Post-transfusion Purpura:  
A Heterogeneous Syndrome

By Zella Zeigler, Scott Murphy, and Frank H. Gardner

Three new patients with post-transfusion purpura (PTP) are described. As the manifestations in two differ significantly from those of previously reported cases, they serve to expand the definition of this syndrome. Although all 14 previously reported cases have occurred in PlA1-negative females, one of our patients was a PlA1-negative male. Moreover, a female whose postrecovery platelets possessed the PlA1 antigen is described. Antiplatelet antibody activity was detected in all three patients by the 51Cr release test; in contrast, only one reacted in the complement (C) fixation assay. Serum obtained during the acute episode from the PlA1-positive patient reacted against platelets from four of 11 normals by C fixation and against platelets from 48 of 53 normals by 51Cr release, including five of nine PlA1-negative platelet samples. This case represents the first instance of PTP in which the platelet isoantibody was not specifically directed against the PlA1 antigen. These observations suggest that PTP may be a more heterogeneous disorder than previously realized.

Post-transfusion purpura (PTP) is a rare clinical syndrome consisting of thrombocytopenic purpura which occurs approximately 1 wk after blood transfusion. Evidence suggests that the syndrome results from the formation of an isoantibody directed against the platelet antigen, PlA1. At present, it is unclear how the isoantibody can cause destruction of the patient's own platelets. Shulman has suggested that antibodies to PlA1 antigen may form immune complexes with circulating soluble PlA1 antigen. These complexes might interact with autologous platelets in a fashion similar to that of the antibody in quinidine purpura. Fourteen cases have been previously described; all have been women who were sensitized by either pregnancies or previous transfusion. In each case, platelets obtained after the patient's recovery lacked the PlA1 antigen; this immunologic determinant is present in 97% of the population. In the present report, three additional patients with PTP are reported. These include a PlA1-negative female with classic manifestations of this disorder, a PlA1-negative male, and a second female whose platelets were positive for the PlA1 antigen. Thus, it appears that PTP is a more heterogeneous disorder than previously recognized.

In most reported cases, the isoantibody has been detected by complement (C) fixation; however, serum from three patients failed to fix C. Antibody from a fourth patient fixed C with some, but not all PlA1-positive platelet
samples. These results suggested that the classic C fixation assay may lack sensitivity in detecting isoantibody activity in all cases. Further, a patient was reported by Cimo and Aster in 1972 whose serum was negative by C fixation but positive by the $^{31}$Cr release assay. Therefore, comparisons were made between the C fixation test of Shulman and the $^{31}$Cr release assay of Aster and Enright in detecting antibody activity in the present three patients. As shown by the data presented in this study, the $^{31}$Cr release assay proved to be more sensitive for detecting antibodies in this syndrome. The sensitivity could be further increased by using platelets from a patient with paroxysmal nocturnal hemoglobinuria (PNH).

CASE REPORTS

Patient I (Fig. 1)

JB, a 34-yr-old nulliparous woman, was evaluated for abdominal pain and found to have a uterine leiomyoma. Her past medical history included surgery for an ovarian cyst 3 yr earlier; at that time she received 5 u of blood. On June 23, 1972, while undergoing a total abdominal hysterectomy, she received 1 u of blood. A second unit of blood was given on June 30, 1972; concomitantly, she developed an episode of fever and chills. Ampicillin therapy was started. Five days later the patient was noted to have widespread purpura and vaginal bleeding. At that time, the platelet count was 25,000/cu mm, and bone marrow smears showed adequate numbers of megakaryocytes. All medications, including ampicillin, pentobarbital, and phenobarbital, were discontinued, and she was started on prednisone. The platelet count gradually returned to normal over a 25-day period, and it has remained normal subsequently.

Fig. 1. Patient 1: Clinical course of PTP in relation to antibody titer using $^{31}$Cr release assay and complement fixation. The latter was positive for 20 days. In the $^{31}$Cr release test, the duration of positive responses was related to the source of platelets. PNH platelets were more sensitive than normal C fixation-positive platelets which, in turn, were more sensitive than those that failed to fix complement (Tx, transfusion).
POST-TRANSFUSION PURPURA

Patient 2
LS, a 53-yr-old male with a 15-yr history of peptic ulcer disease was admitted for evaluation of melena and syncope. Nine years previously, he developed an episode of bloody diarrhea and received 2 u of blood. At the time of his present admission, persistent melena necessitated administration of 6 u of blood. He had fever and chills with the second and third units. Radiographic studies showed a scarred duodenal bulb with an active ulcer crater. He underwent a hemigastrectomy and gastrojejunostomy. No blood was administered during surgery. On postoperative day 3 (8 days after his blood transfusions), he developed profuse purpura and bleeding. His platelet count at that time was 9000/cu mm. Prothrombin time, partial thromboplastin time, and fibrin split products were normal. He received an additional 2 u of blood and 10 u of platelets. In association with the platelet transfusions, he had a febrile reaction.

