high APA concentrations). No correlation was found with sex, age, the degree of thrombocytopenia, or the severity of hemorrhages. ANA tested positive in nine patients (seven women and two men; median age, 63 years) with a stable thrombocytopenia; in seven of them with a homogeneous pattern and in two (a woman aged 62 years and a man aged 68 years) with a speckled pattern. These patients were also investigated for extractable nuclear antigens and double-stranded DNA, which tests were negative. Four of these patients had LA present, two showed elevated ACA-IgG, and one had elevated LA and ACA-IgG. One case was ASMA-positive and had LA present. Increased PAIgG levels were detected in 106 (83%) of the 127 patients in whom this analysis could be performed. Again, no relationship was observed with clinical parameters or with APA levels. However, all cases with elevated APA also had increased PAIgG. Correlation analysis between platelet count, PAIgG, and ACA levels in ACA-positive cases is detailed in Table 3.

Seventy-one asymptomatic patients had a stable disease with a platelet count between 50 × 10^9/L and 120 × 10^9/L. They received no treatment. Six patients recovered within 6 months, and five of them never did relapse. Elevated APA were detected in 30 cases (42.2%) and were not associated with a particular course of the thrombocytopenia. Four cases recovered after more than 6 months and had several relapses. In five cases the platelet count fell below 50 × 10^9/L after more than 12 months, with two of them showing symptoms. They received prednisone (PDN) for 6 months (see below); two achieved a stable safe count, while three had an early recurrence of the disease and received different forms of therapy. Seventy-eight patients (52.3%) presented with a platelet count less than 50 × 10^9/L at diagnosis. Of these, 39 (50%) had high levels of APA. Overall, an initial complete response to PDN therapy was seen in 43 patients (51.8%), with 13 (16.7%) attaining a durable complete response after cessation of steroids. After PDN therapy, 21 cases achieved a stable safe count and were not further treated. After subsequent treatments, 16 patients had a sustained complete response (Table 4). During the period of observation, we observed 10 relapses; 6 of these had previously responded to PDN, while 4 had experienced additional therapies. A new, longlasting response was achieved in five patients. Seventeen patients who were refractory or eventually relapsed after one or more of these second-line treatments needed small doses of PDN (5-15 mg/d) to maintain a safe count (greater than 50 × 10^9/L). The association with elevated APA did not predict treatment outcome. Also, patients with higher values of PAIgG (greater than 50 ng per 10^9 platelets) did the same as patients with lower titers (less than 50 ng per 10^9 platelets). Whereas APA levels were not significantly modified after PDN therapy and on relapse (Tables 5 and 6), treatment

Data are numbers of patients. Abbreviation: IV, intravenous.
Table 6. Behavior of Elevated APA in Relapsed Patients (n = 14)

<table>
<thead>
<tr>
<th></th>
<th>At Diagnosis</th>
<th>After PDN</th>
<th>At Relapse</th>
</tr>
</thead>
<tbody>
<tr>
<td>LA activity</td>
<td>5</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Elevated ACA</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>-IgG</td>
<td>3</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>-IgM</td>
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</tr>
<tr>
<td>LA + ACA-IgG</td>
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<td>1*</td>
<td>1</td>
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<tr>
<td>LA + ACA-IgM</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>LA + ACA-IgG + ACA-IgM</td>
<td>2</td>
<td>0*</td>
<td>1</td>
</tr>
<tr>
<td>ACA-IgG + ACA-IgM</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
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Data are numbers of patients.
* One patient lost LA but retained ACA-IgG.
† In one patient LA activity was lost, in the other one ACA-IgM became normal.

with corticosteroids was associated with a significant decrease of PAIgG in responding patients (P < .001, Fig 1). Differences in PAIgG titers were not significant in those individuals whose platelet counts were unchanged or increased moderately.

DISCUSSION

Nearly half of ITP patients had either LA or elevated ACA at the time of diagnosis. Concordance between the solid-phase and the coagulation tests was seen in just one third of such cases, thus confirming that the combined use of the two assays is necessary to detect different classes of antibodies.16 This result is not subject to comparison because no previous study has determined APA in ITP by both methods. With regard to ACA-IgG, our findings are fairly consistent with those reported by Harris et al.11 and Nomura et al.,16 while the incidence of raised ACA-IgM was remarkably lower in our series.

