Autoantibodies Against Platelet Glycoproteins in Autoimmune Thrombocytopenic Purpura: Their Clinical Significance and Response to Treatment

By Peter Berchtold and Martin Wenger

Autoantibodies against platelet glycoproteins (GP) have been demonstrated in patients with autoimmune thrombocytopenic purpura (ATP). However, their clinical and pathogenetic significance as well as their response to immunosuppressive treatment is unknown. Using an immunobead assay capable of measuring autoantibodies against GPIIb-IIIa and GPIb-IX, we studied 58 adult patients with active ATP (platelet count < 150 x 10^9/L) and 26 patients with ATP in remission (platelet count > 150 x 10^9/L and without any therapy at time of investigation). Platelet-associated autoantibodies were detected in 39 of 53 patients with active ATP (73.6%) and in 2 of 28 patients in remission (7.1%). Circulating plasma autoantibodies were noted in 17 of 58 patients in the group with active disease (29.3%) and in none of the patients in remission. Twelve patients with active ATP and autoantibodies against GPIIb-IIIa were studied prospectively during treatment with corticosteroids. Of eight patients whose platelet count normalized during treatment, platelet-associated and plasma antibodies decreased significantly in two or became undetectable in six.

Autoimmune thrombocytopenic purpura (ATP) is an autoimmune disease characterized by increased platelet destruction caused by antiplatelet autoantibodies that result in increased platelet clearance by the reticuloendothelial system (RES). Several investigators have demonstrated autoantibodies against platelet membrane glycoproteins (GP) in ATP, confirming the autoimmune nature of the disease. Using an antigen-specific immunobead assay, platelet-associated autoantibodies against GPIIb-IIIa and GPIb-IX have been reported recently in 75.0% of thrombocytopenic patients with ATP. Circulating autoantibodies in the plasma against the same GP were noted in 57.6% of the patients. No antibody binding was found when platelets or plasma from either healthy donors or patients with nonimmune thrombocytopenia were analyzed, suggesting disease specificity of the immunobead assay. However, the clinical and pathogenetic significance of these anti-GP autoantibodies in ATP patients is unknown.

Corticosteroids are usually the initial treatment in ATP. About one half of the patients respond to prednisone within 4 weeks but long-term remissions are noted rarely. In patients with significant bleeding or extremely low platelet counts, high-dose intravenous immunoglobulin (IVIgG) is recommended as emergency treatment. This treatment is followed by a rapid but usually transient increase of the platelet count in most patients with ATP. The mechanism(s) of action of corticosteroids as well as of IVIgG in ATP is not known. It has been suggested that prednisone and IVIgG may impair phagocytosis of platelets by the RES. Others have proposed that platelet production is increased by prednisone or that autoantibody synthesis may be inhibited by corticosteroids and IVIgG.

In this study we evaluated autoantibodies against GPIIb-IIIa and GPIb-IX in adult patients either with active ATP or in remission with a past history of ATP. In addition, we prospectively measured anti-GP autoantibodies in patients during treatment with prednisone and administration of IVIgG.

MATERIALS AND METHODS

Patients. From March 1989 to February 1992, we studied 58 patients with active ATP and 26 patients in remission. Patients with platelet counts < 150 x 10^9/L were classified as active ATP. Patients with a past history of ATP and normal platelet counts (>150 x 10^9/L) without any immunosuppressive therapy at time of investigation were classified as ATP in remission. In all patients, the diagnosis of ATP based on clinical diagnostic criteria as thrombocytopenia with normal or increased megakaryocytes in the bone marrow and without evidence of other types of immune thrombocytopenia. All patients in the remission group had been treated with immunosuppressive therapy, ie, corticosteroids (26 patients), azathioprine (2 patients), IVIgG (2 patients), or splenectomy (12 patients), with a mean duration of remission of 8.4 years, range 1 to 28 years. The mean platelet count (range) was 45 x 10^9/L (2 to 125) in patients with active ATP and 266 x 10^9/L (151 to 371) in the remission group. The mean age (range) of active ATP and of remission patients was 49 years (20 to 83) and 47 years (20 to 76), respectively. For all studies of antiplatelet antibody, EDTA-anticoagulated blood was used from ATP patients and from 16 healthy adult controls.

