Pseudo-Bernard-Soulier Syndrome: Thrombocytopenia Caused by Autoantibody to Platelet Glycoprotein Ib

By Dana V. Devine, Mark S. Currie, Wendell F. Rosse, and Charles S. Greenberg

The Bernard-Soulier syndrome is an inherited bleeding disorder that is due to a deficiency in platelet glycoprotein Ib. Bernard-Soulier platelets fail to agglutinate in response to ristocetin despite normal levels of factor VIII:von Willebrand factor. We report a patient who developed severe refractory thrombocytopenia postsurgically while receiving procainamide therapy. Thrombocytopenia was immune mediated since the patient’s platelets bore high levels of antiplatelet antibody. Radioimmunoprecipitation studies demonstrated that the autoantibodies had specificity for platelet glycoproteins Ib and V as well as platelet HLA. The patient’s plasma as well as purified immunoglobulin G completely inhibited the ristocetin-induced aggregation of normal platelets but did not inhibit adenosine diphosphate-induced aggregation. The laboratory studies revealed that this patient suffered from antibody-mediated thrombocytopenia with unusual characteristics that we have called pseudo-Bernard-Soulier syndrome.

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precipitates the GP Ib/GP IX complex. Antigen-antibody complexes were precipitated by incubation with formalin-fixed protein A–bearing Staphylococcus aureus. For complexes containing monoclonal antibody, the bacteria were coated with goat antimouse immunoglobulin antibody (Tago Diagnostics, Inc, Burlingame, CA). The antigen-antibody complexes were removed from the bacteria by boiling in electrophoresis sample buffer. The proteins were separated under nonreducing conditions by discontinuous sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) using a 5 to 15% acrylamide gradient in the resolving gel. The gel was dried, and autoradiography was performed.

For sequential immunoprecipitation experiments, the platelet lysate was treated four times with monoclonal antibodies to GP Ib and GP Ib/IX to remove GP Ib from the platelet lysate. Control lysate was treated four times with monoclonal antibody P3, which does not react with platelet proteins. Both lysates were then reacted with patient serum as described earlier. Densitometric scans of the autoradiographs were made with a Hoefer GS-300 transmittance/reflectance scanning densitometer (San Francisco).

Platelet aggregation studies were performed with a Bio-Data aggregometer according to the manufacturer's recommendations. Ristocetin cofactor activity aggregation assays were performed by using both fresh, gel-filtered platelets and formalin-fixed, washed platelets from normal donors. Some ristocetin aggregation assays were performed with platelets that were incubated with normal donor or patient IgG. Four hundred microliters of gel-filtered normal platelets were incubated with 100 μL IgG at 4.1 or 1.0 mg/mL for five minutes at RT before the addition of ristocetin (1.5 mg/mL final concentration) and pooled normal plasma. ADP (0.4 μmol/L final concentration) aggregation was performed by mixing normal donor platelet-rich plasma with patient platelet-poor plasma or pooled platelet-poor plasma from normal donors.

**RESULTS**

Initial platelet aggregation studies looking for antitheparin antibody in this patient's plasma were confounded by the fact that the patient's platelet-poor plasma lyzed the test platelets as determined both by visual inspection of the assay mixture and the platelet count. However, if the patient plasma was heat inactivated for 30 minutes at 56°C, no lysis was observed. By indirect radioimmunoassay, both IgG (ranging from 9,000 to 12,000 molecules/platelet) and C3 (8,742 molecules/platelet) were detected on the surface of normal platelets reacted with the patient's serum. The patient's IgG bound equally well to P1a-positive and P1a-negative platelets (10,733 and 11,694 molecules/platelet, respectively), thereby excluding a diagnosis of posttransfusion purpura.

Platelet immunoprecipitation experiments showed that the patient's serum contained antibodies that precipitated protein bands with a relative mobility corresponding to platelet GP Ib (mol wt – 168 kilodaltons [kD]), the heavy chain (43 kD) and light chain (14 kD) of HLA, and a glycoprotein of 79 kD (Fig 1). When monoclonal antibodies reactive with GP Ib were used to deplete platelet lysate of GP Ib, the patient's serum no longer precipitated the 168-kD band from the lysate (Fig 2).

