Selection of Histocompatible Apheresis Platelet Donors by Cross-Matching Random Donor Platelet Concentrates

By Bernadette A. O'Connell, Edward J. Lee, Kate Rothko, Mohamad A. Hussein, and Charles A. Schiffer

It can be impossible to identify compatible platelet donors for alloimmunized patients whose HLA type cannot be determined or who have uncommon HLA types. We have previously shown that histocompatible donors can be rapidly identified by "mass screening" of platelet concentrates (PC), which are readily available in all blood banks, using a solid-phase adherence platelet cross-matching technique. Compatible PC were given to five alloimmunized patients with multispecific HLA antibodies refractory to random donor (RD) PC and selected single-donor platelet transfusions. After transfusions which produced satisfactory responses, we identified the original whole blood donors to serve as apheresis donors. Thus, the donors selected were compatible in vitro by cross-matching, and in vivo by transfusion. Only 3% to 13% of PC cross-matched for these alloimmunized patients were potentially compatible and it was necessary to screen large numbers (65 to 205 U) of PC per patient.

The provision of histocompatible platelet transfusions for the alloimmunized patient remains one of the most difficult problems in transfusion medicine. Despite advances in donor identification by HLA typing, 25% to 45% of HLA-matched, single-donor platelet transfusions do not result in successful responses.1,4 Due to the polymorphism of the HLA system, HLA compatible donors are not always available for patients with less common HLA phenotypes.5,6 Furthermore, it is sometimes impossible to determine the patient's HLA type at the time of diagnosis of leukemia because of difficulties in separating leukemia cells from lymphocytes or using blasts for cytotoxic testing. Several antiplatelet antibody assays suitable for platelet cross-matching have been developed using micro-enzyme-linked immunosorbent assay (ELISA), radioimmune, solid-phase adherence, or immunofluorescence techniques. Retrospective studies have indicated that similar results can be obtained with donor selection by either HLA typing or platelet cross-matching,7,10 with the suggestion that a combination of the two approaches might be preferable.12,13 However, there are few large-scale prospective studies selecting apheresis donors by platelet cross-matching.14

We have previously described the results of transfusions selected by cross-matching sera from alloimmunized patients with units of random donor (RD) platelet concentrates (PC).14 Approximately 70% of the in vitro compatible transfusions produced satisfactory posttransfusion count increments. In this report, we demonstrate the feasibility of recruiting the whole blood donor of the cross-match compatible PC to be a plateletpheresis donor for a specific patient. The advantage to this approach is that the apheresis platelets have already been "pretested" both in vitro by cross-matching and in vivo by transfusion. We report the results of such a strategy in the platelet transfusion therapy of five alloimmunized patients with acute leukemia or myelodysplasia and protracted thrombocytopenia.

MATERIALS AND METHODS

Recipients. R.H. was a 60-year-old woman with acute myeloid leukemia (AML) whose transfusion course is illustrated in Fig 1. Eighteen of 22 PC-selected transfusions produced satisfactory increments, allowing selection of 12 donors, all of whom were willing to undergo apheresis. Ten of 12 of these single-donor transfusions were successful; the two unsuccessful transfusions were infused 2 weeks after the initial PC cross-match and were still compatible with the original serum, but incompatible with more recent serum, demonstrating a change in antibody reactivity. The HLA types of the successful single donors selected by PC cross-matching differed widely from the patients' HLA types and, therefore, these donors would not have been selected by standard approaches using HLA typing. Cross-matching large numbers of RD PC for the identification of apheresis donors is helpful in the management of the alloimmunized patient and may be of particular utility for blood centers that do not have access to HLA-typed donor pools.

Due to poor cell viability at admission, her HLA type could not be established. Her lymphocytotoxic antibody (LCTAb) at the start of induction therapy was negative, but became strongly positive 2 weeks later. Initially, she had a good response to transfusions of RD PC, but gradually became nonresponsive 2 weeks after treatment. She failed to achieve remission with initial induction chemotherapy and was retreated with additional chemotherapy, which prolonged her thrombocytopenia. During the next 8 weeks, patient R.H. received multiple transfusions of cross-match compatible PC and selected apheresis donor platelets whose PC had previously been effective. Her HLA type was determined between the two courses of chemotherapy so that some HLA-matched donor platelets could be provided later in her course as well.

