Influence of HLA-A2 on the Effectiveness of Platelet Transfusions in Alloimmunized Thrombocytopenic Patients

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Platelet transfusions from donors selectively mismatched for cross-reactive and certain non-cross-reactive HLA antigens were found to be more effective in HLA-A2 negative than in HLA-A2 positive, alloimmunized thrombocytopenic patients. The two groups of patients responded equally well to platelets matched for antigens of the HLA-A and B loci. Certain alloimmunized patients negative for HLA-A2 continued to respond satisfactorily to platelets selectively mismatched for non-cross-reactive HLA antigens as long as platelets containing HLA-A2 were avoided. The data indicate that platelet transfusion support can be provided within a broader range of donor-recipient HLA antigenic disparity to HLA-A2 negative alloimmunized patients than to those who are positive for this antigen.

A major obstacle to providing long-term platelet transfusion support to thrombocytopenic patients is the development of refractoriness to platelets from randomly selected donors. This refractoriness is due primarily to alloimmunization to platelet antigens, especially the transplantation antigens of the HLA system. Yankee and co-workers were the first to demonstrate that refractory thrombocytopenic patients could be successfully transfused with HLA-matched platelets from related and unrelated donors. Platelets obtained from donors who share two or three HLA antigens with the recipient and whose remaining antigens type as "blanks" or are serologically cross-reactive to those of the recipient also may be effective in such patients.

In recent studies, we have observed that some refractory patients could be transfused successfully with platelets from donors mismatched for certain non-cross-reactive HLA antigens. In the course of these investigations, it was found that alloimmunized thrombocytopenic patients varied greatly in their responsiveness to mismatched platelet transfusions. In an attempt to explain why certain patients responded well to platelets mismatched for certain non-cross-reactive HLA antigens ("major" mismatch) while in others such transfusions were uniformly unsuccessful, we evaluated a number of donor and recipient variables. In this paper we present evidence indicating that HLA-A2, the most common of the HLA antigens, is a major factor influencing the effectiveness of single-donor platelet transfusions in this patient population, and discuss the implementation of this finding in the strategy of selecting platelet donors for alloimmunized, thrombocytopenic patients.

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MATERIALS AND METHODS

The standard NIH microlymphocytotoxicity test was used to determine the HLA types of the platelet donors and recipients. Patients and platelet donors were each HLA-typed on at least two occasions.

A computerized file of more than 8000 HLA-typed blood donors was developed from a pool of "on-call" blood donors who indicated their willingness to donate blood on short notice. The system is capable of sorting HLA-typed blood donors and listing, for each patient, donors of varying degrees of histocompatibility. The following donor-recipient match classifications were used: In A matches the donor and recipient had identical phenotypes for HLA-A and HLA-B locus antigens. B1 matches between donor and recipient were defined as three antigen matches with the fourth antigen in the donor being unknown (B1U) or cross-reactive (B1X). In B2 matches the donor and recipient shared two antigens, while in the donor the third and fourth antigens were unknown (B2U), cross-reactive (B2X), or one unknown and the other cross-reactive (B2UX). C and D matches were "major" mismatches in which the donor typed for one or more non-cross-reactive antigens not present in the recipient. The classification for cross-reactive HLA antigens was based on reports in the literature and our own observations and has been presented elsewhere. Antigens of the HLA-C locus were not considered in this study.

Single-donor platelets were collected in acid-dextrose anticoagulant by platelet pheresis with a Haemonetics Model 30 cell separator as described before. The average number of platelets transfused was $4 \times 10^{11}$. Responses to platelet transfusions were evaluated by performing peripheral blood platelet counts before and 24 hr after transfusion and by comparing the observed post-transfusion platelet increments with that expected on the basis of the total number of platelets transfused.

