HLA-matched Platelet Transfusion Therapy of Severe Aplastic Anemia

By Giovanna Tosato, Frederick R. Applebaum, and Albert B. Deisseroth

In order to define more clearly the importance of the degree of HLA compatibility in the hematologic support of patients refractory to random-donor platelets, we retrospectively analyzed over 1000 platelet transfusions given during stable periods of their disease to 11 thrombocytopenic, severely alloimmunized patients with aplastic anemia: 28% of the donors fully matched with the recipient for HLA A and B loci (A-matched) and 17% of the donors mismatched with the recipient for two or more HLA antigens (D-matched) provided excellent responses, and 18% of A-matched and 46% of D-matched donors provided poor posttransfusion increments. Among all groups analyzed only A-matched donor-recipient pairs had higher increments than predicted by chance ($p < 0.04$); no such increments were observed for donor-recipient pairs with lower degrees of HLA compatibility. Cross reactivity, ABO compatibility, and/or absence of HLA-A2 antigen in the recipient did not appear to influence the outcome of platelet transfusions. While HLA-matched platelets are of benefit in selected cases, HLA matching does not reliably predict platelet transfusion responses even in stable patients, and other as yet unknown factors appear to play an important role in determining transfusion outcomes.

Platelet transfusion therapy of thrombocytopenic patients has reduced the frequency of bleeding complications. Prolonged transfusion support with random donor platelets, however, frequently results in the development of resistance to infused platelets and failure to maintain hemostasis effectively. Resistance is presumed to develop because patients become alloimmunized to antigens on the surface of the transfused platelets. Yankee and co-workers reported that patients refractory to platelet transfusions from random donors could be successfully supported with platelets from HLA-matched family members or unrelated donors. These findings have been extended to the transfusion of platelets from donors mismatched for certain HLA antigens cross reactive with those of the recipient; such platelets may be as effective as those from HLA-matched donors. Moreover, in a few instances, platelets from donors mismatched for HLA antigens not cross-reactive with those of the recipient have been reported to be effective. In these studies the results of a limited number of transfusions to a heterogeneous but selected group of patients with hematologic malignancies or aplastic anemia were examined. The recipients included patients with varying degrees of immunocompetence, hypersplenism, and/or active malignant disease who may have received various therapies that could affect the results of platelet transfusions.

Over the last several years, we have attempted to provide long-term platelet transfusion support to a group of patients with stable aplastic anemia. This

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experience provides an opportunity to examine the results of a large number of platelet transfusions of varying degrees of HLA compatibility given to a homogeneous group of highly alloimmunized patients who are otherwise stable. A retrospective analysis of over 1000 platelet transfusion responses observed in 11 recipients forms the basis for this report.

**MATERIALS AND METHODS**

Eleven patients with aplastic anemia admitted to the National Cancer Institute were chosen for this study. All had severe thrombocytopenia and had been supported for at least 8 wk with various blood components. All were considered refractory to random-donor platelets in that corrected posttransfusion increments of less than 2.5 were observed on at least three consecutive occasions. The following formula was used:

\[
\text{corrected increment} = \frac{\text{postcount} - \text{precount}}{\text{units of platelets} \times \text{BSA} \times 10^{-3}}
\]

Patients' sera were screened against the lymphocytes of selected panels of normal blood donors. Lymphocytotoxic antibodies with reactivity greater than 80% could be detected in each case, confirming the patients' severely alloimmunized state. None of the patients under study developed significant bleeding episodes, splenomegaly, sepsis, fever over 38°C, or disseminated intravascular coagulopathy. Five of the patients had received corticosteroids throughout the course of their disease. Donors were selected from a computerized file of over 25,000 HLA-typed donors. HLA typing was based on a lymphocytotoxicity assay performed by Dr. P. I. Terasaki, University of California, Los Angeles.

Grades of HLA compatibility were defined as follows: A-match: donor and recipient have identical phenotype; B1-match: all of the donor's HLA antigens are present in the recipient, but the donor lacks one of the recipient's HLA antigens; B2-match: all of the donor's HLA antigens are present in the recipient, but the donor lacks two of the recipient's HLA antigens; C-match: the donor has one HLA antigen that is not present in the recipient; D-match: the donor has more than one HLA antigen that is not present in the recipient. Groups of HLA antigens, including HLA-A2, 28; All, 26; A29, 30, 31; A25, 32; B5, 15, 17, 18, 21, 35; B7, 22, 27, 40; B8, 14, 16, 39; and B13, 40 were considered cross reactive according to previous reports.

