Effect of HLA Bw4/Bw6 Compatibility on Platelet Transfusion Responses of Refractory Thrombocytopenic Patients

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Platelet transfusions from donors matched for cross-reactive antigens have been shown to be effective in providing hemostasis in alloimmunized thrombocytopenic patients. A significant number of these transfusions, however, fail to provide posttransfusion platelet recoveries. We investigated incompatibility in the Bw4/Bw6 system as a possible explanation for these failures. The Bw4/Bw6 system is a biallelic antigen system closely associated with HLA-B. HLA-B locus antigens that are cross-reactive frequently differ in their Bw4/Bw6 specificity. Posttransfusion platelet recoveries from 21 alloimmunized thrombocytopenic patients homozygous for Bw4 or Bw6 and transfused with both Bw4/Bw6 compatible and incompatible platelets were analyzed. The mean 1-hr posttransfusion recovery was 84% following Bw4/Bw6-compatible platelets versus 52% with Bw4/Bw6-incompatible platelets (p < 0.02). Twenty-four hours following transfusion, mean recoveries were 44% and 24%, respectively, (p < 0.01). A subgroup of 8 patients (38%) was identified who had markedly lower responses following Bw4/Bw6-incompatible transfusions as compared to Bw4/Bw6-compatible transfusions (mean recoveries: 1 hr—compatible 100%, incompatible 27%, p < 0.001; 24 hr—compatible 45%, incompatible 7%, p < 0.01). These data suggest that the Bw4/Bw6 antigen system has clinical significance for some patients requiring platelet transfusion therapy and, when appropriate, should be considered in the selection of donors.

PLATELET TRANSFUSIONS from donors selectively matched for cross-reactive HLA-A and B antigens have successfully been used in the prevention and treatment of bleeding problems of alloimmunized thrombocytopenic patients refractory to random-donor platelet concentrates. However, a significant proportion of HLA-matched platelet transfusions fail to provide satisfactory posttransfusion increments. Investigations seeking immunologic causes of poor recovery of transfused platelets have shown that donor/recipient matching at the HLA-C locus does not influence platelet transfusion response in refractory patients and that ABO incompatibility of donor and recipient affects platelet recovery only slightly. Other suggested immunologic causes of refractoriness include unrecognized HLA incompatibilities, antibodies against platelet-specific antigens, the presence of circulating immune complexes, and incompatibility in the Bw4/Bw6 antigen system.

The Bw4/Bw6 antigen system is a biallelic system of HLA antigens, closely related to HLA-B, that has been detected on lymphocytes and platelets by serologic methods. The Bw4/Bw6 system was originally described by van Rood and van Leeuwen as 4a/4b. The gene frequencies of Bw4 and Bw6 are 0.35 and 0.65, respectively. Phenotypically, 13% of the population is homozygous for Bw4, 37% homozygous for Bw6, and 50% heterozygous (i.e., Bw4/Bw6). The HLA-Bw4 and Bw6 antigens show very strong association with two mutually exclusive groups of HLA-B antigens. Biochemical evidence indicates that Bw4/Bw6 and HLA-B antigens represent separate antigenic determinants (epitopes) on the HLA-B peptide.

Antigens that are serologically cross-reactive sometimes differ in their Bw4/Bw6 specificities. We have previously reported that incompatibility for Bw4/Bw6 can influence survival of platelets transfused to alloimmunized patients. This study was conducted to further investigate this observation.

MATERIALS AND METHODS

The records of all patients given single-donor, HLA-matched platelet transfusions by the Blood Center of Southeastern Wisconsin between December 1973 and December 1980 were reviewed. The patients had a history of multiple platelet and blood transfusions, and in some cases pregnancies, and had become refractory to random-donor platelet transfusions documented by failure to respond to random-donor platelets on each of two successive occasions. All patients were thrombocytopenic as a result of bone marrow failure or suppression by chemotherapy. Only transfusion episodes not associated with splenomegaly, fever, infection, sepsis, or disseminated intravascular coagulation (i.e., conditions that may decrease survival of transfused platelets) were evaluated.