Therapy. From the group with active ATP, we studied 17 consecutive patients with detectable anti-GPIIb-IIIa or anti-GPIb-IX autoantibodies...
autoantibodies during treatment with either corticosteroids and/or IVIgG.

Twelve patients with newly diagnosed ATP were evaluated prospectively during therapy with corticosteroids (Table 1). Prednisone, between 100 and 150 mg/d, was started initially and tapered when the patient achieved normal platelet counts. In eight patients platelet counts returned to normal (>150 x 10^9/L) without therapy. CR, normal platelet count with prednisone; REL, relapse; NO, no response; Pt-Assoc, anti-GPIIb-IIIa platelet-associated or plasma antibodies expressed as a ratio of patient/control results (positive > 1.4); NEG, negative.

* Study at the end of follow-up, at relapse, or before administration of IVIgG in patient no. 10.

Table 1. ATP Patients Treated With Corticosteroids

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Pct Count</th>
<th>Anti-GP IIb-IIIa</th>
<th>Follow-Up</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>x 10^9/L</td>
<td>Pt-Assoc Plasma</td>
<td>Response</td>
</tr>
<tr>
<td>1</td>
<td>2</td>
<td>2.9</td>
<td>REM</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>10.9</td>
<td>1.7</td>
</tr>
<tr>
<td>3</td>
<td>4</td>
<td>19.0</td>
<td>4.8</td>
</tr>
<tr>
<td>4</td>
<td>30</td>
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</tr>
<tr>
<td>5</td>
<td>66</td>
<td>2.3</td>
<td>2.6</td>
</tr>
<tr>
<td>6</td>
<td>2</td>
<td>85.5</td>
<td>78.0 CR</td>
</tr>
<tr>
<td>7</td>
<td>8</td>
<td>41.3</td>
<td>33.6 REL</td>
</tr>
<tr>
<td>8</td>
<td>5</td>
<td>2.0</td>
<td>NEG</td>
</tr>
<tr>
<td>9</td>
<td>35</td>
<td>5.7</td>
<td>2.5 REL</td>
</tr>
<tr>
<td>10</td>
<td>5</td>
<td>2.4</td>
<td>2.1 NO</td>
</tr>
<tr>
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<td>108</td>
<td>3.3</td>
<td>2.3 NO</td>
</tr>
<tr>
<td>12</td>
<td>64</td>
<td>3.7</td>
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Table 2. ATP Patients Treated With IVIgG

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Pct Count</th>
<th>Anti-GP IIb-IIIa</th>
<th>Follow-Up</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>x 10^9/L</td>
<td>Pt-Assoc Plasma</td>
<td>Response</td>
</tr>
<tr>
<td>10</td>
<td>7</td>
<td>2.5</td>
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<tr>
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<tr>
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<td>11.5†</td>
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</tr>
<tr>
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<td>2</td>
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</tr>
<tr>
<td>15</td>
<td>27</td>
<td>3.2</td>
<td>NEG</td>
</tr>
<tr>
<td>16</td>
<td>3</td>
<td>31.8†</td>
<td>NEG</td>
</tr>
<tr>
<td>17</td>
<td>19</td>
<td>1.7</td>
<td>NEG</td>
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</table>

Abbreviations: REM, normal platelet count (>150 x 10^9/L) without therapy; CR, normal platelet count with prednisone; REL, relapse; NO, no response; Pt-Assoc, anti-GPIIb-IIIa platelet-associated or plasma antibodies expressed as a ratio of patient/control results (positive > 1.4); NEG, negative.

