Thrombocytopenia Caused By Passive Transfusion of Anti-Glycoprotein Ia/IIa Alloantibody (Anti–HPA-5b)

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We describe a patient who developed transient and moderately severe thrombocytopenia (platelet count nadir 35 × 10⁹/L) after the transfusion of plasma. Using the technique of direct radioimmunoprecipitation, we showed that during the thrombocytopenia episode, the patient’s platelets had IgG specifically bound to the glycoprotein (GP) Ia/IIa complex. Indirect radioimmunoprecipitation using serum from the plasma donor confirmed that anti–HPA-5b (anti-Zav⁺) was the cause of the GP Ia/IIa sensitization. The relatively mild thrombocytopenia, compared with passive alloimmune thrombocytopenia caused by anti–HPA-1a (anti–PIA¹), may reflect the low copy number of HPA-5 compared with HPA-1. Direct radioimmunoprecipitation permits the detection of the GPⅠs carrying the known platelet alloantigen systems, and this study suggests that this technique can be used to diagnose passive alloimmune thrombocytopenia. © 1992 by The American Society of Hematology.

For many years, platelet-reactive alloantibodies have been known to cause two important clinical syndromes, neonatal alloimmune thrombocytopenia and posttransfusion purpura. Recently, they have been implicated in refractoriness to platelet transfusions and transplantation-associated thrombocytopenia.

Most platelet alloimmune disorders are caused by alloantibodies to the HPA-1 (PIA, Zw) system, especially anti–HPA-1a (anti–PIA¹, –Zw⁺). Recently, the HPA-5 system (Br, Zav, Hc) has been recognized as the second most frequent cause of neonatal alloimmune thrombocytopenia. Anti–HPA-5 has been implicated in approximately 20% of serologically confirmed cases of neonatal alloimmune thrombocytopenia and was recently implicated in a case of posttransfusion purpura.

The HPA-5 alloantigen system is found on platelet glycoprotein Ia/IIa (putative platelet collagen receptor). There are only approximately 2,000 antigen sites per platelet. Consequently, special techniques such as radioimmunoprecipitation or monoclonal antibody-specific immobilization of platelet antigens (MAIPA) are required to detect these antibodies.

During the 1980s, a new alloimmune platelet syndrome was described by three different groups of investigators. They described transient, but severe, thrombocytopenia resulting from the passive transfusion of anti–HPA-1a alloantibodies via blood products. In this report, we describe a patient who developed transient and moderately severe thrombocytopenia shortly after receiving plasma containing anti–HPA-5b. The diagnosis was made using direct radioimmunoprecipitation of the patient’s platelets.

Case Summary

A 52-year-old man underwent coronary artery bypass surgery. He had never previously received blood transfusions. His preoperative platelet count was 241 × 10⁹/L, and it decreased approximately 50% with institution of cardiopulmonary bypass (Fig 1). In the early postoperative period, the platelet count was stable, but decreased after transfusion with 1 U of packed cells and 2 U of fresh frozen plasma that were given 4, 6.5, and 8 hours after surgery, respectively. The next morning the platelet count had dropped further to 43 × 10⁹/L, repeat 35 × 10⁹/L (Fig 1). Although the bleeding time was prolonged—more than 15 minutes (normal is less than 7 minutes)—no bleeding complications were noted, and platelet transfusions were not given. The platelet counts ranged between 35 and 45 × 10⁹/L for 48 hours, then returned gradually to normal.

The patient received blood products from three donors in the postoperative period. Two were men and one was a woman who was requested to return for review. This donor was a healthy 43-year-old woman with a history of three pregnancies. The final pregnancy 15 years earlier resulted in a stillbirth at 7 months gestation. She had never been transfused.

Heparin-induced thrombocytopenia was excluded by the timing of the thrombocytopenia in relation to heparin administration, as well as by negative testing using the ¹⁴C-serotonin release assay.