Lymphocytotoxicity analysis of the patient's serum uncovered anti-HLA antibody(s) with broad specificity that lysed all target lymphocytes on a 30-donor test panel. Antibodies in the patient's serum also bound to and activated complement on paraformaldehyde-fixed granulocytes at twice normal levels as measured by anti-IgG and anti-C3 radioimmunoassay. The specificity of the antigranulocyte antibody was not determined. It must be noted, however, that the patient was not neutropenic.

The platelet aggregation studies demonstrated that the patient's plasma inhibited the ristocetin cofactor activity of pooled normal plasma when tested with either fresh washed platelets or formalin-fixed platelets. IgG purified from the patient's serum caused the same inhibition. IgG from normal

**Fig 1.** Immunoprecipitation of radiolabeled platelet glycoproteins by patient serum and monoclonal antibody to platelet GP Ib. Solubilized radiolabeled platelets were reacted with monoclonal antibody to GP Ib (lane 1), autologous plasma (lane 2), or the patient's plasma (lane 3). The patient's plasma precipitated proteins corresponding to GP Ib (mol wt, 168 kD), HLA (heavy-chain mol wt, 43 kD; and light-chain mol wt, 14 kD), and GP V (mol wt 79 kD). Lane 4 contains the lysates from the surface-labeled platelets. GP III (mol wt, 89 kD) was a contaminating protein in lanes 1 to 3. Monoclonal antibody to GP Ib3 was the kind gift of Dr Barry Coller.

**Fig 2.** Sequential immunoprecipitation of GP Ib. GP Ib was removed from solubilized radiolabeled platelets by precipitation with monoclonal antibody to GP Ib/IX as described in the text. Precipitations were performed four times, at which time GP Ib was no longer detected in autoradiographs of the precipitates. Control lysates were treated with monoclonal antibody that does not react with platelet proteins (P3). The platelet lysates were then reacted with patient serum and antibody-antigen complexes electrophoresed in SDS-PAGE. The gels were subjected to autoradiography, then scanned with a densitometer. Patient serum precipitated bands corresponding to GP Ib, the heavy chain of HLA, and a protein of 79 kD from lysate treated with P3 (dashed line). Patient serum failed to precipitate the band corresponding to GP Ib from lysate that no longer contained GP Ib (solid line). The arrows at the left and right edges of the tracing mark the top and bottom of the gel, respectively. As in the experiment shown in Fig 1, GP III was a contaminating protein in the immunoprecipitations.
The relevant laboratory findings are summarized in Table 1.

Table 1. Patient's Laboratory Data

<table>
<thead>
<tr>
<th>Test</th>
<th>Patient</th>
<th>Normal Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Factor VIII antigen</td>
<td>150%</td>
<td>50%-150%</td>
</tr>
<tr>
<td>Ristocetin cofactor activity</td>
<td>150%</td>
<td>50%-150%</td>
</tr>
<tr>
<td>Antiplatelet antibody (molecules/platelet)</td>
<td>10,286</td>
<td>&lt;1,200</td>
</tr>
<tr>
<td>Posttransfusion purpura screen</td>
<td>Negative</td>
<td></td>
</tr>
<tr>
<td>Circulating immune complexes</td>
<td>Elevated</td>
<td></td>
</tr>
<tr>
<td>Total serum complement (CH50)</td>
<td>33.8</td>
<td>38-46</td>
</tr>
<tr>
<td>Serum protein electrophoresis</td>
<td>Normal</td>
<td></td>
</tr>
<tr>
<td>Fluorescent antinuclear antibody</td>
<td>1:2,560</td>
<td></td>
</tr>
</tbody>
</table>

