POST-TRANSFUSION PURPURA: IMMUNOLOGIC ASPECTS AND THERAPY

Neil Abramson, M.D., Peter D. Eisenberg, M.D., and Richard H. Aster, M.D.

Abstract To determine the mechanisms of post-transfusion thrombocytopenia, platelet antibodies were studied in a 45-year-old woman in whom severe thrombocytopenia with lingual and buccal hemorrhagic bullae and purpura developed seven days after the transfusion of whole blood. Her platelets were later typed as PIA 1 negative, and her plasma and serum contained anti-PIA 1 and anti-HLA antibodies. Antibody activity was demonstrated by microscopical agglutination and lysis, by aggregation with use of an aggregometer, and by $^{51}$Cr release. Antibody activity, as measured with the aggregometer, seemed restricted to the IgG 3 class of immunoglobulin. Plasmapheresis resulted in clinical improvement associated with disappearance of antibody activity. Thus, this patient, with post-transfusion purpura demonstrated anti-PIA 1, and anti-HLA (IgG 3 ) anti-platelet antibodies. Plasmapheresis with removal of the antibody proved effective therapy. (N Engl J Med 291:1163-1166, 1974)
A diagnosis of post-transfusion purpura was made, and on the 14th hospital day (13 days after the first unit of transfused blood and five days after the onset of purpura), plasmapheresis was begun. Blood was removed one unit at a time into a standard acid citrate dextrose plastic collection bag,* and centrifuged at 2100 rpm at 4°C for 20 minutes. The plasma was expressed, and remaining red cells were resuspended in saline and retransfused. Intravascular volume was maintained by infusion of 5 per cent dextrose in 0.5N saline. Twenty-five grams of albumin was transfused for every 2 units of plasma removed.

At the end of the first day of plasmapheresis, during which 7 units (approximately 1550 ml) were removed, the platelet count was 7000, and an occasional platelet was noted on the blood smear. The next morning the platelet count was 2000, and plasmapheresis was re instituted. Over the next two hospital days, 9 and 6 units of plasma (approximately 2000 and 1350 ml) were removed respectively. The entire procedure was without complication except for transient hypotension during the removal of the last unit of blood on the first day. As shown in Figure 1, over the next several days the platelet level rose to 17,000, 27,000, and 156,000. The patient became asymptomatic, and the platelet level has remained normal during the ensuing 12 months.

**METHODS**

Platelet counts were done by phase microscopy. Platelet antibody was assessed in several ways. Agglutination and lysis were first observed after the addition of one part of normal platelet-rich plasma to four parts of test plasma (citrate or acid citrate dextrose). This mixture was observed by light microscopy immediately and after a 30-minute incubation at 37°C. Agglutination was judged by clumping of platelets, and lysis by the appearance of platelet stromal clumps. Also, aggregation was studied with a platelet aggregometer (Chrono-Log) with use of modified techniques previously reported.* To one part of platelet-rich plasma from a normal person one part of test plasma was added, and observations were made for spontaneous aggregation. After at least three minutes of observation, ADP was added. When aggregation took place during the primary incubation, ADP induced no further aggregation (ADP unresponsiveness). Serial dilutions of the test plasma were made; the antibody titers recorded are the highest dilutions at which aggregation in the primary mixture and ADP unresponsiveness were noted. The IgG subclass of the anti-platelet antibody was evaluated by the addition to the patient’s plasma of purified anti-IgG subclass antiserum (prepared, purified, absorbed, and judged for specificity as previously noted). To five parts of the patient’s plasma or normal plasma, one part of the purified anti-IgG subclass antiserum (prepared, purified, absorbed, and judged for specificity as previously noted) was added. After a two-hour incubation at 37°C the mixture was spun to remove any precipitate and supernatant added to an equal quantity of platelet-rich plasma from a normal person. Aggregation and, subsequently, ADP responsiveness were again judged with use of the aggregometer. Finally, 51 Chromium release, using PIA1-positive and PIA1-negative platelets, was used for identification and measurement of platelet antibody as previously described.5,6

![Figure 1. Hospital Course of Patient.](image)

Ordinate to right refers to antibody levels expressed as a percentage of initial "A-HLA" and "A-PIA" activity as measured by 51Cr release. "Ab (Aggr.)" refers to antibody activity measured by aggregometer. Each arrow under blood denotes a transfusion, and the numbers under plasmapheresis indicate the units of plasma removed.