Materials and Methods

Direct radioimmunoprecipitation. Platelets from the patient and a normal control were prepared from whole blood drawn into acid citrate dextrose (ACD). All blood samples were obtained after receiving informed consent, and with the approval of the hospital ethics committee. The platelets were washed three times with phosphate-buffered saline containing 10 mmol/L ethylenediaminetetraacetic acid (PBS/EDTA pH 6.7), and surface radiolabeled with sodium (¹¹¹)Iodide using lactoperoxidase. The platelets were solubilized with 1% Triton X-100 (Fisher Chemical Co., Rockford, IL), at a concentration of 1 × 10⁹/mL. Lin lysis buffer (10 mmol/L Tris-HCl pH 7.4, 10 mmol/L EDTA, 0.1 mmol/L phenylmethylsulfonyl fluoride, 50 μg/mL leupeptin, and 0.02 μg/mL aprotinin) were added to the radiolabeled platelets, and the platelets were incubated at 37°C for 1 hour with or without the addition of the platelet-specific immobilization of antigen systems (MAIPA) and specific antibody. The reaction was terminated by addition of a 10-fold excess of unlabeled platelet lysate and the mixture was applied to the gel column. The column was washed with buffer containing 0.25 mol/L ethylenediaminetetraacetic acid (PBS/EDTA pH 6.7), and the immunoprecipitated antigens were eluted with 1 mol/L Tris-HCl pH 6.7. The eluted platelet antigen was counted in a liquid scintillation spectrometer.

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Fig 1. Sequential platelet counts and mean platelet volumes observed for the patient with passive alloimmune thrombocytopenia caused by anti-HPA-5b. The patient developed the expected 50% decrease in platelet count associated with cardio-pulmonary bypass (CPB) surgery. However, an unexpected further decrease in the platelet count was observed following the transfusion of blood products. The unit of fresh-frozen plasma (FFP) containing anti-HPA-5b was administered 10 hours before the next available platelet count. The insert shows the sequential mean platelet values observed in this patient.

soybean trypsin inhibitor). The lysate was centrifuged (30,000g for 30 minutes) to remove the detergent-insoluble fraction. To detect preformed platelet-bound IgG/platelet antigen complexes, 1 mL of radiolabeled lysate was immunoprecipitated with 50 μL of Protein A-Sepharose CL-4B (Pharmacia, Baie d'Urfe, Quebec) for 1 hour at 22°C. The beads were washed, the complexes eluted with sample buffer, and then analyzed by 5% to 10% gradient sodium dodecyl sulfate-polyacrylamide gels (SDS-PAGE) and autoradiography. All immunoprecipitations were run under reducing conditions using 5% 2-mercaptoethanol in the sample buffer, unless otherwise stated.

Indirect radioimmunoprecipitation (platelet alloantigen typing). Patient and control platelets were radiolabeled and solubilized as described above. One milliliter of lysate was incubated with 50 μL of Protein A-Sepharose and 30 μL of test or control alloantisera for 1 hour at 22°C. The glycoprotein (GP) Ia/IIa complex was identified by incubating 1 mL of lysate with 50 μL of protein A-Sepharose and 3 μg of monoclonal antibody 12F1 (anti-GP Ia/IIa). After washing, the complexes were eluted and analyzed by SDS-PAGE. Alloantisera with anti-HPA-la, anti-HPA-Sb, and anti-HPA-Sa-3a (anti-Zavb/Baka) reactivities were used to type the test platelets.

Complete blood counts and mean platelet volumes. Complete blood counts and mean platelet volumes were obtained routinely, from EDTA-anticoagulated blood, using a Coulter STKR (Coulter Electronics, Hialeah, FL).

RESULTS

Direct radioimmunoprecipitation of patient platelets. Samples were obtained from the patient during the early recovery phase of thrombocytopenia (platelet count 89 x 10^9/L). The patient’s platelets were compared with control platelets using direct radioimmunoprecipitation. Direct immunoprecipitation of patient platelets showed two major protein bands that migrated under reducing conditions at 162 and 140 Kd, as well as a minor doublet at 53 Kd (Fig 2A). No protein bands were immunoprecipitated from the control platelets (Fig 2A).