L.L. was a 36-year-old man with AML who became refractory to transfusion with RD PC at the end of his induction therapy. He had a common HLA type (A3, B7) (A24, B18) and was readily supported with HLA-matched donors. He failed to achieve complete remission and was retreated with another course of chemotherapy. During this period of thrombocytopenia, he had poor increments following most of the HLA-matched platelet transfusions, even though his clinical status was stable with no evidence of bleeding, infection, or hepatosplenomegaly. Compatible platelets identified by cross-matching techniques were used during this period.

C.D. was a 34-year-old woman with AML who became refractory to RD platelet transfusions at the end of her induction chemotherapy. She had an unusual HLA type (A30, A32) (B14, B63), making
donor selection by HLA matching difficult. During her remission, she underwent plateletpheresis and platelets were cryopreserved for future autologous transfusions as previously described. Her leukemia relapsed 1 year later, requiring frequent transfusions and exhausting the autologous frozen stores, thus necessitating the use of the cross-matched PC approach.

C.G. was a 61-year-old man with severe thrombocytopenia due to myelodysplasia and hemorrhagic esophagitis who was refractory to RD platelet transfusion. He had an acute episode of gastrointestinal bleeding, which required multiple platelet and red blood cell transfusions. Very few HLA-matched donors were available for his particular HLA type (A2, B13) (A32, B62), necessitating the cross-match (X-M) compatible PC approach.

B.M. was a 40-year-old woman with AML who initially responded well to RD platelet transfusions, but became refractory to transfusion 2 weeks after chemotherapy was begun. Only a few poorly HLA-matched donors were available due to her unusual HLA type (A29, A30) (B35, B53).

All five patients had lymphocytotoxic antibodies in their serum that were reactive with greater than 80% of the lymphocytes in a cell panel at the time they were refractory to platelet transfusion. LCTAb assays were repeated frequently during the period of platelet transfusion and remained strongly reactive (Fig 1).

Platelet recovery. Posttransfusion platelet counts were obtained 10 minutes after transfusion. The results were expressed as a corrected count increment (CCI), where CCI = posttransfusion platelet count – pretransfusion platelet count × body surface area (m²)/number of platelets transfused × 10¹¹. All platelet counts were performed using laser technology on an Ortho ELT-15 blood cell counter (Ortho, Raritan, NJ).

Donor selection procedure. Samples of RD PC obtained from the integral tubing of each unit were cross-matched with patient sera by a solid-phase red blood cell adherence assay using the Capture P kits (Immucor, Norcross, GA) as previously described. In vitro compatible PC were administered in packs of three concentrates or less. The American Red Cross, Chesapeake and Potomac Regional Blood Services was then provided with the identification numbers of those transfused platelet concentrates that had produced CCIs greater than 10,000. Ordinarily, a CCI of 7,500 is judged to be a “successful” transfusion; thus, a more rigorous cutoff was used in this study. Whole blood donors who were sources of a compatible PC were recruited to be apheresis donors. Platelets were obtained using a Fenwal CS 3000 cell separator (Fenwal, Deerfield, IL). Blood for HLA typing was drawn at the time of apheresis. Each platepheresis transfusion was administered within 2 weeks of the original PC cross-matched transfusion. Cross-matches were repeated on the apheresis platelets using serum from the original cross-match date and serum obtained just before the administration of the apheresis-derived platelets.

RESULTS

Platelet concentrate transfusions. A total of 205 RD PC were cross-matched for patient R.H. Only 11 PC (5%) were considered compatible. These 11 PC were administered as five different transfusions; three transfusions consisted of three PC and two transfusions of a single PC each (Table 1). Four of the five transfusions produced CCIs greater than 13,000. Of note are the small absolute changes in the platelet counts after the two single-unit PC transfusions.

Patient L.L. had 154 PC cross-matched and 17 (11%) were compatible. A total of six transfusions were administered, with a range of one to four PC per transfusion. All transfusions resulted in CCIs of greater than 12,000.

Patient C.D. only had three PC (3%) compatible of the 109 PC cross-matched for her over a 2-week period. Three transfusions of single units of PC were given, of which two produced CCIs of greater than 15,000. Again of note are the small absolute platelet count increments.

Only three (5%) PC were compatible of the 65 PC cross-matched for patient C.G. The compatible PC were administered as three different transfusions, two of which produced CCIs greater than 16,000.