In mixing experiments, the patient’s plasma caused immediate aggregation of normal platelets. After a 37°C incubation for 30 minutes, lysis occurred. Figure 2 shows the platelet aggregation and ADP responsiveness of normal (ABO-compatible) platelets incubated in the patient’s or in normal plasma. The patient’s plasma caused spontaneous aggregation whereas, as expected, no aggregation occurred with normal plasma and normal platelets until ADP was added. However, when ADP was added to the patient’s plasma and normal platelets after spontaneous
aggregation, no further aggregation could be induced. The aggregating factor in the patient's plasma persisted after heating at 56°C for 30 minutes.

Figure 1 shows the clinical course and the effect of plasmapheresis on antibody levels. Antibody activity is expressed as a percentage of initial activity with use of the aggregometer and ¹¹¹I release measurement methods. Antiplatelet antibody activity decreased notably after the first day's plasmapheresis, during which the platelet count rose; by the next morning the antibody activity rose again and the platelet count returned to approximately 1000. Anti-platelet antibody, as measured by the aggregometer method, paralleled that of anti-PIAI measured by ¹¹¹I release with use of HLA-compatible PIAI-positive platelets. When antibody was evaluated by the ¹¹¹I release technic with use of PIAI-positive and PIAI-negative platelets, two antibodies were noted to be present. The major one reacted in high dilution with PIAI-positive but not with PIAI-negative platelets and therefore appeared to be a typical anti-PIAI antibody. The second antibody reacted undiluted with platelets from PIAI-negative donors and with the lymphocytes of 22 of 25 donors in conventional lymphocytotoxicity assay, suggesting that a mixture of anti-HLA isoantibodies was present. Three weeks after the thrombocytopenia occurred, the patient's platelets were typed as PIAI negative, and the pre-plasmapheresis plasma was found to have no antibody activity against her platelets.

Absorption experiments were performed with use of purified anti-IgG subclass antisera as shown in Table 1. Anti-IgG₁, IgG₂, and IgG₃ did not affect platelet antibody activity, whereas anti-IgG₄ removed it completely. At a 1:10 dilution of anti-IgG subclass antiserum, no effect was observed.

Table 1. Antibody Activity after Addition of Anti-IgG Subclasses or Saline.*

<table>
<thead>
<tr>
<th>PLASMA</th>
<th>A-IgG₁</th>
<th>A-IgG₂</th>
<th>A-IgG₃</th>
<th>A-IgG₄</th>
<th>A-IgG₄</th>
<th>SALINE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>A</td>
<td>A</td>
<td>A</td>
<td>A</td>
<td>A</td>
<td>A</td>
</tr>
<tr>
<td>Patient</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>0</td>
<td>0</td>
<td>+</td>
</tr>
</tbody>
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* Denotes undiluted, & B 1:10 dilution.

Soluble PIAI antigen was sought in the serum and plasma by inhibition of platelet lysis with use of an antibody of known anti-PIAI specificity. No evidence of soluble PIAI antigen was found in the serum of any of the donors whose blood was transfused to this patient.⁶,⁷

**DISCUSSION**

The thrombocytopenia that occurred in this patient provides yet another example of "post-transfusion purpura."³ The patient's serum contained an anti-PIAI antibody 1½ weeks after initial transfusions, and the platelets were later found to be PIAI negative. Like all but one of the previous patients,² she was a woman who had previously been pregnant. Upon transfusion after the onset of symptoms, the characteristic chill and fever of a platelet-antibody reaction developed. The antibody was a heat-stable IgG globulin with specificity directed against PIAI. In addition, a second antibody was present in this case, which reacted with platelets and lymphocytes from a number of donors, suggesting that it had HLA activity; it is likely that this antibody (or antibodies) was unrelated to the thrombocytopenia, since it persisted after clinical remission.