Donors were selected from a computerized file of over 8000 HLA-typed volunteer donors. HLA typing by the NIH microlymphocytotoxicity technique included identification of HLA-A, B, and C locus antigens as well as the Bw4/Bw6 antigens. This technique was also used to screen patient sera for HLA antibodies.

The following donor/recipient match classification was used: In A matches, the donor and recipient had identical HLA-A and B locus antigens. B1 matches were defined as three A or B locus donor antigens identical with those of the recipient, with the fourth being unknown (B1U) or cross-reactive (B1X). In B2 matches, two donor antigens are unknown (B2U), two or more donor antigens are cross-reactive (B2X), or two or more antigens are both unknown and unknown.
cross-reactive (B2UX). The remaining donor antigens in each of these match categories are identical with those of the recipient. Major mismatches (C or D matches) between donor and recipient were excluded from analysis.

Sixty-one refractory patients who were homozygous for Bw4 or Bw6 were identified. Bw4/Bw6 “compatibility” is defined as donor and recipient homozygous for the same specificity. “Incompatibility” is Bw4/Bw6 heterozygosity in the donor or donor homozygosity for the Bw4/Bw6 specificity not present on the recipient’s cells. The 21 patients selected for analysis received at least one Bw4/Bw6-compatible transfusion that contained a cross-reactive antigen and at least one Bw4/Bw6-incompatible transfusion that contained a cross-reactive antigen. Of patients not satisfying the criteria, 21 patients received only Bw4/Bw6-compatible transfusions, 9 received only Bw4/Bw6-incompatible transfusions, and for 10 patients, all Bw4/Bw6-compatible transfusions were A, B1U, or B2U matches. Hence, all study patients received both Bw4/Bw6-compatible and Bw4/Bw6-incompatible B1X, B2X, or B2UX match-grade platelet transfusions.

Platelets were obtained by intermittent flow centrifugation with the Haemonetics Model 30 Cell Separator (Haemonetics Corporation, Braintree, Mass.) or by continuous flow centrifugation using the IBM 2997 Blood Cell Separator (International Business Machines, Princeton, N.J.). Platelet concentrates were centrifuged at 800 rpm for 10 min at room temperature in a Sorvall RC-3 Centrifuge (Sorvall Instruments, Newton, Connecticut) to remove red cells. Platelet counts were performed on a sample from each platelet concentrate, with an average of $4 \times 10^7$ platelets being obtained from each plateletpheresis.

Platelet counts were performed on blood samples obtained from the recipient immediately prior to the platelet transfusion and 1 and 24 hr posttransfusion. Recovery of transfused platelets was calculated as:

$$\text{% Recovery} = \frac{(\text{Posttrans PIt Ct} - \text{Pretrans PIt Ct})}{(\text{patient's blood vol})} \times 100$$

$$(\text{Number of Platelets Transfused}) (0.67)$$

The figure 0.67 is used to account for obligatory splenic pooling of one-third of the transfused platelets. This correction factor was omitted if patients had undergone splenectomy. Platelet recoveries from multiple transfusions for a donor/recipient pair were averaged, and the mean value for the pair was used for analysis. Comparison of platelet recoveries between Bw4/Bw6-compatible and incompatible transfusions was performed using the paired t test.

RESULTS

The 21 patients (Table 1) received 203 transfusions from 159 donors of B1X, B2X, or B2UX match-grades and received an average of 11 transfusions (range 2–36). There were 105 Bw4- or Bw6-compatible transfusions and 98 Bw4- or Bw6-incompatible transfusions. A comparison of the mean platelet recovery for each patient showed that the 1-hr recovery was significantly less for Bw4/Bw6-incompatible than Bw4/Bw6-compatible transfusions (84% versus 52%, $p < 0.02$). Similar data were also obtained 24 hr following transfusion: mean recoveries were 44% and 24%, respectively ($p < 0.01$).

