A 38-year-old woman (JT) was diagnosed with posttransfusion purpura and significant post hysterectomy vaginal bleeding 9 days after the transfusion of 2 U of packed red blood cells. Analysis of JT's serum by a monoclonal antibody-antigen capture enzyme-linked immunosorbent assay method showed the presence of anti-HPA-5b (anti-Br*) antibodies directed against an epitope on platelet glycoprotein (GP) Ia of the GPIa/IIa complex. The patient's serum immunoprecipitated two proteins from [%1-labeled HPA-5b positive platelets that migrated under both nonreducing and reducing conditions on sodium dodecyl sulfate polyacrylamide gels at molecular weights characteristic of GPIa (160 Kd and 165 Kd, respectively) and GPIIa (120 Kd and 145 Kd, respectively).

**POSTTRANSFUSION purpura (PTP) is a severe immunohematologic disorder that typically occurs 7 to 10 days after transfusion of packed red blood cells (RBCs) in patients with an otherwise normal platelet count.** Patients sensitized previously to a platelet-specific antigen, such as HPA-1a (originally termed Pl*), see ref 2 for details of the new nomenclature system for platelet-specific antigens that will be used throughout this report) through pregnancy or transfusion develop an "anamnestic" antibody response to this antigen that is carried by the platelets present in the unit of transfused RBCs. The striking feature of this disorder is that the patient's own nonallogeneic platelets are destroyed, resulting in profound thrombocytopenia with platelet counts typically falling below 10 × 10^9/L (normal range, 150 to 450 × 10^9/L). The condition is frequently accompanied with significant morbidity due to hemorrhage.

The antibody most often associated with PTP is anti-HPA-1a, which is directed against an alloantigen epitope located on glycoprotein (GP) IIIa of the GPIIb/IIIa complex. In addition to anti-HPA-1a, anti-HPA-1b, or anti-Pl* antibodies, anti-HPA-3a or anti-Bak, anti-HPA-3b or anti-Bak, and anti-HPA-4a or anti-Pen have been found to occasionally induce PTP. All of these alloantigens are located on GPIIb or GPIIa, of which there are ~40,000 to 50,000 molecules present on the platelet surface. In contrast, no clear evidence for PTP induced by antibodies directed against the newly described alloantigens, HPA-5a (Br*) and HPA-5b (Br*), has been reported. Unlike the previously characterized alloantigens associated with PTP, HPA-5a and HPA-5b are located on GPIa of the GPIa/IIa complex, for which there are ~1,000 to 2,000 molecules expressed on the platelet surface. Due to the considerably lower density of GPIa/IIa as compared with GPIIb/IIIa, questions have been raised as to whether antibodies produced in adult patients that are directed against HPA-5a and HPA-5b can provoke clinically significant hemorrhagic symptoms. In this report we describe a patient who developed PTP that was provoked by anti-HPA-5b and was associated with significant postsurgical bleeding.

These bands were not precipitated when [%1-labeled HPA-5b negative platelets were used. Platelet typings performed on JT and her three children showed that the patient was HPA-5b negative and one of her children was HPA-5b positive. Platelets obtained from one of the donors who provided blood for the inciting transfusion also typed as HPA-5b positive. These findings demonstrate that posttransfusion purpura may be induced by antibodies directed against an alloantigenic epitope, namely HPA-5b (Br*), located on GPIa/IIa. Moreover, clinically significant bleeding can be associated with antibody reactions directed against this GP complex.

**MATERIALS AND METHODS**

**Patient history.** JT, a 38-year-old white woman (gravida 3ipara 3) was admitted for elective hysterectomy on November 17, 1988. She was iron deficient and intolerant of oral iron. Laboratory tests at the time of admission showed a hemoglobin count of 8.7 g/dL, hematocrit of 28.8%, white blood cells (WBCs) at 15.2 × 10^9/L, and platelets at 203 × 10^9/L. The patient's gynecologist elected to transfuse her with 2 U of packed RBCs (unwashed) the evening (November 18, 1988) before surgery, even though she was young and had no cardiorespiratory problems. She was discharged 4 days later without complications. On November 27, 1988 the patient was readmitted with heavy vaginal bleeding, including large clots. There were no other sites of bleeding and no petechiae or ecchymoses except at previous venipuncture sites. Laboratory tests then showed a hemoglobin count of 8.3 g/dL, hematocrit of 33.9%, WBCs at 14.4 × 10^9/L, and platelets at 26 × 10^9/L. Prothrombin time was 12.6 seconds, partial thromboplastin time (PTT) was 24.8 seconds, and the fibrinogen level was 339 mg/dL, all of which were within normal ranges. Peripheral smear was remarkable only for large platelets and small pale RBCs. Due to continued bleeding, the patient received 8 U of random donor platelets on the night of November 27, 1988 (Fig 1). The following morning, the platelet count was 40 × 10^9/L. No adverse reaction occurred with these transfused platelets. Prednisone (initially at 80 mg/d and then rapidly tapered to 40 mg/d) was administered to the patient on...
November 28, 1988 and the platelet count increased to 89 x 10^9/L by November 30, 1988. Prednisone was discontinued on December 5, 1988, at which time the patient’s platelet count was 287 x 10^9/L. It has remained normal since then.

