Elevated Platelet-Bound IgG Associated With an Episode of Thrombotic Thrombocytopenic Purpura

By P. J. Sims and E. B. Boswell

The level of platelet-associated IgG (PAIgG) was measured during the successful treatment of a patient with thrombotic thrombocytopenic purpura. Prior to therapy, PAIgG was found to be markedly elevated to 195 fg/cell (normal range 0–3.5 fg/cell). The institution of combined therapy with intensive plasma exchange transfusions, high-dose steroids, and antiplatelet drugs resulted in a complete recovery and a decline in PAIgG to the normal range. The possible role of platelet antibody in the pathogenesis of this disorder is discussed.

THE ASSOCIATION of thrombotic thrombocytopenic purpura (TTP) with disease states of altered immunity or with prior antigenic challenge have led to numerous suggestions that immunologic injury to platelets by antibody or immune complexes can be a precipitating event in this syndrome. Nevertheless, only rarely have elevated circulating immune complexes been detected in serum obtained during the acute phase of the disorder, and in only a single case has elevated platelet-associated immunoglobulin (PAIgG) been measured.

In the present study we describe a case of TTP associated with a profound elevation of PAIgG that is detected prior to therapeutic intervention and in the absence of a significant elevation of circulating immune complexes.

MATERIALS AND METHODS

Case Report

A 51-yr-old female presented to her local physician with complaints of nausea, vomiting, and abdominal pain, and she was begun on cimetidine. Hematocrit (Hct) was 46% and platelets normal. Gastrointestinal symptoms persisted for 11 days, and she was admitted to her local hospital where she was found to have a Hct of 21% and was transfused with whole blood. She was mildly febrile and treated with antibiotics. Two days later, she suffered a sudden rise of blood pressure, a left hemiperesis, and the onset of seizures; platelet count dropped from a normal range to 30,000/cu mm, and she was transferred to the University of Virginia Hospital (UVH) for evaluation.

Physical exam at UVH revealed an obtunded woman responding to pain only. The skin and oral mucosa had multiple areas of ecchymoses and petechiae, and retinal hemorrhages were noted. Serum chemistries revealed a mildly elevated creatinine (1.8 mg/dl), total bilirubin (1.6 mg/dl), and SGOT (74 IU/liter). LDH was markedly elevated (2259 IU/liter) and haptoglobin decreased (5.0 g/dl). Prothrombin time was 13.8/13.3 sec; partial thromboplastin time 29.2 sec (normal 26.0–40.0); fibrinogen 500 mg/dl (normal 170–340), and fibrinogen degradation products 4 μg/ml (normal 0.5–8.0). Urinalysis revealed brown urine strongly positive for blood and protein. Stool was trace positive by guaiac. Erythrocyte Coombs tests for immunoglobulin and complement were negative and fluorescent antinuclear factor (ANF) and latex rheumatoid factor (RF) were both negative. Total hemolytic complement (CH50) was 46 U/ml (normal 34–48) and the serum was negative for immune complexes by Raji cell radioimmunoassay (10 μg/ml, normal <15). A bone marrow biopsy showed erythroid hyperplasia and increased numbers of megakaryocytes. A gingival biopsy revealed scattered capillary thrombi consistent with the diagnosis of TTP.

Special Studies

Assay for membrane-bound IgG. PAIgG was measured by a quantitative antiglobulin consumption assay as described by Dixon et al. with the modification of Kaden et al. All assays were performed on washed platelets prepared by differential centrifugation of freshly drawn venous blood, anticoagulated by a 10% EDTA solution. Assays were performed in duplicate within 12 hr of phlebotomy. Controls included 97 healthy adult volunteers at UVH.

Assay for serum antiplatelet IgG. Pooled platelets were freshly prepared by differential centrifugation of venous blood obtained from healthy adult volunteers, as for the direct assay. The washed platelets were then suspended at approximately 300,000/cu mm in 0.15 M NaCl. Two-hundred microliter aliquots of this suspension were mixed with 400 μl volumes of the patient’s sera diluted 1/4 (v/v) in 0.15 M NaCl and incubated for 60 min at 37°C. The cells were then washed 3 times with 10-ml volumes of 0.15 M NaCl, resuspended in saline, and assayed for PAIgG as described above. Controls were similarly prepared with the pooled autologous sera from the platelet donors, sera from randomly selected healthy adults, and serum from a patient known to contain elevated levels of circulating immune complexes.

RESULTS

Our patient presented with the classical pentad of findings associated with the clinical syndrome TTP, namely a hemolytic anemia with fragmentation, thrombocytopenia, fever, renal disease, and neurologic symptoms. In the course of our laboratory investigation of thrombocytopenia in this patient, we undertook a quantitative measurement of platelet-associated IgG (PAIgG). The results of these studies are presented in Fig. 1, with platelet count and PAIgG plotted on a
ELEVATED PLATELET-BOUND IgG

logarithmic scale as suggested by Karpatkin. PaIgG measured at the time of initial presentation (prior to therapy) was markedly elevated at 195 (±8) fg/cell, as compared to a normal range (mean ± 2 SD) of 0–3.5 fg/cell, as determined for a population of 97 healthy adult volunteers at UVH. Platelet count at this time was 17,000/cu mm. No antiplatelet immunoglobulin was detected in the patient’s serum, as measured in the indirect assay, using pooled platelets from normal donors (Table 1).