Two distinct groups of patients were apparent: eight patients had markedly lower posttransfusion platelet recoveries following Bw4/Bw6-incompatible transfusions than after compatible transfusions (Fig. 1) (mean 1 hr recoveries: compatible 100%, incompatible 27%, $p < 0.001$; mean 24 hr recoveries: compatible 45%, incompatible 7%, $p < 0.01$). In each instance, recovery of incompatible platelets was less than one-third that of compatible platelets at 1 hr and/or 24 hr posttransfusion. Each patient had a mean 1-hr posttransfusion platelet recovery of at least 50% for compatible transfusions. In 13 patients, posttransfusion platelet recoveries were unrelated to Bw4/Bw6-compatibility.

### Table 1. Patient Profile

<table>
<thead>
<tr>
<th>Patient Number</th>
<th>Diagnosis</th>
<th>HLA-A, B, C, Bw4/Bw6 Loci</th>
<th>Antibody Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Aplastic Anemia</td>
<td>26.33, 14.y.z.z.w6</td>
<td>A2</td>
</tr>
<tr>
<td>2</td>
<td>Lymphoma</td>
<td>2, 11, 40.y,cw2, cw3.w6</td>
<td>None</td>
</tr>
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<td>3</td>
<td>Preleukemia</td>
<td>1.2, 17.51.z.w4</td>
<td>N.D.</td>
</tr>
<tr>
<td>4</td>
<td>AML</td>
<td>1.2, 15.18, cw3.z.w6</td>
<td>Bw4, multi</td>
</tr>
<tr>
<td>5</td>
<td>Aplastic anemia</td>
<td>2.3, 15.40, cw3.z.w6</td>
<td>A1, A9, B27</td>
</tr>
<tr>
<td>6</td>
<td>Breast cancer</td>
<td>3.7, y.z.z.w6</td>
<td>Multi</td>
</tr>
<tr>
<td>7</td>
<td>AML</td>
<td>1.33, 7.18, cw4.z.w6</td>
<td>Bw4.A2</td>
</tr>
<tr>
<td>8</td>
<td>Histioctyoma</td>
<td>2.25, 27.y.z.z.w4</td>
<td>Bw6, multi</td>
</tr>
<tr>
<td>9</td>
<td>CML</td>
<td>1.2, 27.37, z.w4</td>
<td>A24, B12, B21</td>
</tr>
<tr>
<td>10</td>
<td>Breast cancer</td>
<td>2.11, 38.49, z.z.w4</td>
<td>Nonspecific</td>
</tr>
<tr>
<td>11</td>
<td>AML</td>
<td>1.2.44, 51.z.z.w4</td>
<td>Nonspecific</td>
</tr>
<tr>
<td>12</td>
<td>AML</td>
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<td>None</td>
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<tr>
<td>13</td>
<td>AML</td>
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<td>A1, A3, A11, B8, B14</td>
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<td>A2, B27, B44</td>
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<td>Aplastic anemia</td>
<td>11.24, 7.22, cw3.z.w6</td>
<td>Bw4, A2</td>
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<tr>
<td>17</td>
<td>AML</td>
<td>2.25, 17.44, z.z.w4</td>
<td>Multi</td>
</tr>
<tr>
<td>18</td>
<td>Aplastic anemia</td>
<td>2.15, y.cw3.z.w6</td>
<td>Multi</td>
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<td>19</td>
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<td>Bw4, multi</td>
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<td>20</td>
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<td>None</td>
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<tr>
<td>21</td>
<td>CGL</td>
<td>3.33, 7.18, z.z.w6</td>
<td>A2, A9, B8</td>
</tr>
</tbody>
</table>
Patients 1–8 demonstrated significantly lower recovery of transfused platelets when given Bw4/Bw6-incompatible platelets compared to Bw4/Bw6-compatible platelets. This effect was not seen in patients 9–21.

Serum analysis for lymphocytotoxic antibody did not identify those patients whose posttransfusion platelet increments were affected by Bw4/Bw6 incompatibility. A specific Bw4/Bw6 antibody was demonstrated serologically in only three of seven patients tested from the group of eight patients with poor responses to Bw4/Bw6-incompatible transfusions. Three patients in this group showed lymphocytotoxic antibody without Bw4/Bw6 specificity. Antibody directed against Bw4 was detected in the serum of 2 of 13 patients showing comparable recoveries with Bw4/Bw6-compatible and incompatible transfusions. Lymphocytotoxic antibody was shown in 11 of these 13 patients.

