Platelet Support in Polysensitized Patients: Role of HLA Specificities and Crossmatch Testing for Donor Selection

By J. Gmürr, A. von Felten, and P. Frick

Twenty-four thrombocytopenic patients refractory to random-donor platelet support were given 106 platelet transfusions obtained from 57 different HLA-typed single donors. For critical evaluation of the transfusion responses, “fullhouse” HLA typing of donors and a precise measurement of the number of infused platelets were performed. Posttransfusion platelet increments were related to the HLA matching of donor-recipient pairs and to leukocyte crossmatch tests in vitro. The transfusion response displayed a striking difference depending on the absence (type-0 match) or presence (type-1 or -2 match) of donor HLA antigens differing from the recipient. Whereas 95% of transfusions from type-0 match donors resulted in a compatible platelet increment, only 20% successful transfusion responses could be achieved with type-1 or -2 match donors. In 77% of type-1 or -2 match transfusions with poor posttransfusion platelet increments, the lymphocyte cytotoxicity test (incubation time 180 min at room temperature) was positive. This test therefore seems to be a useful tool in predicting the outcome of transfusion responses in type-1 and -2 match pairs. In contrast, the lymphocyte cytotoxicity tests were of no predictive value in type-0 match pairs. The influence of specific antigranulocyte antibodies on type-0 match platelet transfusions was assessed in four recipients. Although severe transfusion reactions were observed, no detrimental influence on platelet increments could be observed, at least following the initial transfusion.

INCIDENCE OF HEMORRHAGE in thrombocytopenic patients with bone marrow aplasia can be reduced by platelet transfusions. Multiple transfusions of platelets from unselected, random donors, however, have usually resulted in alloimmunization with subsequent refractoriness to random donor platelet support. Yankee and co-workers showed that such patients will later respond to platelets compatible for loci A and B of the HLA system. On the other hand, it was claimed recently that only 70% of alloimmunized patients responded to standard platelet concentrates from HLA-identical donors. Responses in the remaining 30%, however, can usually be improved by removing contaminating leukocytes in such platelet concentrates. Non-HLA antigen-antibody reactions involving platelets as “innocent bystanders” have been postulated to explain poor posttransfusion platelet increments despite HLA identity.

The present study was carried out in patients highly refractory to random donor platelets in order to evaluate (1) the transfusion response of such patients to platelets from single donors of different degrees of HLA identity, (2) the value of different leukocyte crossmatch tests in vitro in predicting the response...
to single-donor transfusions, and (3) the influence of antigranulocyte antibodies upon transfusion response in vivo.

**MATERIALS AND METHODS**

**Platelet collection** from single donors was performed by three different methods: (1) by use of the Aminco Celltrifuge (American Instrument, Silver Spring, Md.11) 56 collections with a mean of $4.8 \times 10^{11}$ platelets; (2) by use of the Haemonetics Model 30 (Haemonetics, Natick, Mass.12) 36 collections with a mean of $4.1 \times 10^{11}$ platelets; (3) by the standard “split-ACD” (acid citrate dextrose) method,13 with preparation of two to four consecutive platelet concentrates per donation 14 collections with a mean of $2.0 \times 10^{11}$ platelets per donation. For preparation of leukocyte-poor platelet concentrates, an additional centrifugation of 178 g for 3 min was performed before platelet transfusion.9

Platelets were kept at room temperature and administered within 6 hr of collection. Effectiveness in vivo of platelets collected by the three different methods proved to be identical with regard to 24-hr posttransfusion recoveries in compatible transfusion responses (see also Ref. 14).

Platelet counts were done by phase-contrast microscopy15 of the collected platelet concentrates as well as of the recipient prior to and 24 hr after transfusion.

**Histocompatibility testing** was done by the standard microlymphocyte cytotoxicity method16 with antisera detecting 13 HLA specificities of locus A and 17 of locus B. In all recipients and donors included in this study four HLA antigens of the two loci A and B were defined without exception. Homozygosity of either locus was regularly verified by HLA typing of the parents.