The main objective of this study was a clinical one, namely to define the characteristics and outcome of ITP cases with elevated APA. Contrary to some early reports,1,26 individuals with elevated APA neither showed nor had a history of thrombosis or clinical symptoms of other autoimmune diseases. We must emphasize, however, that the period of observation was relatively short. Response to treatment was not different in APA-positive patients, and although the circulating levels of these antibodies may become normal in some after therapy, in the majority of cases they are not influenced by treatment and may remain elevated in these in complete remission. This behavior is quite different from that of PAIgG and of autoantibodies to GP Ib/IX and GP IIb/IIIa,22-23 and is also different from the behavior of APA in some hematologic malignancies, where their levels mirror disease activity,18 or in systemic lupus erythematosus, where their presence correlates strongly with disease manifestations.24

The lack of correlation between the levels of antiplatelet antibodies and ACA and with the LA ratio is consistent with the results of other investigations.15-9,25 The ELISA for PAIgG that we used had no specificity and did not distinguish between autoantibodies and immune complexes or other immunoglobulins nonspecifically bound to the platelet membrane through their Fc receptors. As a matter of fact, although no significant correlation was found at diagnosis between the absolute values of APA and PAIgG, all cases with elevated APA also presented high PAIgG, suggesting that both tests may in part detect the same antibodies. On the other hand, it should be considered that our PAIgG assay measured total platelet IgG, approximately two thirds of which may actually be within the platelets.26 Much of this IgG may be taken up nonspecifically, as increased PAIgG is also associated with increased levels of platelet-associated albumin.27-28 Thus, it is theoretically possible that ITP patients with APA have increased plasma IgG levels that are reflected in the measurement of total platelet IgG, providing an alternative explanation to the fact that all patients with elevated APA had elevated PAIgG.

The precise role played by increased serum concentrations of APA in the mechanism of thrombocytopenia is still unde-
determined, but the data collected here suggest that APA are not apparently involved in the pathogenesis of ITP. Indeed, a causal relationship between APA and the thrombocytopenia has never been adequately proven, and crucial points regarding the interaction between platelets and APA remain controversial. The first topic to clarify, and probably the most important, is whether the binding of APA to platelets is the primum movens, i.e., sufficient to initiate the thrombocytopenia. This binding is significantly increased only after platelet activation or aggregation, because in these conditions greater proportions of anionic phospholipids are exposed on the outer leaflet of the platelet membrane. Quite recently Shi et al. have shown that the binding of immunoglobulins with LA activity and of ACA to platelets is dependent on thrombin activation; ACA also require the presence of \( \beta_2 \) glycoprotein I. Although it has been suggested that patients with APA have enhanced platelet activation,11-15 no definite laboratory demonstrations have been provided. The only study evaluating an activation-specific lysosomal membrane protein in patients with APA has shown a pattern of activation comparable with controls. In agreement with this finding, our preliminary data on the expression of CD62, a membrane platelet activation marker, have not shown significant differences in the percentage of activated platelets between ACA-positive ITP patients and controls (R.S. and E.S., unpublished observation, January-May 1994). Another cardinal question to be answered is why APA levels remain, for the most part, unmodified despite an achieved and maintained clinical remission. In a proportion of cases APA may not be relevant for the pathogenesis of the thrombocytopenia and are present simply as part of the spectrum of autoantibodies of many specificities that become more evident with increasing age. Another possible explanation is that in some patients the binding of APA to platelets results in increased platelet clearance balanced by increased platelet production,66 this balance may be altered by the presence of antibodies against platelet glycoproteins, which play a relevant role in platelet destruction, but may be re-established after these latter antibodies disappear. This hypothetical mechanism might explain the persistence of mild thrombocytopenia with high APA and low PAIgG after PDN therapy in cases with severe thrombocytopenia and elevated APA and high PAIgG at diagnosis. Finally, recent findings have shown the picture of a very heterogeneous population of autoantibodies that can be divided into subpopulations directed against different combinations of phospholipids and phospholipid-binding proteins.2-4,12-14,38,43 Suppression of one or few of these clones reacting with platelets will not be detected by standard methods.

In conclusion, elevated APA are a common finding in ITP, with patients with normal and elevated APA sharing similar clinical profiles. The fact that immunosuppressive therapy with steroids and disease activity is not accompanied by significant changes of APA levels validates the concept that these antibodies do not play a major role, if any, in the mechanism of the thrombocytopenia. However, the investigation of the influence of APA on platelet function is far from being completed, and more studies are warranted.

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