Proteins directly immunoprecipitated from the patient’s platelets were compared with GP Ia/IIa immunoprecipitates obtained from control platelets, using either monoclonal antibody 12F1 or anti-HPA-5b alloantiserum (Fig 2B). Under both reducing and nonreducing conditions, the major protein bands directly immunoprecipitated from the patient platelets comigrated with GP Ia/IIa. A minor doublet band was also seen at 53 Kd when patient platelets were used for direct radioimmunoprecipitation under reducing conditions (Fig 2, A and B). Under nonreducing conditions, this protein had an M, of 165 Kd. These findings indicate surface-bound IgG on the patient platelets that was coprecipitated with GP Ia/IIa.

Direct immunoprecipitation of patient and control platelets was repeated 6 weeks after the patient had recovered from the acute thrombocytopenia. No platelet GPs were immunoprecipitated.

Identification of anti-HPA-5b in plasma donor serum. Serum was obtained from the blood donor suspected to possess the platelet-reactive alloantibody. The serum was screened for alloantibodies by indirect immunoprecipitation using test platelets carrying known alloantigens. The donor serum immunoprecipitated protein bands at 162 and 140 Kd (reduced) from platelets that were heterozygous HPA-5a/b, but not from platelets that were homozygous HPA-5a/a (Fig 3). These bands were also immunoprecipitated when the donor serum was reacted with lysate prepared from the patient platelets (Fig 3), confirming the results of the direct immunoprecipitation. In both cases the immunoprecipitated bands comigrated with GP Ia/IIa recognized by the known anti-HPA-5b antiserum (Fig 3).
Fig 2. (A) An autoradiogram showing results of direct radioimmunoprecipitation of patient (P, lane 1) and control (C, lane 2) platelets. Proteins that migrated at 162 and 140 Kd (reduced) were directly immunoprecipitated from patient but not control platelets. The doublet at 53 Kd corresponds to IgG heavy chain, which was coprecipitated from the patient but not control platelets. (B) An autoradiogram characterizing the proteins directly immunoprecipitated from patient platelets. Bands obtained by direct radioimmunoprecipitation of patient platelets (lanes 1 and 3) were compared with bands obtained from control platelets indirectly immunoprecipitated using the anti-GP IIb/IIIa MoAb 12F1 (lanes 2 and 4) or anti-HPA-5b alloantiserum (lane 5).

Fig 3. An autoradiogram demonstrating typing of the patient platelets and presence of anti-HPA-5b alloantibody in the plasma donor serum. Indirect radioimmunoprecipitation was performed using platelet proteins obtained from the patient (lanes 1 to 5), an HPA-5a/b heterozygous control (lanes 6 through 10), and an HPA-5a/a homozygous control (lanes 11 through 15). Anti-HPA-1a alloantisemum immunoprecipitated GP IIb/IIIa from the patient and both control platelets (lanes 1, 6, and 11). Anti-HPA-5b alloantisemum immunoprecipitated GP IIa/IIIa from the patient (lane 2) and an HPA-5a/b heterozygous donor (lane 7), but not an HPA-5a/a homozygous donor (lane 12). Anti-HPA-5a-3a alloantisemum immunoprecipitated both GP IIa/IIIa and GP IIb/IIIa from all three target platelets (lanes 3, 8, and 13). A normal serum control also was used (lanes 4, 9, and 14). Serum obtained from the plasma donor demonstrated immunoprecipitation of GP IIa/IIIa from the patient (lane 5) and the HPA-5a/b control platelets (lane 10), but not from HPA-5a/a control platelets (lane 15).
No other alloantibodies were detected in the donor’s serum.

**Alloantigen typing of patient and plasma donor platelets.** The platelets from the patient were allotted by indirect radioimmunoprecipitation (Fig 3). The patient typed HPA-1a-3a-5a/b. As expected, the plasma donor platelets were homozygous for the HPA-5a allele.