**Leukocyte crossmatch tests** between donor white blood cells and pretransfusion recipient sera were performed simultaneously by two microlymphocyte cytotoxicity tests [standard incubation time of 60 min at room temperature (standard LT),16 or incubation time of 180 min at room temperature (long-LT)] as well as by the microgranulocyte cytotoxicity test (GT).17 All crossmatch tests were repeated later with donor leukocytes being tested simultaneously against the pretransfusion as well as weekly collected posttransfusion recipient sera.

**Absorption studies** of the granulocytotoxic antibodies were performed with either granulocytes ($10^9$/ml serum), lymphocytes ($2.5 \times 10^9$/ml), or thrombocytes ($5 \times 10^9$/ml), each incubated for 10 min at 37°C followed by 60 min on ice.

The recipients of single-donor platelet transfusions were 6 patients with aplastic anemia and 18 with acute leukemia, all with pretransfusion platelet counts below 6 $\times 10^9$/liter owing to bone marrow failure. All had multiple random transfusions in their past histories leading to refractoriness to further platelet support from random donors as judged by failure to increase circulating platelet counts after transfusion of six to ten random platelet concentrates on at least three occasions. Patients with either fever above 38°C, sepsis, suspected disseminated intravascular coagulation, or splenomegaly at the time of single-donor transfusion were excluded from this study.

**Platelet donors** were 57 normal healthy adults selected from among family members of the patients or from a computerized file of over 3000 ABO- and HLA-typed unrelated blood donors from Swiss Red Cross blood banks.

**Transfusion responses** were expressed as “corrected platelet increments” in order to evaluate the posttransfusion increments independently of the recipients and various doses of transfused platelets. Platelet increments 24 hr after transfusion were therefore multiplied by body surface area (sq m) and divided by the calculated number of so-called platelet units transfused, one platelet unit being $0.85 \times 10^{11}$ platelets. The number of units could be calculated by dividing the total number of platelets collected by $0.85 \times 10^{11}$. In accordance with Yankee et al., transfusion responses were defined as compatible if the corrected 24-hr posttransfusion increments were at least $4.5 \times 10^9$ sq m/liter $\times$ U.9 All transfusions were ABO and crossmatch compatible.

**Matching of donor-recipient pairs** was classified into three types according to the number of nonidentical HLA antigens: type-0, -1, and -2 matching corresponded to zero, one, or two donor antigens differing from those in the recipient. Twenty-four patients were supported 106 times with platelets from 57 different donors, thus resulting in 57 different donor-recipient pairs. With respect to our classification of the HLA matching between donor and recipient, 18 pairs were type 0, 21 pairs type 1, and 18 pairs type 2. In addition to responses to the initial transfusion in these
57 different pairs, responses to subsequent transfusions of the same donor could be analyzed in 11 of the 18 type-0 match pairs.

RESULTS

Response to Initial Transfusion

Results of the initial transfusion of each donor-recipient pair are shown in Fig. 1. Type-0 match platelet transfusions (Fig. 1A) were compatible in 17 of 18 pairs, with a mean corrected 24-hr increment of 8300 platelets sq m/cu mm x U. Eight pairs were genotype-identical siblings, four were nonrelatives with identical HLA phenotypes and in the remaining six pairs nonrelated donors were homozygous for HLA loci A and/or B. Transfusion responses of nonrelated donors did not differ significantly from those of HLA-identical siblings (mean corrected increment 8725 versus 7750). The sole incompatible platelet transfusion originated from a genotype-identical, MLC-nonreactive sibling. Type-1 match platelet transfusions (Fig. 1B) were compatible in 4 and incompatible in 17 pairs, and type-2 match platelet transfusions (Fig. 1C) were compatible in 5 and incompatible in 13 pairs.

Response to Repeated Transfusions From the Same Donor in Type-0 Match Pairs

Repeated transfusions of single-donor platelets were performed in 11 pairs of type-0 match only. In five pairs, all subsequent transfusions (minimum 2, maximum 19, mean 9) continued to reveal compatible responses. Four were HLA-identical, MLC-nonreactive sibling pairs; one was a nonrelated pair. In contrast, in the remaining six pairs compatible responses to the initial transfusion shifted to incompatibility after one to three subsequent transfusions. One of
Table 1. Transfusion Response to Initial Single-Donor Platelet Transfusion in Donor-Recipient Pairs of Different HLA Match Grade and Corresponding Leukocyte Crossmatch Tests In Vitro

