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.

**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 those 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

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**From the Blood Center of Southeastern Wisconsin, Inc., Milwaukee, Wis.**

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

By Margaret C. McElligott, Jay E. Menitove, Rene J. Duquesnoy, Jay B. Hunter, and Richard H. Aster
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 and Bw4/Bw6-incompatible transfusions were A, B1U, or B2U matches. Hence, all study patients received both Bw4/Bw6-compatible and Bw4/Bw6-incompatible BIX, 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 x 10^11 platelets being obtained from each platelethropheresis.

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:

\[
\text{Percent Recovery} = \frac{\text{Posttrans PIt Ct} - \text{Pretrans PIt Ct}}{\text{(patient's blood vol) } \times 0.67} \times 100
\]

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 com-

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<th>Table 1. Patient Profile</th>
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Fig. 1. 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.

Patibility (Fig. 1) (mean recoveries: 1 hr—compatible 74%, incompatible 68%, p = NS; 24 hr—compatible 44%, incompatible 34%, p = NS).

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-
fusions from donors homozygous for Bw6 were signifi-
cantly better than those obtained from donors positive
for the Bw4 antigen.

DISCUSSION

Although HLA-matched platelet transfusions are
effective in the treatment of alloimmunized thrombo-
cytopenic patients refractory to random-donor platelet
concentrates, they do not always provide satisfactory
platelet recoveries. Immunologic explanations for poor
posttransfusion platelet recoveries include: unrecog-
nized HLA incompatibilities, the presence of circulating
immune complexes, an unknown effect of antibod-
ies 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 posttransfu-
sion recoveries with Bw4/Bw6-incompatible transfu-
sions but experienced satisfactory recoveries when trans-
 fused 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 lymphocyto-
toxic antibody in 3 of the 21 patients is only a slightly
greater proportion than that reported by Dutcher et
al., and is perhaps due to the presence of noncytotoxic
antibodies in some patients. Patients 16 and 19 demon-
strated antibodies to Bw4 in their serum, but showed
no difference in transfusion response between Bw4/
Bw6-compatible and incompatible transfusions. Pa-
tient 19 was highly alloimmunized and responded
poorly to B match-grade platelet transfusions regard-
less of Bw4/Bw6 compatibility. Patient 16 responded
well to both Bw4/Bw6-compatible and incompatible
transfusions. We are unable to explain this observa-
tion.

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 distri-
bution 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.14 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 posi-
tive for B7, Bw26, and Bw35 and weak on platelets
carrying B8 and B14. Our data base was insufficient to
determine whether this observation has clinical signifi-
cance.

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 signifi-
cance of Bw4/Bw6 sensitization. Patients are trans-
 fused 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 trans-
 fusions prior to abandoning a patient as refractory to
all forms of platelet support therapy.

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