Platelets. Platelets for use in antibody testing were obtained from ABO type O normal volunteers, the patient (JT, ABO type A), her three children (RT, AT, AB0 type A), and a donor (AB0 type AB), and a donor (AB0 type A) who had provided blood for the initial transfusion. All blood samples were obtained after receiving informed consent and with the approval of the University of Minnesota Committee on the Use of Human Subjects in Research.

Whole blood was anticoagulated with 4 mmol/L EDTA and platelets were isolated by centrifugation as previously described. Platelets were washed three times in phosphate-buffered saline (PBS)-EDTA, pH 7.4) and used for antigen typing and antibody detection as described below.

Monoclonal antibody-specific immobilization of platelet antigens (MAIPA) assay. The MAIPA assay was performed essentially as described. The murine monoclonal antibodies (MoAbs) used for platelet GP detection were: 10E5 and 6D1 (gifts from Dr Barry Coller, SUNY, Stony Brook, NY) directed against the GPIib/IIIa complex and GPIbα, respectively; AP3 (a gift from Dr Peter Newman, The Blood Center of Southeastern Wisconsin, Milwaukee) directed against GPIIIa; FMC25 (a gift from Dr Michael Berndt, University of Sidney, Sydney, Australia) directed against GPIIX; and B1.515 (a gift from Clare Isacke, Ian Trowbridge, and Tony Hunter, The Salk Institute, La Jolla, CA) directed against GPIa (see Fig 3). This latter antibody has similar specificity to 12F1. Antibodies for platelet typing were obtained from patients with PTP, individuals after receiving multiple platelet transfusions, or women alloimmunized through pregnancy (all identified by the University of Minnesota Platelet Serology Laboratory). Anti–HPA-3a (anti-Bak*) was kindly provided by Dr R. Nordhagen (Oslo, Norway) and anti–HPA-5a (anti-Br*) by Dr C. Mueller-Eckhardt (Giessen, Germany).

Other antibody assays. Preliminary analysis of JT’s serum for platelet antibodies and HPA-3a platelet typings were performed by immunofluorescence as previously described. Platelet-associated Igs (PAIgG and PAIgM) were measured by electroimmunoassay as previously described. Immunoprecipitation studies for HLA antibodies were performed by the University of Minnesota Immunology Laboratory, Minneapolis.

Immunoprecipitation. Washed platelets were radiolabeled in PBS, pH 7.8, with 125I (Amersham, Arlington Heights, IL) using lactoperoxidase (Sigma, St Louis, MO) as previously described. Immunoprecipitation of platelet GPs was performed as described. Solubilized platelet GPs (with or without 5% 2-mercaptoethanol) were subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) on 7.5% gels and dried. Autoradiography of the resulting gels was performed with Kodak XAR-5 film (Eastman Kodak, Rochester, NY) and an intensifying screen (Dupont, Wilmington, DE) at −80°C for 1 to 4 days. Molecular weight standards (Life Technologies, Inc, Gaithersburg, MD) labeled with 12C consisted of myosin (200 Kd), phosphorylase B (97.4 Kd), bovine serum albumin (68 Kd), and ovalbumin (43 Kd).

RESULTS

Analysis of acute-phase serum from patient JT showed the presence of lymphocytotoxic antibodies reactive with 41 of 48 panel cells and positive immunofluorescence with two of four platelets with and without chloroquine stripping to remove HLA class I antigens. By immunofluorescence, the patient’s serum did not react with her own platelets. Two months after the initial episode of thrombocytopenia, PAIgG was 11.3 fg/platelet (normal range: 1.9 to 10.3 fg/platelet) and PAIgM was 2.6 fg/platelet (normal range: 0 to 3.4 fg/platelet). The apparent platelet-specific reactions did not correlate with antigens of either the HPA-1 (P10) or HPA-3 (Bak) systems located on the GPIb/IIIa complex, and further attempts by immunofluorescence to determine an antibody specificity were unsuccessful. However, by using the MAIPA procedure, JT’s serum was found to react with platelets that were positive, but not negative, for the HPA-5b antigen located on the GPIa/IIa complex (Fig 2). No reactivity against antigens located on GPIb, GPIIb, GPIIIa, or GPIX was detected. Serum obtained from JT 2