**DISCUSSION**

In this report, we describe a patient who developed unexpected thrombocytopenia shortly after transfusion with plasma containing anti–HPA-5b (anti-Zav⁺). The case is unusual for several reasons. First, previous reports describing passive alloimmune thrombocytopenia have implicated the HPA-1 (PIA) system. Our patient had only moderately severe thrombocytopenia (platelet count nadir 35 × 10⁹/L) and did not show any clinical consequences attributable to the thrombocytopenia. In contrast, all six recipients of blood products containing anti–HPA-1a developed severe thrombocytopenia (platelet count ≤20 × 10⁹/L), and five developed bleeding symptoms. The less severe thrombocytopenia, and corresponding lower risk of hemorrhage, could reflect the lower number of GP Ia/IIa molecules on the platelet surface compared with GP IIb/IIIa (approximately 2,000⁻¹⁰⁹ compared with 60,00⁻¹⁰⁹). This parallels the situation in neonatal alloimmune thrombocytopenia and posttransfusion purpura: the severity of thrombocytopenia is less with anti–HPA-1 compared with anti–HPA-5 alloantibodies (summarized in Table 1).

A second unusual aspect of this case is the use of direct radioimmunoprecipitation as the screening investigation is that it permits simultaneous screening for all alloantibodies that have been described to date. Further, radioimmunoprecipitation is capable of detecting alloantigens present in low copy number, such as the HPA-5 and the Gov alloantigen systems.

Concomitant with the onset of the thrombocytopenia, our patient developed an increase in the mean platelet volume (MPV), from 7.5 to 11.3 fl (50% increase). This exceeds the 9% increase in MPV (upper 95% confidence level) described in patients after heart surgery. However, similar marked increases in MPV (25% to 50% at 8 to 24 hours) have been observed in experimental animals rendered severely thrombocytopenic using heterologous platelet antiserum. Our observations are consistent with the animal data, and show that MPV can increase rapidly in humans following acute, moderately severe, immune thrombocytopenia. Although the explanation for the rapid increase in MPV is believed to be release of larger-than-normal platelets from stimulated megakaryocytes, we cannot exclude the possibility that alloimmune platelet destruction causes preferential clearance of small platelets.

In summary, we have observed that the passive transfusion of an HPA-5b alloantibody can cause transient, moderately severe thrombocytopenia. In comparison with passive alloimmune thrombocytopenia caused by anti–HPA-1a, the severity of the thrombocytopenia is consistent with other alloimmune thrombocytopenia, posttransfusion purpura, and is consistent with the concept that the alloimmune thrombocytopenic syndromes differ in severity largely on the basis of the number of antigen sites per platelet. Our studies also show the usefulness of direct platelet radioimmunoprecipitation in the investigation of alloimmune thrombocytopenic disorders.

**ACKNOWLEDGMENT**

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**Table 1. Mean Platelet Count (×10⁹/L) Nadirs in Three Alloimmune Platelet Syndromes, as Related to Platelet Alloantigen System Involved**

<table>
<thead>
<tr>
<th>Alloantigen Typing</th>
<th>Neonatal Alloimmune Thrombocytopenia</th>
<th>Posttransfusion Purpura</th>
<th>Passive Alloimmune Thrombocytopenia</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti–HPA-1 (PIA⁺)</td>
<td>(n = 81) 17</td>
<td>(n = 43) 6</td>
<td>(n = 6) 10</td>
<td>1, 13, 21-23</td>
</tr>
<tr>
<td>Anti–HPA-5 (Zav⁺)</td>
<td>(n = 43) 26</td>
<td>(n = 6) 35</td>
<td></td>
<td>13, 16, present report</td>
</tr>
</tbody>
</table>

Data shown are from the literature and include the patient described in this report. In general, the thrombocytopenia is more severe when the HPA-1 rather than the HPA-5 system is involved, probably because of the higher number of alloantigen sites per platelet. The platelet count nadir (35 × 10⁹/L) in the present report may have been influenced by the mild thrombocytopenia (135 × 10⁹/L) before transfusion with plasma containing anti–HPA-5b.

*Data derived from Kunicki and Beardsley, as follows: the mean platelet count was determined for all patients listed for whom the minimum platelet count was given, and who had been demonstrated to be PIA⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻~-~-


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Thrombocytopenia caused by passive transfusion of anti-glycoprotein Ia/IIa alloantibody (anti-HPA-5b)

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