<table>
<thead>
<tr>
<th>Leukocyte Crossmatch Test</th>
<th>Type-1 and -2 Match</th>
<th>Type-0 Match</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Compatible</td>
<td>Incompatible</td>
</tr>
<tr>
<td>Standard LT†</td>
<td>0/8</td>
<td>17/26</td>
</tr>
<tr>
<td>Long LT†</td>
<td>0/8</td>
<td>20/26</td>
</tr>
<tr>
<td>GT†</td>
<td>2/8</td>
<td>14/24</td>
</tr>
</tbody>
</table>

*Number of pairs with positive test/number of pairs tested.
†Lymphocyte cytotoxicity tests (see Materials and Methods).
‡Granulocyte cytotoxicity test.

them was a MLC-nonreactive sibling pair, and five were nonrelated pairs. However, compatible responses were again achieved by reduction of contaminating leukocytes in the platelet preparation in three of four type-0 match pairs tested.

Correlation of Initial Transfusion Responses and Leukocyte Crossmatch Tests In Vitro

The value of leukocyte crossmatch tests in vitro in predicting the outcome of platelet transfusion responses was analyzed in 51 donor-recipient pairs.

Lymphocyte cytotoxicity tests. Crossmatch tests were performed in 34 type-1 and -2 match pairs prior to the initial transfusion (Table 1). Eight pairs with compatible transfusion responses consistently had negative tests. In 26 situations with poor posttransfusion platelet increments, 20 long LT were positive, whereas only 17 showed simultaneously a positive standard LT. Six tests were false negative before transfusion; three of them converted to positive 7–19 days after transfusion.

Pretransfusion crossmatch tests were performed in 17 type-0 match pairs. Of the 16 pairs with compatible transfusion responses, 13 had negative tests and three displayed a positive long LT, twice in combination with a positive GT. The only incompatible transfusion response was observed in a sibling pair in which a strongly positive long LT as well as GT were obtained in spite of HLA identity and MLC nonreactivity.

Granulocyte cytotoxicity tests. Sixteen type-1 and -2 match pairs showed a positive GT. In 11, standard LT was also positive; all showed an incompatible transfusion response. The remaining 5 pairs had an exclusively positive GT; two of them had a compatible transfusion response in spite of this positive GT and a high number of contaminating leukocytes in the platelet concentrates (0.8 x 10⁶ and 1.1 x 10⁷ per transfusion), and three showed an incompatible response.

Of special interest were four type-0 match pairs with an exclusively positive GT. All four showed a compatible transfusion response, although severe febrile transfusion reactions occurred.

Specificity of Granulocyte Cytotoxic Antibodies

Absorption studies were performed in type-0 match pairs with exclusively positive GT and compatible transfusion responses. In three sera tested, anti-granulocyte antibodies could not be absorbed either with a mixture of platelets
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originating from 120 random donors or with platelets and lymphocytes obtained from the respective donors. However, elimination of the antibodies was achieved with the corresponding donor granulocytes.

DISCUSSION

To date, no general consensus has emerged concerning the percentage of successful transfusion responses that can be achieved in polysensitized patients or to what degree HLA specificities should be matched. In order to answer these questions, special attention was given to the exact calculation of the number of transfused platelets and to the precise determination of HLA differences between platelet donors and recipients.

According to Yankee and co-workers, transfusion responses may be expressed as posttransfusion platelet increments corrected per sq m of recipient body surface area and per platelet unit infused. The platelet units collected from random donors represent an average of \(0.85 \times 10^{11}\) platelets per concentrate. However, preparation of multiple units from a single donor reveals a highly variable number of platelets depending on the platelet count at the time of collection. If calculation of corrected increments to single-donor platelets is based only on the average number of platelets usually found in random platelet concentrates, the transfusion responses may be over- or underestimated. Therefore to calculate platelet increments after transfusion of platelets collected from one donor the exact number of platelets infused was always determined and expressed in standardized units of \(0.85 \times 10^{11}\) platelets.