He was treated with corticosteroids, gentamicin, and cephalothin. The platelet count returned to normal within 11 days and has subsequently remained normal.

Patient 3
MK, a 51-yr-old GIV PIV woman was admitted for evaluation of profuse rectal bleeding. She had a history of previous transfusion 27 yr earlier in association with one of her pregnancies. On admission, platelet count was 451,000/cu mm. On November 8, 1971, she received 4 u of blood while undergoing resection of a rectal adenocarcinoma. An additional 1 u of blood was given the following day. On November 15, 1971, she developed profuse purpura and bleeding and was found to have a platelet count of 17,000/cu mm. Bone marrow smears were normal, with adequate numbers of megakaryocytes. Prothrombin time, partial thromboplastin time, and fibrinogen levels were normal. She was treated with prednisone, ampicillin, platelet transfusions on November 15 and November 18, and whole blood transfusions on November 18. Her platelet count returned to normal by November 24 and has remained normal subsequently.

MATERIALS AND METHODS

Serum samples were stored at -20°C and heated at 56°C for 30 min prior to use. Quantitative complement fixation studies and assays for platelet agglutination and inhibition of clot retraction were carried out as previously described by Shulman et al.4 Further, lymphocytotoxic antibody screening was kindly performed by Dr. Paul Terasaki on serum from patient 1.

Platelets isolated from the patients after recovery, as well as donor platelets, were typed by C fixation as P1M-positive or -negative using anti-P1M1 serum obtained from the original patient (PK) with PTP. This serum was kindly supplied by Dr. N. R. Shulman. In addition, all the donors of P1M-negative platelet samples had clinical syndromes known to occur with this antigen discrepancy; two had PTP, and two were mothers of children with neonatal thrombocytopenia.

The 51Cr release assay was performed by a modification of the technique previously described by Aster et al.15 It was modified by the use of autologous PPP rather than ABO-compatible PPP, and in the calculation of per cent immune lysis. For this, sera from ten normal subjects were simultaneously evaluated in each assay to determine spontaneous release of 51Cr. This value, used in the formula below, was expressed as the mean cpm + 2 SD of the radioactivity of the supernatants of the ten normal sera. Per cent immune lysis was calculated as follows:

\[
\text{Per cent immune lysis} = \frac{\text{cpm, experimental sample} - \text{cpm (spontaneous release)}}{\text{cpm, 0.1 ml labeled platelets} - \text{cpm (spontaneous release)}} \times 100
\]

A result was considered to be positive if it was reproducibly positive on assays performed on two different days.

Platelets from one patient with PNH, whose cells were P1M-positive,* were also used in this assay system. He was clinically stable, and the diagnosis of PNH was substantiated by positive sucrose hemolysis14 and acid hemolysis tests.15

Previous reports of Dubarry10 and of Gockerman and Shulman12 showed that some anti-P1M sera do not fix C with all P1M-positive platelet samples. Therefore, sera obtained from the two

*Defined by C fixation using serum supplied by Dr. N. R. Shulman.
Table 1. Clinical Summary

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age</th>
<th>Sex</th>
<th>Previous Pregnancy</th>
<th>Previous Transfusion</th>
<th>Onset Purpura After Tx* (days)</th>
<th>Time to Recovery (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>34</td>
<td>F</td>
<td>0</td>
<td>+</td>
<td>11</td>
<td>35</td>
</tr>
<tr>
<td>2</td>
<td>53</td>
<td>M</td>
<td>—</td>
<td>+</td>
<td>8</td>
<td>11</td>
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<tr>
<td>3</td>
<td>51</td>
<td>F</td>
<td>+</td>
<td>+</td>
<td>9</td>
<td>9</td>
</tr>
</tbody>
</table>

*Tx, transfusion.

Table 2. Serologic Data

<table>
<thead>
<tr>
<th>Antibody Tests</th>
<th>Pt.</th>
<th>PiA1 Antigen</th>
<th>C Fixation</th>
<th>Platelet Agglutination</th>
<th>Inhibition of Clot Retraction</th>
<th>51 Chromium Release</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>Positive</td>
<td>Positive</td>
<td>Weakly positive</td>
<td>Negative</td>
<td>1:4</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
<td>1:32</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>Negative</td>
<td>Inconsistent</td>
<td>Weakly positive</td>
<td>Negative</td>
<td>1:4</td>
</tr>
</tbody>
</table>

*Detected with patients' platelets obtained after recovery by complement fixation using serum obtained from Dr. N. R. Shulman.