B.M. had 99 PC cross-matched over a period of 2½ months, of which only 13% were compatible. Five transfusions were administered, of which four produced CCIs of greater than 11,000.
These five patients were transfused between January 1990 and June 1991. During that 1½-year period, 12 other alloimmunized patients were evaluated by platelet cross-matching. No compatible units could be identified for three patients; six patients with less broadly reactive antibody had CCIs greater than 10,000 following transfusion of multiple cross-match selected PC, but did not have single donors selected because of the ready availability of HLA-compatible donors; three patients had poor increments following transfusion of the selected PC. Complicating clinical factors may have influenced the results of transfusion in two of these latter patients.

**Apheresis donor transfusions.** A total of 14 PC from eight different transfusions administered to either patient R.H., L.L., C.D., C.G., or B.M. were identified as potential candidates for apheresis recruitment. In general, we selected PC transfusions that produced the highest CCI or the most recent successful transfusion. The blood center was provided with the identification numbers of each of the platelet concentrates and was able to recruit 14 donors for apheresis. Twelve of these donors were plateletpheresed within 2 weeks of their whole blood donation and selection of their platelets by cross-matching. One donor was disqualified due to a low hematocrit; the other had poor venous access precluding apheresis.

Patient R.H. received six transfusions from five different donors (Table 2, Fig 1). Four transfusions from three donors produced satisfactory increments, with CCIs between 10,900 and 28,500. Two of these four transfusions were from the same donor. Transfusions from donors A and B produced poor platelet responses. Repeat cross-matches with fresh serum obtained before the apheresis transfusions were incompatible with these two donors. The repeat cross-matches with the original serum obtained at the time of the initial platelet concentrate cross-match remained compatible with each donor’s apheresis platelets, suggesting the development of new antibody over the 2-week period.

Patient L.L. received two plateletpheresis transfusions from two different donors. The absolute changes in the platelet counts were not large in either transfusion due to low platelet yields in the apheresis products. However, both had acceptable CCIs of 8,800 and 8,700. The original serum from the day of the PC transfusions and serum from the day of the apheresis transfusions were compatible with the apheresis platelets from both donors. This was also the case with patients C.D., C.G., and B.M. C.D. and C.G. each received one apheresis transfusion selected from a single PC transfusion, with excellent CCIs of 17,300 and 22,600, respectively. The donor for patient C.G. underwent plateletpheresis again 1 month later, producing a CCI of 13,600. The donor selected for B.M. donated within 2 weeks of the initial cross-match, but this transfusion was not administered because bone marrow recovery had begun, making transfusion unnecessary. Two months later, the same individual donated again, resulting in a CCI of 30,800 (Table 2). Thus, in some patients, this approach may permit longer term platelet support.

Overall, 10 of the 12 apheresis donor transfusions produced satisfactory CCI 10 minutes posttransfusion. Furthermore, six of seven transfusions for which data were available resulted in CCIs greater than 5,000 at 24 hours after transfusion. Although patient C.G. had a CCI of only 2,200 at 24 hours, the transfusion produced a marked decrease in his gastrointestinal bleeding.

HLA types were determined on all but one of the apheresis donors. The blood was drawn on the day of the plateletpheresis, so the HLA types were unknown at the time of the transfusion. As shown in Table 3, the donors selected by PC cross-matching had different HLA types.

<table>
<thead>
<tr>
<th>Donor</th>
<th>Platelet Count Increment</th>
<th>CCI</th>
<th>Cross-match*</th>
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<tr>
<td>A</td>
<td>0</td>
<td>0</td>
<td>Neg/pos</td>
</tr>
<tr>
<td>B</td>
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<td>0</td>
<td>Neg/pos</td>
</tr>
<tr>
<td>C</td>
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<td>D</td>
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<td>28,500</td>
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<td>26,900</td>
<td>Neg/neg</td>
</tr>
<tr>
<td>F</td>
<td>11,000</td>
<td>8,900</td>
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<tr>
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</tr>
<tr>
<td>L</td>
<td>72,000</td>
<td>30,800</td>
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*The apheresis platelets were cross-matched with both the original and most recent sera.
of which were serologically cross-reactive with L.L.'s anti-HLA matching alone. For example, patient R.H. was typed as (A31, A32) (B27, B40). Donors C and E, whose transfusions produced excellent increments, had no antigens in common with patient R.H. Similarly, the donors identified for patient L.L. both had two mismatched antigens, neither of which were serologically cross-reactive with L.L.'s antigens. The only PC-selected apheresis transfusion administered to patient C.D. was not HLA-typed. The other donor recruited was plateletpherased and HLA-typed, but the platelets were not transfused because the patient's count had begun to increase concurrent with bone marrow recovery.