Antibody activity was demonstrated by aggregation of platelets. The aggregometer provided a convenient and rapid means of determining antibody activity. Our data suggest that the technic can also be used to identify the IgG subclass of the antibody causing platelet aggregation. It is of interest that other workers have suggested that platelet autoantibodies of idiopathic thrombocytopenic purpura are also of the IgG₂ subclass.⁸

In all the previous cases of post-transfusion purpura, with one exception, the patients' platelets have been negative for the PIAI antigen. PIAI antigen is absent in only 2 per cent of the general population. Since antibody is formed in PIAI-negative persons when PIAI-positive platelets are infused, one would expect this disease to occur more frequently than reports indicate. A unique feature of this disorder is the destruction of recipient platelets, which are PIAI negative and presumably do not react directly with anti-PIAI isoaantibody. It has been hypothesized that destruction of autologous platelets is related to quantity of PIAI antigen eluting from donor platelets or to antigen-antibody complexes absorbing to recipient platelets. Serum of donors whose blood was transfused to our patient was tested for soluble PIAI antigen, but none was detected by the method used.

In previous reports of post-transfusion purpura, severe, life-threatening thrombocytopenia has lasted for as long as five weeks and has not been affected by treatment with corticosteroids. Remission has been induced in at least two cases by exchange transfusion with whole blood;¹,³; however, these were attended by severe chill-fever reactions and, in one case, severe hypotension. With plasmapheresis, of course, in vivo antigen-antibody reactions are avoided. As shown in Figure 1, after the first day's plasmapheresis, antibody activity was definitely decreased; however, by the next morning, levels increased. In this connection, it is of interest that platelets were visible on the peripheral smear after the first day's
plasmapheresis, but disappeared by the next morning. Since the anti-platelet antibody was of the IgG class, presumably only 50 per cent was present in the intravascular compartment. It is likely, therefore, that the increase in plasma antibody that appeared overnight was related to a compartmental shift. Also, synthesis may have occurred. It can be noted that the antibody activity decreased to an even greater degree over the next several days and, at the same time, the platelets rose to normal levels. Although clinical improvement might have occurred after only two days of plasmapheresis, a third day of this therapy was undertaken as a precaution, in view of the safety of the procedure. This method of therapy appears to be effective and safe and circumvents the potential hazards of exchange transfusion.

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SPECIAL ARTICLE

ADVOCACY AND COMPLIANCE IN GENETIC SCREENING

Behavior of Physicians and Clients in a Voluntary Program of Testing for the Tay–Sachs Gene

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Abstract The relation between advocacy and compliance was evaluated in a voluntary testing program to identify carriers of the Tay–Sachs gene among 30,000 eligible subjects. The overall compliance rate was 88 per cent, and among subgroups of clients it was 27.6 per cent in final-year high-school students, 20 per cent in college students, 10.8 per cent in newlyweds, and 8.1 per cent in “random” eligible adults. Poor specific knowledge and lack of motivation, despite equivalent exposure to the screening option, greatly influenced the nonparticipant group by comparison with compliant subjects.

The physician is the most powerful potential advocate of testing, for the Tay–Sachs gene, but he performs poorly in this role. Compliance rates can be improved in the program when its advocates are well informed and motivated. Our findings indicate a need for improved perception of the personal relevance of heredity, not only among clients of genetic screening programs but also among the personnel who function as advocates. (N Engl J Med 291:1166-1170, 1974)

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REFERENCES