RESULTS

Table 1 shows the clinical data on each of the three patients. All had received prior transfusions, but only patient 3 had a history of previous pregnancy. Two of the three had received the putative unit of blood during surgery; the male had been transfused prior to, but not during, surgery. The onset of purpura occurred 8–11 days after transfusion, and recovery ranged from 9 to 35 days following the onset of purpura. Autologous platelets obtained from patients 2 and 3, following recovery, were PiA1-negative, but platelets obtained from patient 1 were found to possess the PiA1 antigen.

Serologic data are presented in Table 2. During the acute phase of their illnesses, all three patients had antiplatelet antibodies readily detected by the 51Cr release test. The titer of their antibodies was consistently higher when PNH platelets were used, but PNH platelets were not necessary for detection of this activity. Using the chromium release assay, sera from all three patients, obtained during the period of thrombocytopenia, were nonreactive with their own platelets after recovery. Within several months, antibody activity was no longer detectable using either normal or PNH platelets.

In view of the previous report by Gockerman and Shulman of the failure of an anti-PiA1 serum to react with all PiA1-positive samples, sera from patients 2 and 3 were tested against four PiA1-positive platelet samples and four PiA1-negative platelet samples. As expected, sera from patients 2 and 3 did not yield 51Cr release from any of the PiA1-negative samples. Serum from patient 3 reacted

*Defined by C fixation using serum supplied by Dr. N. R. Shulman.
with all four PI^A^1-positive samples, whereas serum from patient 2 was reactive with only three of the four PI^A^1-positive platelet samples.

In addition to the ^3^H release assay, sera from all three patients were evaluated by the quantitative C fixation technique. A sample from the first patient fixed C with four of 11 normal platelet samples; the second patient’s serum failed to fix C with any platelet suspension, and serum from the third patient gave borderline and inconsistent results. Sera from patients 1 and 3 had weak platelet agglutinin activity; none of the three inhibited clot retraction.

The first patient, whose platelets were PI^A^1 positive, has been studied most extensively. The clinical course as related to antibody titer is shown in Fig. 1. Two units of blood from two different donors had been administered prior to the onset of purpura. The patient’s acute-phase serum was tested against ^3^H-labeled platelets prepared from each donor. Positive results were obtained with samples from the first donor, but not the second. In contrast, C fixation could not be detected with platelets from either donor. As ^3^H release occurred only with platelets from the first donor, it appears likely that this was the causative unit.

The patient’s serum possessed presumed antibody activity which was detectable in the following assays: (1) C fixing activity was demonstrated with four of 11 platelet samples. (2) Acute-phase serum caused ^3^H release from 48 of 53 platelet samples, including five of nine PI^A^1-negative samples. The patient’s serum promoted ^3^H release strongly (greater than 30% immune lysis) with one of four PI^A^1-negative platelet samples in our laboratory. Dr. Richard Aster kindly confirmed that her serum promoted ^3^H release from four of five PI^A^1-negative samples in his laboratory.

The patient’s serum reacted with 10% of the panel in the lymphocytotoxic assay, but no HL-A specificity could be demonstrated. In contrast, positive results were obtained in the ^3^H release test with 48 of 53 platelet samples, 32 of which were strongly positive with PNH platelets (greater than 30% immune lysis). Therefore, it seems unlikely that the activity in ^3^H release can be accounted for solely by the presence of anti-HL-A antibodies.

The ^3^H release assay was more sensitive than C fixation because radioisotope was released from three of seven platelet samples which did not consume complement. In all instances where complement was fixed, ^3^H release also occurred. Moreover, the ^3^H release test remained positive for a longer period of time than did C fixation.

The use of PNH platelets enhanced the sensitivity of the ^3^H release test. Antiplatelet activity was present in serum dilutions of 1:32 as compared to 1:4 with normal platelets. Prolonged detection of this activity was seen with PNH platelets. However, 8 mo after the onset of purpura, antibody activity was no longer detectable.

DISCUSSION

Post-transfusion purpura (PTP) is a syndrome characterized by acute transient thrombocytopenia which follows blood transfusion. Current concepts suggest that the transfusion recipient develops an antibody to a major platelet antigen (the PI^A^1 antigen) which, in some as yet undefined manner, causes the
rapid destruction of autologous platelets. The data presented in this study indicate that the syndrome is a more heterogeneous disorder than previously recognized; it can occur in men as well as women and is not limited to 

\( \text{P1A}^1 \)

negative individuals. Comparison of two available assays for detection of antiplatelet factors, \( ^{51} \text{Cr} \) release, and quantitative C fixation, indicates that \( ^{51} \text{Cr} \) release is a more sensitive test. The latter is capable of detecting antiplatelet activity in sera which show no reactivity in the C fixation assay.