ABO typing was performed by standard agglutination methods. In most cases 4 units platelets (1 unit = 500 ml blood processed) were collected from individual donors by the split bag ACD technique. The concentrates were kept at room temperature and transfused within 12 hr. Recipient platelet counts were performed before (precoun) and 12-20 hr after (postcount) transfusion. The response to platelet transfusions was measured using the corrected increment. When a single donor was repeatedly used for the same recipient, the mean increment was utilized for analysis in order to prevent bias toward the favorable responses. Increments (or mean increments) were defined as excellent when >10, good when >5, fair when >2.5, and poor when <2.5.

The nonparametric Mann-Whitney U test and \( \chi^2 \) analysis were used to determine the significance of transfusion responses to platelets of different categories.

**RESULTS**

Eleven patients with aplastic anemia, "refractory" to random-donor platelets, were supported with 1280 platelet transfusions from 256 HLA-typed donors. Table 1 lists ABO and HLA types of the patients and the numbers of platelet transfusions and of donors for each recipient. The length of time each patient was supported after a refractory state developed varied from 6 wk to 40 mo (Table 1).

Evaluated were 518 transfusions among 49 A-matched pairs, 214 among 63 B1-matched pairs, 252 among 47 B2-matched pairs, 197 among 69 C-matched pairs, and 99 among 28 D-matched pairs. Table 2 shows the numbers
Table 1. Summary of Clinical and Platelet Transfusion Data

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Age (yr)/Sex</th>
<th>ABO</th>
<th>RH</th>
<th>HLA</th>
<th>No. of Transfusions</th>
<th>No. of Donors</th>
<th>Period of Support (mo)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>9/M</td>
<td>A</td>
<td>+</td>
<td>(2.32)(5.8)</td>
<td>291</td>
<td>43</td>
<td>40</td>
</tr>
<tr>
<td>2</td>
<td>18/M</td>
<td>A</td>
<td>+</td>
<td>(2.24)(35.40)</td>
<td>40</td>
<td>20</td>
<td>3.5</td>
</tr>
<tr>
<td>3</td>
<td>24/F</td>
<td>O</td>
<td>+</td>
<td>(2.6)(7.12)</td>
<td>142</td>
<td>34</td>
<td>22</td>
</tr>
<tr>
<td>4</td>
<td>5/M</td>
<td>A</td>
<td>+</td>
<td>(1.8)(14.32)</td>
<td>88</td>
<td>24</td>
<td>2</td>
</tr>
<tr>
<td>5</td>
<td>24/F</td>
<td>O</td>
<td>+</td>
<td>(2.3)(4.12)</td>
<td>87</td>
<td>36</td>
<td>8.5</td>
</tr>
<tr>
<td>6</td>
<td>14/M</td>
<td>O</td>
<td>+</td>
<td>(2.24)(12.40)</td>
<td>21</td>
<td>17</td>
<td>1.5</td>
</tr>
<tr>
<td>7</td>
<td>24/F</td>
<td>O</td>
<td>+</td>
<td>(2.6)(3.7)</td>
<td>51</td>
<td>10</td>
<td>6</td>
</tr>
<tr>
<td>8</td>
<td>14/F</td>
<td>A</td>
<td>+</td>
<td>(1.2)(27.--)</td>
<td>64</td>
<td>10</td>
<td>15</td>
</tr>
<tr>
<td>9</td>
<td>42/M</td>
<td>O</td>
<td>+</td>
<td>(2.10)(8.40)</td>
<td>290</td>
<td>14</td>
<td>46</td>
</tr>
<tr>
<td>10</td>
<td>52/F</td>
<td>A</td>
<td>+</td>
<td>(2.90)(12.40)</td>
<td>100</td>
<td>23</td>
<td>20</td>
</tr>
<tr>
<td>11</td>
<td>47/F</td>
<td>O</td>
<td>+</td>
<td>(2.11)(12.--)</td>
<td>106</td>
<td>25</td>
<td>19</td>
</tr>
</tbody>
</table>