![Fig 1. Platelet counts of patient JT before and after receiving packed RBCs from two donors, one of whom typed as HPA-5b positive. JT received a pool of eight random donor platelets (8 RP) on day 12 that failed to cause a significant increase in the platelet count when measured 24 hours later. Prednisone was started on day 14 and platelets returned to normal levels by day 21. The abcissa represents days from the patient’s initial laboratory work before surgery.

![Fig 2. MAIPA analysis of JT’s serum reacted against donor platelets that typed either as HPA-1b/b, HPA-3b/b, and HPA-5a/a ( ), or HPA-1a/a, HPA-3a/a, and HPA-5a/b ( ). Experiments were performed with MoAbs directed against various platelet glycoproteins as described in Materials and Methods. JT’s serum only reacted with HPA-5b positive platelets in the presence of an MoAb to GPIa and did not react with autologous platelets ( ). Optical density readings less than 0.3 (dashed line) were considered negative.]
months and 14 months following the episode of PTP still possessed potent anti–HPA-5b antibodies that titered greater than 1:50 in the MAIPA procedure. Additional studies by immunoprecipitation demonstrated that the alloantibody present in JT’s acute and convalescent sera, as well as MoAbs 12F1 and B1.515, reacted with two protein bands with nonreduced molecular weights of 150 Kd and 120 Kd (lanes 2 and 3) and reduced molecular weights of 165 Kd and 145 Kd (lanes 9 and 10), which were characteristic of this gel system and consistent with the previously reported molecular weights for GPIa and GPIIa,15,26 respectively (Fig 3). Both AP3 and anti-HPA-1a precipitated two bands consistent in mobility with those of GPIIib and GPIIIa (lanes 4, 7, 11, 14, 18, and 21) from each of the two donors under reduced and nonreduced conditions. No specific reactions with protein bands characteristic of GPIIib and/or GPIIIa or other surface molecules were detected using JT’s serum. In agreement with the MAIPA results, the patient’s antibody immunoprecipitated only with HPA-5b positive (lanes 2, 3, 9, and 10) and not with HPA-5b negative (lanes 16 and 17) platelets.

Platelet-typing studies were performed for the HPA-1a, HPA-1b, HPA-3a, HPA-3b, HPA-5a, and HPA-5b antigens with platelets from patient JT, her three children (RT, AT, and BT), and one of the original two RBC donors. Patient JT typed as HPA-1a/b, HPA-3a/a, and HPA-5a/a (Table 1). Antigen incompatibility was detected in two of the children: RT typed as HPA-3a/b, and, thus, could have sensitized JT to the HPA-3b antigen, and AT typed as HPA-5a/b and, thus, could have sensitized JT to the HPA-5b antigen. Antigen incompatibility was also detected with the donor’s platelets that typed as HPA-5a/b. Further evidence for HPA-5b incompatibility between JT and AT and the donor was obtained by performing cross-matches between JT’s serum and platelets from her children and the donor. Using the MAIPA procedure and the GPIa/IIa-specific MoAb B1.515, positive reactions were only detected with platelets from AT, the donor, and a known HPA-5b positive control (Fig 4).

**DISCUSSION**

Two important findings emerge from this study: (1) PTP may be induced by anti–HPA-5b (anti-Br") antibodies; and (2) reaction of an alloantibody with the GPIa/IIa complex may be accompanied by significant hemorrhagic symptoms. Several lines of evidence support these conclusions, including the onset in patient JT of severe thrombocytopenia (26 x 10^9 platelets/L) 9 days after infusion of 1 U of packed RBCs from an HPA-5b positive donor; the presence of potent IgG anti–HPA-5b antibodies in the patient’s acute-phase serum that reacted with the original donor’s platelets and those of one of her three children; and the presence of substantial posthysterectomy vaginal bleeding (loss of ~1,000 mL of blood), which coincided with the patient’s thrombocytopenia, and was resolved when the platelet count returned to normal (287 x 10^9/L). Moreover, platelets from JT typed as HPA-5a/a and her anti–HPA-5b antibody did not react with platelets that were negative for HPA-5b, nor were other platelet-specific alloantibodies or autoantibodies detected in her serum. HLA antibodies were found in JT’s serum that reacted in the MAIPA assay with platelets from the donor who had provided blood for the inciting transfusion (data not shown), and thus it is possible that HLA antibodies may also have played a role in provoking PTP in this particular case. However, we are
aware of only one publication attributing PTP to HLA antibodies, and no assays for detection of platelet-specific antibodies were performed in that case.27 Importantly, although the role of HLA antibodies in PTP is not clearly understood, all true cases of PTP appear to be associated with platelet-specific antibodies that may or may not include the presence of HLA antibodies.12,28,29 There is a single previous report of anti-Zavb (identical to anti-HPA-Sa) in association with PTP. However, it is significant that the patient also possessed anti-HPA-3a antibodies,30 which are known to be associated with PTP; consequently, the role of anti-Zavb in this disorder could not be ascertained. Thus, the present study appears to provide the first definitive evidence for platelet-specific alloantibodies reacting only with the GPIIa/IIa complex involved in precipitating PTP, and that were also associated with clinically significant problems.