All of the 14 previously reported patients with PTP and two in the present series were found to have 

\( \text{P1A}^1 \)-negative platelets. However, as illustrated by the first patient in this series, a similar syndrome can occur in an individual who has 

\( \text{P1A}^1 \)-positive platelets. This patient fulfills the following criteria needed to establish the diagnosis of PTP: (1) The clinical sequence of events was consistent with previously reported cases; (2) the antiplatelet factor present in her serum during the acute phase of her illness reacted with normal platelets, including those of the donor of the blood administered 11 days prior to the onset of purpura, but did not react with her own platelets obtained following recovery; and (3) the presumed antibody activity as detected by C fixation and by 

\( ^{51} \text{Cr} \) release using normal platelets was no longer detectable 6 wk after the acute episode. This is compatible with the time course of detection of antibody in previously reported cases. An alternative possibility would be that the patient may have received an isoantibody in the second transfusion. This is thought to be unlikely, as the donor of the second unit was a male who had never been transfused and who therefore lacked a reason for possessing isoantibodies. Since her platelets were 

\( \text{P1A}^1 \)-positive and her serum promoted \( ^{51} \text{Cr} \) release from five of nine 

\( \text{P1A}^1 \)-negative platelet samples, she is the first example of PTP in which the 

\( \text{P1A}^1 \) antigen is not involved.

Using this patient's serum, \( ^{51} \text{Cr} \) release was classified as weakly positive with 16 platelet samples and strongly positive with 32. Since her serum promoted \( ^{51} \text{Cr} \) release with 90% of platelet samples and was reactive with only 10% of the panel in the lymphocytotoxic assay, it seems unlikely that the activity in \( ^{51} \text{Cr} \) release can be accounted for by anti-HL-A antibodies. Perhaps the differential release of \( ^{51} \text{Cr} \) reflects differences between homozygous and heterozygous platelets for an as yet undefined antigen. It has been previously shown that 

\( \text{P1A}^1 \)-positive platelets can be classified into two groups by quantitative C fixation tests; homozygous platelets fix approximately twice as much C as heterozygous platelets. Alternatively, the differences in \( ^{51} \text{Cr} \) release might represent a summation effect of multiple antibodies.

In all three patients, \( ^{51} \text{Cr} \) release was appreciably more sensitive in detecting antibody activity than C fixation. Only one patient was unequivocally positive in the C fixation test. In contrast, all three showed significant isotope release from radioactively labeled platelets. The sensitivity of the \( ^{51} \text{Cr} \) release assay could be increased by using PNH platelets. As shown in Table 2, the serum titer was higher (fourfold or greater) than with normal platelets. C fixing antibody activity in patient 1 disappeared before that activity promoting \( ^{51} \text{Cr} \) release using either normal or PNH platelets.

As shown in the \( ^{51} \text{Cr} \) release assay, the anti-\n
\( \text{P1A}^1 \) antibody from patient 2 did not react with one of the four 

\( \text{P1A}^1 \)-positive platelet samples. A similar phenom-
enon has been observed in the C fixation assay. Gockerman and Shulman showed that all PI\textsuperscript{A1}-positive platelets would adsorb antibody activity, but not all would fix C. A similar adsorption pattern has not been tested in the \textsuperscript{51}Cr release assay. However, it appears by either test that some PI\textsuperscript{A1}-positive platelets are not suitable for antibody detection in PTP. These data demonstrate that it is important to employ a panel of platelets in evaluating serum from patients suspected of having PTP.

In summary, it appears that not all patients with the clinical syndrome of PTP develop C-fixing antibodies. The results obtained in the present study, as well as in the patient of Cimo and Aster, suggest that the \textsuperscript{51}Cr release assay is a more sensitive test for detection of isoantibodies in this syndrome. In addition, PTP is a more heterogeneous disorder than previously appreciated. Our findings further indicate that the syndrome of PTP is not confined to females or to PI\textsuperscript{A1}-negative individuals.

ACKNOWLEDGMENT

We wish to thank Dr. Peter Cassileth, Dr. Francis Rosato, Dr. Abraham Frumin, and Dr. Frank DeMaio for referring these patients for study and Ms. Dolores Piperno for technical assistance. Dr. N. Raphael Shulman kindly provided us with complement-fixing anti-PI\textsuperscript{A1} serum and advice. Dr. Richard Aster assisted us by extending results obtained in the \textsuperscript{51}Cr release assay in patients 1 and 2. Dr. Paul Terasaki kindly performed lymphocytotoxic antibody screening on serum from patient 1.

REFERENCES

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