Table 2. Number and Percentage of Donors Providing Excellent, Good, Fair, and Poor Corrected Increments Within Each Group of Compatible Donor-Recipient Pairs

<table>
<thead>
<tr>
<th>Match Grade</th>
<th>No. of Donors</th>
<th>No. of Transfusions</th>
<th>Excellent (28.57%)</th>
<th>Good (32.65%)</th>
<th>Fair (10.40%)</th>
<th>Poor (18.36%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>49</td>
<td>518</td>
<td>14</td>
<td>16</td>
<td>10</td>
<td>9</td>
</tr>
<tr>
<td>B1</td>
<td>63</td>
<td>214</td>
<td>17</td>
<td>9</td>
<td>7</td>
<td>30</td>
</tr>
<tr>
<td>B2</td>
<td>47</td>
<td>252</td>
<td>9</td>
<td>14</td>
<td>7</td>
<td>17</td>
</tr>
<tr>
<td>C</td>
<td>69</td>
<td>197</td>
<td>14</td>
<td>15</td>
<td>12</td>
<td>28</td>
</tr>
<tr>
<td>D</td>
<td>28</td>
<td>99</td>
<td>5</td>
<td>7</td>
<td>3</td>
<td>13</td>
</tr>
</tbody>
</table>
 increments observed in the two groups (4.52 in HLA-A2-positive versus 5.27 in HLA-A2-negative recipients) were not significantly different (Fig. 1).

Pairs with the same degree of HLA compatibility provided variable increments. As can be seen from Fig. 2, this was evident for all five groups of donors. In addition, differences in transfusion increments obtained from the A, B1, B2, C, and D groups were not statistically significant ($p > 0.05$).

A large variation in corrected increments was also observed in single donor-recipient pairs. In order to analyze this result, standard deviations were calculated for increments observed in single donor-recipient pairs and averaged according to degree of compatibility. Corrected increments from 33 donors who donated more than ten times for single recipients were analyzed. Results are shown in Table 4. The variation in corrected increments observed in single donor-recipient pairs of every degree of compatibility was surprisingly high and
could not be clearly ascribed to techniques of collection or clinical status. Two typical patterns are shown in Figs. 3 and 4. The corrected increments obtained in the case of a B2-matched donor who supported patient 1 for more than 3 yr varied in subsequent transfusions from 10 to 24, and those obtained in the use of a D-matched donor who supported patient 10 for almost 2 yr varied in subsequent transfusions from 0 to 10.

Eighty-four donors were ABO incompatible with their recipients. Within the

<table>
<thead>
<tr>
<th>Match Grade</th>
<th>No. of Donors</th>
<th>No. of Transfusions</th>
<th>Mean SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>9</td>
<td>237</td>
<td>5.58</td>
</tr>
<tr>
<td>B1</td>
<td>7</td>
<td>109</td>
<td>4.70</td>
</tr>
<tr>
<td>B2</td>
<td>7</td>
<td>178</td>
<td>4.44</td>
</tr>
<tr>
<td>C</td>
<td>7</td>
<td>66</td>
<td>5.44</td>
</tr>
<tr>
<td>D</td>
<td>3</td>
<td>58</td>
<td>3.62</td>
</tr>
</tbody>
</table>
Fig. 3. Posttransfusion increments over 38-mo period in single donor-recipient pair; donor was HLA B-2-matched with recipient.

Fig. 4. Posttransfusion increments over 21-mo period in single donor-recipient pair; donor was HLA D-matched with recipient.
Table 5. Mean Posttransfusion Increments and Standard Deviations for ABO-compatible and
ABO-incompatible Donors Within Each Group of HLA-matched Donor-Recipient Pairs

<table>
<thead>
<tr>
<th>Match Grade</th>
<th>ABO Compatible</th>
<th>ABO Incompatible</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of Donors</td>
<td>Increment</td>
<td>SD</td>
</tr>
<tr>
<td>A</td>
<td>27</td>
<td>8.92</td>
</tr>
<tr>
<td>B1</td>
<td>36</td>
<td>3.87</td>
</tr>
<tr>
<td>B2</td>
<td>39</td>
<td>5.89</td>
</tr>
<tr>
<td>C</td>
<td>47</td>
<td>5.87</td>
</tr>
<tr>
<td>D</td>
<td>23</td>
<td>5.10</td>
</tr>
</tbody>
</table>

different groups of donor-recipient HLA compatibility, there were no significant
differences ($p > 0.05$) in the posttransfusion platelet increments whether the
donors were ABO compatible or incompatible (Table 5).