Institution of therapy including daily plasma exchange transfusions of 3 liters fresh frozen plasma, prednisone (200 mg/day), and antiplatelet drugs (aspirin, 20 grains/day and dipyridamole, 400 mg/day) was followed by an immediate improvement in her clinical symptoms as well as an elevation in platelet count to the normal range and a decline in PAIgG to normal levels during the second week of hospitalization (Fig. 1). An initial attempt to taper therapy at 2 wk was followed by a decline in the platelet count to below the normal range, accompanied by a small but significant elevation in PAIgG. The patient improved after reinstitution of plasma exchange transfusions and therapy was then successfully tapered after the fourth week of treatment. Following cessation of therapy, PAIgG has remained within normal limits (not shown).

DISCUSSION

Our findings of elevated PAIgG in a patient with TTP confirm a previous report\(^1\) of elevated platelet-bound immunoglobulin in this disorder and provides direct evidence of an immunologic abnormality of platelets at the time of acute onset of the syndrome. The profound elevation in PAIgG (195 fg/cell) measured at the time of presentation of this patient is to be compared with the slight elevation (3.762 fg/cell) reported by Morrison and McMillan.\(^2\)\(^3\)\(^4\) In their study, platelets for determination of PAIgG were first obtained following successful therapy with steroids and splenectomy. It is likely, therefore, that PAIgG at the time of onset of the disorder may have been elevated to levels significantly above the values observed in their study.

Elevated PAIgG may reflect immune complexes adherent to the platelets or immunoglobulin directed against a platelet membrane antigen.\(^5\)\(^6\)\(^7\) The association of TTP with states of altered immunity\(^1\)\(^8\) and reports of immunoglobulin and complement deposited in vascular lesions\(^14\)\(^15\) have raised suggestions that circulating immune complexes may be an important precipitating factor. Although elevated circulating immune complexes have been reported in this disorder,\(^4\)\(^6\) in the present case, circulating immune

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### Table 1. Circulating Antiplatelet Immunoglobulin (Indirect Assay)

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<tr>
<th></th>
<th>IgG Bound to Normal Platelets (×10^15 g/Platelet)</th>
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</thead>
<tbody>
<tr>
<td>Patient</td>
<td>1.5 ± 0.2</td>
</tr>
<tr>
<td>Controls</td>
<td></td>
</tr>
<tr>
<td>Autologous control (donor pool)</td>
<td>2.1 ± 0.6</td>
</tr>
<tr>
<td>Normal controls</td>
<td>1.1 ± 0.5</td>
</tr>
<tr>
<td>Immune complex serum*</td>
<td>5.3 ± 0.1</td>
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Washed platelets obtained from healthy blood group O adults were incubated (60 min, 37°C) with serum obtained from the patient on day of admission to UVH and then assayed for PAIgG as described in Materials and Methods. Results given as mean ± 1 SD of 4 determinations.

Controls: Results obtained using pooled sera of platelet donors (autologous controls) and sera from healthy adults selected at random (normal controls).

*Results obtained using human serum known to contain elevated levels of immune complexes (41 μg/ml by Raji cell radioimmunoassay).
complexes were not detected, and we were unable to detect increased levels of immunoglobulin bound to normal platelets after exposure to the patient’s serum (Table 1). These results suggest that elevated PAIgG observed in this patient may have been due to a limited autoimmune episode rather than to adherence of immune complexes to the platelet membrane. In this context, it is of interest to note that in one report of elevated circulating immune complexes measured in a case of TTP, the circulating antigen was stated to be a platelet membrane antigen.

The etiology of TTP remains uncertain, and it is likely that the syndrome can be initiated by many different events. Furthermore, this rare disorder usually presents as an acute, life-threatening medical emergency, for which multiple modalities of therapy are generally instituted, precluding definitive conclusion as to how specific treatment relates to the pathogenesis of the disorder. In the present case, no elevation in either circulating immune complexes or serum antiplatelet immunoglobulin (Table 1) were noted, suggesting that plasma exchange transfusion per se may not have directly contributed to the abrupt decline in PAIgG. This response is, on the other hand, consistent with the immunosuppressive activity of high-dose steroids, as well documented in classical immune thrombocytopenias. Nevertheless, the contribution of a circulating thrombogenic agent to the pathogenesis of this disorder is by no means excluded by the present study, and it would have been premature to have withheld plasmapheresis in light of its documented efficacy in the management of TTP. Most interesting in this context is recent evidence that alteration in platelet function with thrombotic consequences can arise from immune damage to the platelet membrane, possibly by an alteration of the arachidonic acid transformation pathway that stimulates production of thromboxane-A2 and the release of agents that induce platelet aggregation. Our finding of markedly elevated PAIgG coincident with the onset of this disorder provides evidence that immunologic damage to platelets may play a causal role in the evolution of TTP and suggests that future laboratory studies directed towards the action of immunoproteins in platelet function may contribute significantly to the clinical management of this disorder.

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REFERENCES

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