The original report of anti-HPA-5b involved four cases of children born with neonatal alloimmune thrombocytopenia.28 Although platelet counts in the affected newborns ranged from 19 × 10^9/L to 75 × 10^9/L, none of these patients experienced hemorrhagic symptoms. In another study, anti-Hca, which is identical to anti-HPA-5b, was detected in the serum of two patients with systemic lupus erythematosus.29 Neither of these patients was any bleeding associated with the presence of the alloantibodies. However, clinical significance for anti-HPA-5a and -5b has been demonstrated in several polytransfused patients31,32 who developed these antibodies and subsequently became refractory to platelet transfusion. Although these reports do not document the presence of bleeding in the affected patients, it is well known that prolonged lack of response to platelet transfusion is frequently associated with major bleeding complications.

### Table 1. Platelet Antigen Typings of JT, Her Three Children, and One of the Donors Providing Blood for the Inciting RBC Transfusion

<table>
<thead>
<tr>
<th>Platelets</th>
<th>Antigen Typing*</th>
</tr>
</thead>
<tbody>
<tr>
<td>JT</td>
<td>HPA-1a/b, HPA-3a/a, HPA-5a/a</td>
</tr>
<tr>
<td>RT</td>
<td>HPA-1a/a, HPA-3a/b, HPA-5a/a</td>
</tr>
<tr>
<td>AT</td>
<td>HPA-1a/b, HPA-3a/a, HPA-5a/b</td>
</tr>
<tr>
<td>BT</td>
<td>HPA-1a/b, HPA-3a/b, HPA-5a/a</td>
</tr>
<tr>
<td>Donor</td>
<td>HPA-3a/a, HPA-5a/b</td>
</tr>
</tbody>
</table>

*Platelet antigen typings were performed by the MAIPA assay using defined alloantiserum and MoAbs directed against GPIIa (AP3) for the HPA-1a, HPA-1b, HPA-3a, and HPA-3b antigens and GPIb (B1.51B) for the HPA-5a and HPA-5b antigens. HPA-3a typings with RT, AT, and BT platelets were performed by immunofluorescence.

The donor was only typed for the HPA-3 and HPA-5 antigens because JT typed as HPA-1a/b.

All of the platelet-specific alloantibodies, including HPA-1a, HPA-1b, HPA-3a, HPA-3b, and HPA-4a, that have been reported until now in cases of PTP react with GPIIa/IIa. The frequencies of the corresponding antigens among Caucasians are 0.979 and 0.265 (HPA-1a and HPA-1b), 0.877 and 0.641 (HPA-3a and HPA-3b), and 0.992 (HPA-4a). The frequencies of HPA-5a and HPA-5b among Caucasians are 0.992 and 0.206, respectively.33 In a recently published review,34 195 cases of presumed neonatal alloimmune thrombocytopenia were examined and anti-HPA-5b was the second highest in occurrence (4.1%) next to anti-HPA-1a (23.6%). It is estimated that 200 to 300 molecules of bound antibody per platelet are sufficient to provoke platelet destruction in vivo; thus, 1,000 to 2,000 molecules of HPA-5b per platelet should provide adequate antigen density for attachment of anti-HPA-5b and subsequent initiation of thrombocytopenia. Drawing on these observations and the fact that sensitive techniques such as antigen-capture methods are needed for detection of alloantibodies against GPIIa and/or IIa, it seems reasonable to anticipate that additional cases of PTP due to anti-HPA-5b may occur. It is even possible that anti-HPA-5b could account for prior cases of PTP in which no platelet-specific antibodies were detected.

### ACKNOWLEDGMENT

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Posttransfusion purpura due to an alloantibody reactive with glycoprotein la/IIa (anti-HPA-5b)

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