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 postoperatively 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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THE BERNARD-SOULIER SYNDROME (BSS) is an inherited bleeding disorder characterized by moderate thrombocytopenia, prolonged bleeding time, giant platelets on the peripheral blood film, normal platelet aggregation in response to adenosine diphosphate (ADP), epinephrine, and collagen, but no aggregation in response to ristocetin. These platelets are deficient in the membrane glycoprotein (GP) Ib, which is the binding site for factor VIII:von Willebrand factor. The membrane proteins GP V and GP IX have also been reported to be deficient in BSS platelets.

We report a patient who developed refractory thrombocytopenia and an IgG autoantibody that selectively inhibited ristocetin-induced platelet aggregation. Biochemical analysis of purified immunoglobulin demonstrated autoantibodies to platelet GP Ib, GP V, and nonpolymorphic determinants on HLA molecules.

MATERIALS AND METHODS

Case report. The patient was a 76-year-old white female who was admitted to Duke University Hospital for open heart surgery to replace the mitral and tricuspid valves. Before the open heart surgery, she had a cardiac catheterization via a right femoral approach from which she developed a right groin hematoma. Preoperative coagulation studies demonstrated a normal prothrombin time (PT) and partial thromboplastin time (PTT) and a platelet count of 105,000/μL. The patient had received procainamide for a year before surgery to treat ventricular tachycardia. During surgery procainamide was continued as an intravenous infusion. The patient received 7 units of packed red cells and 10 units of platelets in the operating room. Postoperative coagulation testing revealed a PT of 12 seconds (normal, 9 to 10 seconds), PTT of 39 seconds (normal, 23 to 34 seconds), thrombin clotting time (TCT) of 22.5 seconds (control, 20), fibrin degradation products (FDP) level less than 10 μg/mL, and fibrinogen level of 330 mg/dL. The patient developed petechiae and suffered from repeated nose bleeds. Her platelet count progressively declined after surgery, with a nadir of 6,000/μL occurring on the eighth postoperative day. At this time, her WBC count was 4,700/μL, with 60% granulocytes, 26% lymphocytes, and 14% monocytes. Repeat coagulation studies performed on the ninth postoperative day showed a PT of 11 seconds, PTT of 35 seconds, TCT of 23.7 seconds, fibrinogen at 360 mg/dL, FDP less than 10 μg/mL, and no fibrin monomer. The patient received several platelet transfusions from which she never obtained an increment in her platelet count. In accordance with institutional guidelines, the patient was advised of procedures and attendant risks and gave informed consent for a bone marrow biopsy. The bone marrow biopsy sample revealed the presence of megakaryocytes in the marrow. Her stained peripheral blood smear did not demonstrate giant platelets. Prednisone therapy was started; however, her thrombocytopenia never corrected. The patient subsequently developed a pneumonia that was unresponsive to parenteral antibiotics. She died on the 35th postoperative day.

Methods. Blood samples were anticoagulated in sodium citrate (1 part 3.2% sodium citrate:9 parts blood) or were allowed to clot in glass tubes. IgG was purified from normal or patient serum by ammonium sulfate precipitation and diethyl aminoethyl cellulose chromatography as described. IgG was purified from patient serum that had been diluted 1:9 with normal serum. After nonidet P-40 (NP-40) treatment of normal platelets, the platelets were solubilized in buffer containing the nonionic detergent of ammonium sulfate precipitation and diethyl aminoethyl cellulose chromatography. The patient serum was treated similarly. The patient serum that had been diluted 1:9 with normal serum was reacted with monoclonal antibody to GP Ib (6D1) or monoclonal antibody that binds to platelet GP Ib, GP V, and nonpolymorphic determinants on HLA molecules.

either direct or indirect radioimmunoassays for platelet-bound IgG were performed as described with mouse monoclonal anti-IgG (Fc) (Miles, Elkhart, IN). For indirect assays, washed normal platelets at 5 x 10^9/mL in phosphate-buffered saline (PBS) containing 0.015 mol/L EDTA (PBS-EDTA) were reacted with an equal volume of patient or autologous serum for 30 minutes at room temperature (RT), washed twice in PBS-EDTA, and incubated with 125I-labeled monoclonal anti-IgG. Bound radiolabel was separated from unbound by centrifugation through phthalate esters. Indirect assays for C3 deposition were performed by incubating normal platelets washed into veronal-buffered saline with an equal volume of patient serum that had been diluted 1:9 with normal serum. After three washes in PBS-EDTA, the platelets were reacted with 125I-labeled monoclonal anti-C3d (Cytotech, San Diego). A similar indirect binding assay was performed for antigranulocyte antibodies. Microlymphocytotoxicity assays were performed by using the method of Amos et al.

Immunoprecipitation studies were carried out with 125I-labeled washed normal platelets as described. Briefly, surface-labeled platelets were solubilized in buffer containing the nonionic detergent Nonidet P-40 and reacted with the patient serum or autologous serum. Immunoprecipitations of GP Ib were performed by using monoclonal antibody to GP Ib (6D1) or monoclonal antibody that
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 antiepiphrin antibody in this patient’s plasma were confounded by the fact that the patient’s platelet-poor plasma lysed 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 P1α-positive and P1α-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
donor serum did not affect the ristocetin aggregation assay, nor did plasma from patients with polyspecific anti-HLA antibodies that lysed all donor cells in the lymphocytotoxicity panel. Effective inhibition of ristocetin cofactor activity required a brief (ten-minute) incubation of test plates 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.

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>1:2,560,000, speckled pattern, specificity for anti-ribonucleoprotein</td>
<td></td>
</tr>
</tbody>
</table>

Platelet aggregation studies

<table>
<thead>
<tr>
<th>Study</th>
<th>Patient</th>
<th>Normal Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal platelets incubated with patient plasma and ADP</td>
<td>57% aggregation</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>
<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>
<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>
<td>(buffer control, 100%)</td>
</tr>
</tbody>
</table>

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.12 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 al13 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.14 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),15 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 ofprocainamide 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

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