Furthermore, all platelet donors and recipients whose HLA typing did not reveal two specificities for each of the loci A and B were excluded from this study. Thus we were able to characterize precisely the grade of HLA match (type 0, 1, or 2) in all donor-recipient pairs. Under these conditions, almost 95% of the patients refractory to random-donor platelet support continued to demonstrate good transfusion responses to type-0 match donors, either MLC-nonreactive siblings or unrelated individuals. In contrast to our results, it was recently claimed that only 50%-70% of polysensitized patients will respond to initial platelet transfusions from “HLA compatible” single donors. However, since in that study “fullhouse” typing was available in only 71% of matched donor-recipient pairs, some of their poor transfusion responses may have been caused by sensitization of recipients to undetected HLA specificities of the donors.

In sharp contrast to the highly successful transfusion responses to type-0 match platelets, our results with donors of type-1 or -2 match were very poor. Only 20% of these transfusions produced platelet increments that could be called compatible, and no significant differences could be observed between donor-recipient pairs with either one or two donor HLA antigens lacking in the recipient. Obviously, the transfusion success using platelets with donor antigens not present in the recipient will depend on the degree of polysensitization of the recipients. Therefore our patients were selected on the basis of incompatible posttransfusion platelet increments to at least two random-donor transfusions a few days before performance of the first single-donor transfusion. A much higher rate of compatible transfusion responses to type-1 match donors was ob-
served by others in bone marrow transplant recipients.\textsuperscript{18} This finding could be explained by the extensive immunosuppressive treatment in connection with bone marrow transplantation.\textsuperscript{22}

The high proportion of compatible transfusion responses in type-0 match pairs (95\%) and the poor responses in type-1 and -2 match pairs (20\%) confirmed the outstanding role of HLA antigens of loci A and B with respect to platelet support in sensitized patients.\textsuperscript{6,9} Since the most effective donors, i.e., type-0 match, often are not available owing to the complexity of the HLA system, sensitive crossmatch tests are required to pick out compatible donors of type-1 or -2 match. Compared with the various crossmatch tests,\textsuperscript{23} lymphocyte cytotoxicity tests meet this demand to a considerable extent, particularly if the incubation time with complement is extended to 180 min at room temperature. In 77\% of type-1 and -2 match pairs, a poor posttransfusion increment was predicted by a positive test. On the other hand, no false positive lymphocyte cytotoxicity tests were observed in all eight compatible type-1 or -2 match transfusions tested. However, in type-0 match pairs with compatible responses, 3 of 16 tests were false positive. They were observed in two patients with aplastic anemia who had received excessive numbers of platelet transfusions, prepared first from random donors and later, repeatedly, from several type-0 match donors. Both developed antilymphocyte antibodies that react with type-0 match donor lymphocytes, indicating that antigens of other than HLA-A or -B specificity might have been involved, e.g., B lymphocyte alloantigens not shared by thrombocytes.\textsuperscript{24} Moreover, antibodies against specificities not being coded for in the major histocompatibility region may have occurred as well, since in one patient antilymphocyte antibodies were found reacting with cells of his HLA- and MLC-identical sister. Thus in contrast to type-1 or -2 match pairs the predictive value of positive lymphocyte cytotoxicity tests applied to type-0 match pairs is questionable.

The role of specific antigranulocyte antibodies suspected of influencing platelet transfusions to a remarkable extent\textsuperscript{9} can be assessed only in type-0 match pairs, where concomitant influences of HLA antibodies can be excluded. Among our patients, pure antigranulocyte antibodies were present in four such situations. In all of them, compatible transfusion responses with platelets not deprived of granulocytes were observed, at least initially. Thus antigranulocyte antibodies seem not to influence initial platelet support.

From these studies in vitro, we conclude that antibodies directed against non-HLA-A or -B antigens do not necessarily cause poor platelet transfusion responses, at least initially. After repeated platelet transfusions from the same type-0 match donors, however, transfusion responses may shift to incompatibility. Reduction of contaminating leukocytes in the platelet concentrates can restore compatible responses, thus confirming the role of contaminating leukocytes in such situations.\textsuperscript{9}

\textbf{ACKNOWLEDGMENTS}

We would like to thank Dr. M. Metaxas and Dr. M. Frey-Wettstein, heads of the Red Cross Blood Transfusion Centers of Zürich, for making available blood donors. The skillful technical assistance of C. von Felten, S. Hegetschweiler, and H. Hirvonen is gratefully acknowledged.
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