DISCUSSION

Although HLA matching has been widely used for the selection of platelet
donors, few systematic studies of the results of this approach have been reported.
In the present study we retrospectively analyzed 1280 platelet transfusions from
256 donors to 11 severely alloimmunized thrombocytopenic patients with aplastic
anemia, administered during stable periods of their disease. All recipients
were refractory to random-donor platelets; thus all transfusions analyzed in this
series were given with the hope of providing good increments, and D-matched
donors were usually matched for one or two common antigens and cannot be
considered to be totally random. Our data indicate that among all of the groups
analyzed only A-matched donors provided significantly ($p < 0.04$) higher incre-
ments than the rest; no such differences were observed for B1-, B2-, C-, or
D-matched donor-recipient pairs. However, donors providing excellent as well
as poor posttransfusion increments were found among A-matched pairs as well
as among pairs with different degrees of HLA compatibility. In our series,
A-matched donors provided excellent or good transfusion responses 60% of the
time but failed to provide any meaningful increment 18% of the time; in contrast,
C or D matches provided excellent or good responses about 40% of the time and
failed in 40% of cases. Because of the wide spread of results within any one
group of HLA compatibility, differences in mean posttransfusion increments
between groups were not statistically different. These findings suggest that fac-
tors in addition to HLA compatibility for the first and second loci are important
in determining the response to platelet transfusions.

Others have suggested that platelets mismatched for selected HLA antigens
"cross reactive" with those of the recipient were often useful in supporting
alloimmunized patients.14-16 Our experience in this group of heavily alloim-
munized patients showed no significant benefit of using cross-reactive as op-
posed to non-cross-reactive antigenic matches. However, this could conceiv-
ably be explained by avoidance of common antigens in the non-cross-reactive
group. Nonetheless, we could not detect any discernable benefit using donors
who had antigens cross reactive with those of the recipients.

Platelet transfusions from donors mismatched for HLA antigens were found
to be significantly more effective in HLA-A2-negative than in HLA-A2-posi-
tive recipients. Although only two of our patients were HLA-A2 negative, the response to platelet transfusions from HLA C- and D-mismatched donors was not significantly better than that observed in the group of HLA-A2-positive recipients.

In agreement with previous reports, ABO compatibility between donor and recipient did not appear in our series to influence the outcome of platelet transfusions. Several antigenic systems other than HLA and ABO are known to be present on human platelets. Wu et al. reported nonlymphocytotoxic platelet-aggregating antibodies in the sera of alloimmunized thrombocytopenic patients, suggesting a role for platelet-specific antibodies in the outcome of isologous platelet transfusions.

HLA antigens are believed to be expressed on the surface of platelets, however, occasionally they are partially expressed or absent on platelets but present on lymphocytes of the same individuals. Immunogenicity of single HLA antigens of the A and B loci has been shown to be variable, and immune response to transplantation antigens is variable in different individuals, presumably because of genetic or acquired factors. In addition, other still unidentified antigens of the HLA system may be expressed on the platelets. Development of platelet-specific antibodies, absence of HLA antigens on the platelets, varying immunogenicity of HLA antigens, and/or "immunologic competence" of the recipient are factors that may provide an explanation for our findings. Increments within each group of compatible pairs and even within single donor-recipient pairs were surprisingly variable and could not be explained as a function of collection technique or clinical status of the recipient. In our experience, a poor posttransfusion increment was not necessarily followed by similarly poor increments using donors with the same degree of HLA compatibility or even the same donor. Thus poor platelet recoveries did not always establish the presence of a permanently refractory state. It is apparent that HLA matching for A and B loci does not reliably predict platelet transfusion responses and that other, still unknown factors may play an important role in determining transfusion responses.

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