**DISCUSSION**

These results show that platelet cross-matching can select apheresis donors who would otherwise not have been chosen by HLA matching alone. For example, patient R.H. was typed as (A31, A32) (B27, B40). Donors C and E, whose transfusions produced excellent increments, had no antigens in common with patient R.H. Similarly, the donors identified for patient L.L. both had two mismatched antigens, neither of which were serologically cross-reactive with L.L.'s antigens. The only PC-selected apheresis transfusion administered to patient C.D. was not HLA-typed. The other donor recruited was plateletpherased and HLA-typed, but the platelets were not transfused because the patient's count had begun to increase concurrent with bone marrow recovery.

Successful transfusions also provided in vivo confirmation of the suitability of potential apheresis donors. It was then possible to select additional donors with HLA types similar to the successful PC-selected donors. All whole blood donors were willing to donate again in a relatively short period when the need for their platelets was explained. Many of these whole blood donors have now become regular apheresis donors at the blood center.

To date, we have used this approach when HLA-matched donors cannot be identified. When perfectly HLA-matched donors are not available, we currently choose HLA-mismatched antigens based on the specificity of the lymphocytotoxic antibodies present in the patient's serum and the cross-reactivity of the recipient's and donor's antibodies. All of the patients in this report had lymphocytotoxic antibody reactive with greater than 80% of the lymphocytes in the test panel. Because at least two and sometimes all of the donor antigens were mismatched and not cross-reactive with the patients' antigens, none of the donors selected by PC cross-matching would have been identified by HLA-matching. Furthermore, in some instances, the patient had specific lymphocytotoxic antibody directed against these mismatched antigens. Nonetheless, several of the cross-match selected transfusions produced CCIs greater than those of the HLA-matched transfusions. There is no obvious explanation for the good results obtained with the grossly HLA-discrepant platelets in these patients with broadly reactive anti-HLA antibody. It is known that there can be wide variability in the degree of HLA antigen expression between the platelets and lymphocytes from the same individual (particularly for HLA B44), but it is unlikely that such variable expression occurs for all of the mismatched antigens in these donors. Lymphocytotoxic cross-matches were not performed to further explore this possibility. It is also possible that some of the widely reactive antibodies were directed against common "public" antigens not shared by the "successful" donors. However, the antibodies in this study were not characterized in this fashion.

One disadvantage to this selection process is that cross-matches with 100 to 200 PC may be required to find enough donors for adequate platelet support for heavily alloimmunized patients. This may take several days depending on the size of the platelet inventory available. Although this approach should theoretically also be applicable to selection among inventories of stored single-donor platelets, few blood centers have sufficiently large stores of such products available to allow for such large-scale donor screening. Whether this is a viable option for alloimmunized patients with less broadly reactive antibody remains to be determined in further clinical experiments.

At times, only one or two PC may be compatible for transfusion. These few concentrates may provide an apparently "acceptable" CCI, but the absolute change in the platelet count may not be large. The calculation of the CCI is obviously dependent on careful and accurate platelet counts in both the platelet bag and pre- and posttransfusion. However, as demonstrated by the results using donors, D, E, I, and K (absolute count increments following 1 U PC transfusions of 4,000 to 9,000/μL), accurate and clinically meaningful assessments of increments are feasible using current technology.

The approach we have described is capable of rapidly identifying compatible PC for use in emergency situations, as well as providing options for long-term support of alloimmunized patients with prolonged thrombocytopenia. It may be even more successful in patients who are less markedly alloimmunized than the patients described in this report. In addition, this strategy may be of particular value in the many blood centers that do not have ready access to HLA-typed donor pools. The discrepancy between the
donor/recipient HLA types is intriguing and suggests that a comparative trial of this approach compared with donor selection by HLA typing and perhaps antibody characterization would be of interest, particularly for less heavily sensitized recipients.

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REFERENCES


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BA O'Connell, EJ Lee, K Rothko, MA Hussein and CA Schiffer