The results of platelet transfusions given to patient 7 are illustrated in Fig. 2. This patient failed to respond to transfusions of random platelets and to single-donor platelets bearing a major mismatched antigen (C match). The response to B match-grade platelet trans-

![Fig. 1](image_url)

![Fig. 2](image_url)
fusions from donors homozygous for Bw6 were significantly better than those obtained from donors positive for the Bw4 antigen.

**DISCUSSION**

Although HLA-matched platelet transfusions are effective in the treatment of alloimmunized thrombocytopenic patients refractory to random-donor platelet concentrates, they do not always provide satisfactory platelet recoveries. Immunologic explanations for poor posttransfusion platelet recoveries include: unrecognized HLA incompatibilities, the presence of circulating immune complexes, an unknown effect of antibodies to platelet-specific antigens, and incompatibility in the Bw4/Bw6 antigen system. Our study shows that 8 of the 21 (38%) alloimmunized patients homozygous for Bw4 or Bw6 failed to obtain adequate posttransfusion recoveries with Bw4/Bw6-incompatible transfusions but experienced satisfactory recoveries when transfused with Bw4/Bw6-compatible platelets.

The lymphocytotoxic antibody screening identified Bw4 or Bw6 antibodies in less than half of the patients who showed poor responses to Bw4/Bw6 incompatible transfusions. Failure to demonstrate any lymphocytotoxic antibody in 3 of the 21 patients is only a slightly greater proportion than that reported by Dutchen et al., and is perhaps due to the presence of noncytotoxic antibodies in some patients. Patients 16 and 19 demonstrated antibodies to Bw4 in their serum, but showed no difference in transfusion response between Bw4/Bw6-compatible and incompatible transfusions. Patient 19 was highly alloimmunized and responded poorly to B match-grade platelet transfusions regardless of Bw4/Bw6 compatibility. Patient 16 responded well to both Bw4/Bw6-compatible and incompatible transfusions. We are unable to explain this observation.

Homozygosity for Bw4 or Bw6 did not appear to increase the probability that a patient would become alloimmunized in our series. We found no deviation in our alloimmunized patients from the expected distribution of Bw4/Bw4, Bw6/Bw6, or Bw4/Bw6 typing of the random population. Hence, homozygosity for Bw4 or Bw6 may create difficulty in finding a compatible donor, but does not appear to increase the incidence of alloimmunization.

Although our study provides in vivo evidence that the biallelic Bw4/Bw6 antigen system has clinical significance for some patients who require platelet transfusion therapy, the mechanism of Bw4 or Bw6 sensitization remains elusive. Szatkowski and Aster have shown that the expression of HLA-Bw4 or Bw6 on platelets is variable. HLA-Bw4 is strongly expressed on platelets positive for HLA-B specificities B5 and B27 and weakly expressed on platelets carrying B13. HLA-Bw6 expression is strong on platelets positive for B7, Bw62, and Bw35 and weak on platelets carrying B8 and B14. Our data base was insufficient to determine whether this observation has clinical significance.

Another possible mechanism of sensitization may involve the biochemical and spatial arrangements of the Bw4/Bw6 antigen. Polypeptide backbones with folding and conformational changes (perhaps due to hypervariable regions) may alter the molecule so as to be recognized as foreign by some individuals and not others.

Currently, our strategy for selection of donors for alloimmunized patients recognizes the possible significance of Bw4/Bw6 sensitization. Patients are transfused with platelets from donors selected on the basis of their HLA-A and B locus antigens. Response to transfusion is carefully monitored. When Bw4 or Bw6 homozygous patients transfused with platelets from donors incompatible for Bw4 or Bw6 do not realize satisfactory responses, Bw4 or Bw6-compatible donors are then selected for further transfusions. We suggest that this strategy should be attempted by centers supplying single-donor, HLA-matched platelet transfusions prior to abandoning a patient as refractory to all forms of platelet support therapy.

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**REFERENCES**


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