Platelet transfusion responses were analyzed in 59 patients who had thrombocytopenia as a result of bone marrow failure or suppression: 12 had aplastic anemia, 46 had leukoproliferative malignancy, and 1 had amegakaryocytic thrombocytopenia of unknown etiology. All had a history of multiple platelet transfusions and had been shown to be unresponsive to random platelet transfusions on at least two occasions (24-hr posttransfusion platelet recovery less than 10%). Studies on patients with fever greater than 100°F, sepsis, severe splenomegaly, or disseminated intravascular coagulation were excluded from the analysis because these conditions may nonspecifically alter the response to platelet transfusions. When a single donor was repeatedly used for the same patient because of consistently good transfusion responses, the results of only two transfusions were utilized for analysis to avoid biasing the data toward individual donor characteristics. In such instances, the best and the worst responses were selected.

The Mann-Whitney U test and the $\chi^2$ test (with Yate's correction) were used to determine the levels of significance of differences between responses to platelets of different match grades.

RESULTS

Figure 1 depicts the 24-hr posttransfusion platelet recoveries observed in 193 single-donor platelet transfusions given to 28 HLA-A2 positive patients and 254 single-donor platelet transfusions given to 31 HLA-A2 negative refractory thrombocytopenic patients. Transfusion responses to platelets of similar degrees of histocompatibility were subdivided into three groups. The first consisted of transfusions from donors who shared four, three, or two HLA antigens with the patient and had no other detectable antigens in their HLA type (A, B1U, and B2U matches). The second included transfusions from donors mismatched for cross-reactive HLA antigens (B1X, B2X, and B2UX matches). The third included transfusions from donors incompatible for non-cross-reactive HLA antigens (C and D matches). Each patient received one or more transfusions from donors of the first two match groups. Transfusions administered to 28 HLA-A2 positive patients consisted of 30 A matches, 26 B1U
matches, and 22 B2U matches. Those given to 31 HLA-A2 negative patients consisted of 17 A matches, 19 B1U matches, and 21 B2U matches.

HLA-A2 positive and negative patients did not differ significantly in their responses to platelets of match grades A, B1U, and B2U (Fig. 1). In contrast, transfusions from donors selectively mismatched for cross-reactive HLA antigens (match grades B1X, B2UX, and B2X) were significantly less effective in HLA-A2 positive patients than in HLA-A2 negative patients (median 24-hr posttransfusion increments: 33% versus 25%, p = 0.0026).

Platelets of match grades C and D were transfused to 14 HLA-A2 positive patients on 26 occasions and to 18 HLA-A2 negative patients on 65 occasions. Ten of the latter received platelets mismatched for HLA-A2. C and D matched platelets (“major” mismatches) were frequently successful in this group of 32 refractory patients, none of whom responded to pooled platelet concentrates prepared from random donors. HLA-A2 negative patients responded particularly well to such platelets, especially when HLA-A2 was absent in the donor (Fig. 1). The difference in 24-hr posttransfusion increments observed in HLA-A2 negative and HLA-A2 positive patients was statistically significant (median values: 36% versus 9%, p = 0.0009).

The responses of an HLA-A2 negative patient to transfusions of platelets of differing match categories are shown in Fig. 2. H.F. was a 57-yr-old male with idiopathic amegakaryocytic thrombocytopenia who had become totally unresponsive to platelets from random donors after being transfused with more than 100 platelet concentrates over a period of 3 mo. His HLA type was HLA-A1, A11; Bw22, w35. Since no A-matched platelets were available, platelets were transfused from 10 B1 and B2 donors, six of whom fell into the B1X or B2UX match categories. As shown in Fig. 2A, excellent transfusion responses were observed with each of the B-matched platelets. C-matched platelets from donors positive for HLA-A2 or HLA-Aw24 (a subspecificity of HLA-A9) were completely ineffective. In contrast, platelets from donors mismatched with the patient for HLA-B8, HLA-B12, HLA-B13 (and HLA-Aw30, not shown) produced excellent posttransfusion platelet increments (Fig. 2B). This patient failed to become refractory to donor platelets containing HLA-B8 despite repeated transfusions over a 1-yr period with platelets from eight different donors who typed for B8.
These studies demonstrated that platelet transfusions from donors selectively mismatched for cross-reactive and certain non-cross-reactive HLA antigens were more effective in HLA-A2 negative than in HLA-A2 positive individuals in the patient population studied. However, the two groups of patients responded equally well to platelets from fully histocompatible donors. Patients negative for HLA-A2 responded as well to platelets containing cross-reactive HLA antigens as to fully histocompatible platelets and, in many instances, could be successfully transfused even with C- and D-matched platelets as long as the antigen HLA-A2 was avoided. In contrast, transfusions from donors selectively mismatched for cross-reactive HLA antigens were less effective in HLA-A2 positive, alloimmunized patients. C- and D-matched platelets were rarely effective in this group.