Detection of anti-GP antibodies. The immunobead assay was performed with minor modifications as described in detail elsewhere. To evaluate platelet-associated antibodies, patient platelets were separated by differential centrifugation. For measurement of circulating plasma autoantibodies, washed normal platelets (10^8) were incubated with either patient or control plasma (1 mL). Immunobeads were prepared by incubating ¼-inch polystyrene beads with murine monoclonal antibody (MoAb) against either GPIIb-IIIa (2A9; provided by Dr Virgil Woods, University of California, San Diego, CA) for detection of anti-GPIIb-IIIa autoantibodies or human IgG (HB43; American Type Culture Collection, Rockville, MD) for measurement of anti-GP-IgG autoantibodies. For the assay, patient platelets (10^8) or normal platelets sensitized with patient plasma were solubilized in 1% Triton X-100 (BDH Chemicals Ltd, Poole, England). The lysate was then incubated for 60 minutes with immunobeads coated with either anti-GP-IIb-IIIa or anti-human IgG. After washing, any
Fig 1. Platelet-associated (A) and circulating plasma (B) autoantibodies against GPIIb-IIIa (dots) and GPIb-IX (circles) in patients with active ITP, in remission, and in controls. Values above the dotted line (>1.4 antibody binding ratio) are considered positive.

immunobead-bound autoantibody–GPIIb-IIIa complexes were detected by radiolabeled monoclonal antihuman IgG (HB43). Autoantibody–GPIb-IX complexes were detected by radiolabeled murine MoAb against GPIb-IX (6D1; provided by Dr Barry Coller, State University of New York, Stony Brook). Results are expressed as a binding ratio of the patient counts per minute-value divided by the mean counts per minute of at least three control samples. Patient samples with a binding ratio of >1.4 (> mean of controls + 3 SD) are considered positive.

Statistical analysis. The significance of the difference in antibody binding ratio between different groups of patients as well as normal controls was determined by a Wilcoxon rank-sum test.

RESULTS

Autoantibodies in active ATP and remission. Of the 58 patients with active ATP, we were able to measure platelet-associated autoantibodies in 53 patients (Fig 1A). Of these, 39 (73.6%) were positive, 26 with anti–GPIIb-IIIa, 6 with anti–GPIb-IX, and 7 with antibodies to both GP. The antibody-binding ratios of the positive patients ranged from 1.5 to 120.5. In the 26 patients in remission, platelet-associated antibodies were noted in 2 (7.7%) patients, 1 with anti-GPIb-IX and 1 with antibodies against both GP. In these 2 patients, antibody-binding ratios were 2.0, 4.0, and 4.5, respectively.

Circulating plasma autoantibodies were studied in all patients (Fig 1B). In the group with active ATP, 17 (29.3%) were positive, 10 with anti–GPIIb-IIIa, 3 with anti–GPIb-IX, and 4 with antibodies to both GP. None of the patients in remission had circulating plasma antibodies. All samples obtained from healthy adults gave antibody-binding ratios < 1.4 for platelet-associated as well as circulating plasma antibodies.

Corticosteroid treatment. Twelve patients of the group with active ATP and detectable autoantibodies against GPIIb-IIIa were studied prospectively during treatment with corticosteroids (Table 1). In the eight patients who responded with normalization of the platelet count (>150 × 10^9/L), we found, parallel to the increase of platelets, a significant decrease in anti–GPIIb-IIIa autoantibodies. Platelet-associated and plasma antibodies became undetectable in 6 and 8 patients, respectively (Fig 2). After a follow-up of 3 to 24 months, six patients had normal platelet counts without detectable autoantibodies (Table 1). In two of the patients (patients no. 7 and 8) who responded initially but relapsed when corticosteroids were reduced or withdrawn, platelet-associated antibodies reappeared in both. In four patients who showed only a moderate (patient no. 9) or no increase (patients no. 10, 11, and 12) in platelet count during prednisone treatment, platelet-associated and plasma antibodies were without substantial change throughout the follow-up (Table 1).
IVIg. Six patients with active ATP and detectable autoantibodies were studied during IVIgG administration (Table 2). The one patient who developed sustained remission (patient no. 10) was the only patient whose platelet-associated and plasma autoantibodies became undetectable during this therapy. No significant change in autoantibodies was noted in two responding patients whose platelet counts normalized (patient no. 13) or increased moderately (patient no. 14A) during IVIgG infusion but relapsed afterwards. In three nonresponders as well as during the second unsuccessful course of IVIgG in a fourth (patient no. 14B), there was no substantial fall in autoantibodies. In one patient (patient no. 16), this treatment was followed by a transient decrease in platelet-associated antibodies to GPIb-IX without effect on the platelet count.