Platelet aggregation studies

<table>
<thead>
<tr>
<th>Normal platelets incubated with patient plasma and ADP</th>
<th>57% aggregation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(control, 62%; normal range, 45%-85%)</td>
</tr>
<tr>
<td>Normal platelets incubated with patient plasma and ristocetin</td>
<td>1% aggregation</td>
</tr>
<tr>
<td></td>
<td>(normal plasma control, 97%)</td>
</tr>
<tr>
<td>Normal platelets incubated with patient IgG (4.1 mg/mL) and ristocetin</td>
<td>3% aggregation</td>
</tr>
<tr>
<td></td>
<td>(buffer control, 100%)</td>
</tr>
<tr>
<td>Normal platelets incubated with patient IgG (1.0 mg/mL) and ristocetin</td>
<td>69% aggregation</td>
</tr>
<tr>
<td></td>
<td>(buffer control, 100%)</td>
</tr>
</tbody>
</table>

The patient reported here produced an antibody that lysed all donor cells in the lymphocytotoxicity panel. Effective inhibition of ristocetin cofactor activity required a brief (ten-minute) incubation of test platelets with patient IgG before the addition of ristocetin and pooled normal plasma. When the IgG preparation was added to the cuvette last, only 14% inhibition was achieved. No inhibition of ADP-induced aggregation by the patient plasma was seen. The relevant laboratory findings are summarized in Table 1.

DISCUSSION

One of the diagnostic criteria for BSS is the inability of BSS platelets to agglutinate in response to ristocetin in the presence of a normal factor VIII: von Willebrand factor level. The failure to agglutinate to ristocetin is due to the absence of GP Ib, the binding site for von Willebrand factor, on the platelet membrane.13 The patient reported here produced an antibody that interacted with GP Ib and inhibited ristocetin agglutination. The detection of this antibody was hampered because the patient also had anti-HLA antibody. In addition, the antiplatelet antibodies she made activated sufficient complement to bring about platelet lysis. Complement-mediated destruction of her own and transfused platelets may have contributed to the thrombocytopenia since her plasma lysed platelets in vitro. The anti–GP Ib antibody was responsible for the thrombocytopenia since despite having anti-HLA antibodies the patient was not leukopenic.

One other case has been documented of a patient who developed autoantibodies that caused “acquired” BSS. The patient reported by Stricker et al8 differed significantly from the one reported here owing to the fact that their patient maintained a normal platelet count and that, with one exception, the patient did not demonstrate clinical bleeding.

The antiplatelet autoantibodies in this patient's serum contained reactivity not only with platelet HLA and GP Ib, but also with a 77-kD membrane protein that has a relative mobility similar to that of GP V. GP V has been reported to be deficient in the platelets of patients with BSS.4 The spatial relationship of GP Ib and GP V in the platelet membrane is not known. It is possible that the two molecules are closely associated and the antibody produced by this patient precipitated them as a complex.

Development of anti–GP Ib antibodies has been described in three of 106 patients with chronic idiopathic thrombocytopenic purpura (ITP),10 although no platelet function studies were performed with those antibodies. Interestingly, Szatkowski et al11 reported a patient with ITP who had an anti–GP Ib antibody that significantly enhanced ristocetin aggregation and ADP aggregation but did not, by itself, induce platelet aggregation. It is possible that individual anti–GP Ib antibodies recognize different determinants and may either enhance or inhibit agglutination or cause thrombocytopenia.

The patient reported here had received procainamide therapy; therefore drug-induced thrombocytopenia must be considered. The antiplatelet antibody was not drug dependent, however, since samples of serum obtained 30 days after discontinuation of procainamide treatment contained antibody that precipitated GP Ib in the absence of the drug. The induction of autoantibodies in procainamide-treated patients is well documented.12,13 Although these autoantibodies are predominantly against histones,14 ribonucleoprotein,15 and denatured DNA,16 antilymphocyte antibodies17 and rheumatoid factor18 have also been reported. The patient reported here had antilymphocyte antibodies that appeared to be directed, in part, against HLA antigens. Whether procainamide therapy induced the production of anti-HLA and platelet-specific autoantibodies cannot be determined in this case.

ACKNOWLEDGMENT

We thank the Coagulation Laboratory of Duke University for bringing this patient to our attention and Dr Emily G. Reisner for performing the microlymphocytotoxicity assays. Monoclonal antibodies to GP Ib and IX were the kind gifts of Dr Barry Coller, State University of New York, Stony Brook, and Dr John G. Kelton, McMaster University, Hamilton, Ontario.

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