These findings have implications for the strategy of selecting platelet donors for refractory patients because they suggest that in 50% of all patients being transfused (the HLA-A2 positive group) histocompatibility between donor and recipient is much more critical than in the remainder. The excellent transfusion responses achieved with C- and D-matched platelets in many HLA-A2 negative patients suggest that such patients develop restricted antibody responses against HLA antigens following multiple transfusions so that C- and D-matched platelets from single donors are often effective. The hypothesized restrictive response is, however, sufficiently broad to produce refractoriness to pooled, random donor concentrates.

Variability in immune responsiveness to non-self-HLA antigens has been demonstrated, and appears to depend upon both the immunogenicity of individual HLA specificities and the phenotype of the transfused patient. HLA antigens cross-reactive to those of the transfused patient appear to be less immunogenic than non-cross-reactive antigens. Since HLA-A2 is the most
common HLA determinant and is strongly immunogenic in HLA-A2 negative patients, it is to be expected that most patients (except perhaps those typing for HLA-A9 and HLA-A28, which cross-react with HLA-A2) will readily become immunized to HLA-A2 following transfusions. Opelz and co-workers have observed that anti-HLA-A2 is the antibody most commonly encountered in renal dialysis patients with a history of blood transfusions. The good responses of HLA-A2 negative patients to C- and D-matched platelets which lack HLA-A2 may be evidence of a restricted pattern to alloimmunization to HLA. In such patients, lymphocytotoxic antibody analysis of sera may provide additional clues about restricted immunization to HLA and about the predictability of the transfusion of single-donor platelets.

The continued excellent responses of certain refractory patients to selectively mismatched platelets provides support for the concept that these patients may have relative immunologic unresponsiveness, or tolerance, to certain non-self-HLA specificities. An example is provided by H.F., who failed to become alloimmunized to HLA-B8 despite repeated transfusions with platelets mismatched for this antigen. H.F. was highly alloimmunized to HLA-A2 and HLA-A9, as evidenced by his poor response to transfusions mismatched for these antigens. We have encountered several other HLA-A2 negative patients who appear to have similar immunologic unresponsiveness to certain HLA specificities. Opelz and co-workers have observed the absence of lymphocytotoxic antibodies in the sera of one-half of renal dialysis patients who have received 30 or more blood transfusions. In some patients, lymphocytotoxic antibodies were often transient, and then sometimes disappeared despite repeated transfusions. Apparent unresponsiveness to HLA has also been demonstrated by Ferrara and co-workers, who have conducted studies of planned immunization against HLA by blood transfusion to volunteer recipients and found that apparent unresponsiveness to certain HLA antigens was unaffected by changing the frequency and intensity of immunogenic stimulations, even when different donors were used.

No explanation for the apparent effect of HLA-A2 on the survival of selectively mismatched platelets is readily apparent. It is possible that the HLA-A2 allele is associated with a gene controlling immune responsiveness to HLA. Alternatively, HLA-A2, because of its strong immunogenicity in HLA-A2 negative patients, may suppress the immune response to other HLA antigens by antigenic competition.

REFERENCES
6. Duquesnoy RJ, Filip DJ, Aster RH:
Histocompatible platelet transfusion therapy of refractory thrombocytopenic patients. Semin Thromb Hemostasis (in press)


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