DISCUSSION

Autoantibodies against platelet membrane GP have been described in patients with ATP by several investigators using antigen-specific assays. However, the clinical and pathogenetic importance of these antibodies as well as their response to treatment have not yet been reported.

Our study demonstrates that the presence of autoantibodies against GPIIb-IIIa and GPIIb-IX in ATP seems to be related to the activity of the disease: in the group with active ATP, 74% of patients were positive for platelet-associated and 29% for circulating plasma autoantibodies, whereas only two (8%) patients in remission showed platelet-associated antibodies and none had circulating plasma antibodies. The two patients in the latter group with detectable platelet-associated antibodies had long-lasting remissions and no other disease, medication, transfusion history, or preceding infection at the time of this study. Therefore, these results most likely reflect binding of autoantibodies rather than alloantibodies (ie, posttransfusion purpura, drug-induced antibodies).

The mechanism of action of corticosteroids in ATP or other autoimmune diseases is not clear. Corticosteroid-mediated impairment of the phagocytic function of monocytes or stimulation of the effective platelet production may account for the initial effect of prednisone in ATP. Long-term remissions after prednisone treatment may be caused by an inhibitory effect on antiplatelet antibody production. The latter is supported by recent reports demonstrating decreasing autoantibody titers during prednisone treatment in other autoimmune diseases and by our observations that platelet-associated and plasma autoantibodies decreased significantly or became undetectable in eight patients whose platelet counts normalized during treatment. Because an increase in platelet count only by blocking the phagocytic capacity of the RES would be expected without significant change in levels of anti-GP autoantibodies, the effect of prednisone in these patients may be caused by other mechanisms, ie, stimulation of platelet production, or inhibition of antibody formation, or both. A recent study has shown an increase in effective platelet production during prednisone treatment in patients with ATP. Our findings may be explained by this mechanism, at least for the early response to prednisone, because a greater number of platelets from increased platelet production would reduce the amount of antibody per platelet. However, long-term remission and lacking autoantibodies after discontinuation of prednisone in four of our patients suggest that another mechanism of corticosteroids in ATP may be the reduction of autoantibody production.

Studies demonstrating slowed destruction of anti-Rh-sensitized RBCs during IVIgG suggests that the short-term effect of IVIgG in ATP is caused by an inhibition of the clearing function of the RES by blocking its Fc receptors, preventing the elimination of antibody-coated platelets. This is consistent with our findings in two patients who responded to IVIgG with a transient increase of the platelet count but showed no substantial change in autoantibody. Our findings further suggest that the mode of action of the short-term effect of IVIgG is different from the mechanism observed in our patients during treatment with prednisone. In the one patient who developed long-term remission after IVIgG treatment, anti–GPIIb-IIIa autoantibodies became undetectable. Because blockade of the RES is transient, other mechanisms must be operative in this long-term effect of IVIgG. This may be caused by the presence of antiidiotype antibodies against antiplatelet autoantibodies in IVIgG.

In summary, this study provides evidence that occurrence of autoantibodies against platelet glycoproteins in patients with ATP is related to the activity of the disease. Moreover, disappearance of autoantibodies correlated well with the response to prednisone in these patients. Although our data do not demonstrate that these anti-GP autoantibodies are responsible for platelet destruction and further study is required, it is tempting to speculate that these autoantibodies are relevant for the pathogenesis of ATP and for the mode of action of prednisone.

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REFERENCES


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