Correction of Poor Platelet Transfusion Responses With Leukocyte-poor HL-A-matched Platelet Concentrates


Matching donor-recipient pairs for HL-A antigens provides a logical starting point for selecting donors for recipients with extensive prior transfusion histories. However, during the course of continued exposure to even HL-A-matched platelet concentrates, further sensitization occurs, as indicated by progressively poorer post-transfusion increments and transfusion reactions. There is evidence that such sensitization may be due to non-HL-A antigens. Finally, it is postulated that the poor post-transfusion platelet increments obtained when standard platelet concentrates are used result from the leukoagglutinin antigen-antibody reaction involving the platelet as an "innocent bystander." The standard platelet concentrate can be purified by a simple method of centrifugation (178 g × 3 min), removing about 96% of the contaminating white blood cells with concomitant loss of about 21% of the platelets. The use of these leukocyte-poor platelet concentrates can restore compatible transfusion increments in highly alloimmunized thrombocytopenic recipients. The leukocyte-poor concentrates can diminish undesirable transfusion reactions following incompatible platelet transfusions.

Hemorrhagic complications due to thrombocytopenia can be reduced by prophylactic platelet concentrate transfusions. However, prolonged transfusion support becomes impossible because of recipient alloimmunization to donor histocompatibility antigens. It was initially reported that satisfactory post-transfusion increments and reduced thrombocytopenic hemorrhage could be obtained when patients refractory to random donor platelets were transfused with platelets matched for HL-A lymphocyte antigens. Continued investigation has revealed that, despite the use of HL-A phenotypically matched donors, not all alloimmunized recipients achieve compatible post-transfusion platelet increments, and some may suffer transfusion reactions.

In an effort to determine the utility of HL-A-typed platelets, and to define the factors which limit their usefulness, we have reviewed our experience with histocompatible platelet transfusions in a population of alloimmunized recipients. It was observed that a significant number of "matched" platelet transfusions failed to produce satisfactory transfusion responses. Subsequent studies revealed that these poor transfusion responses could be corrected by the ad-
ditional processing of the platelet concentrates to remove contaminating leukocytes.

MATERIALS AND METHODS

HL-A typing of donors and recipients was performed on at least two occasions by the lymphocytotoxicity method of Mittal.12

HL-A compatibility was classified using the following nomenclature11: (1) A Match: donor and recipient have identical HL-A phenotype (or genotype). (2) B Match: all donor HL-A antigens are present in the recipient phenotype, but the donor lacks one (B-1) or two (B-2) of the recipient antigens. (3) C Match: the donor possesses one or more HL-A antigen(s) not found in the recipient. The C-matched donors described in this report were originally identified as B matches following the first typing, but upon subsequent typing were found to be mismatched by only one antigen.

Six patients with aplastic anemia and 11 patients with hematologic malignancy, all of whom had thrombocytopenia refractory to platelet transfusions from mismatched (random) donors, were chosen for study. Platelet concentrates from HL-A-matched donors were administered prophylactically to maintain the recipients' platelet count above a level at which signs of hemorrhage were known to occur (usually requiring twice weekly 4-unit platelet transfusions). Transfusions of platelet concentrates to patients with conditions which may alter the response to platelet concentrate transfusions in the absence of alloimmunization, such as fever, sepsis, disseminated intravascular coagulation, or splenomegaly, were excluded from analysis. When consistently good transfusion responses were obtained from a particular donor-recipient pair, the pair was considered only once to prevent bias of the data toward favorable responses. The “initial” time in the data refers to the first transfusion of a donor-recipient pair. The “final” time indicates the latest follow-up for each pair, which ranged from 1 to 60+ mo (more than 50% have been followed over 3 mo).

Three or four unit platelet concentrates were collected from individual donors by the “split-ACD” technique.13 The concentrates were kept at room temperature and transfused within 12 hr of collection. Leukocyte-poor platelet concentrates were prepared in the same manner, except for final centrifugation of 178 g for 3 min at room temperature followed by removal of the leukocyte-poor platelet-rich supernatant. Platelet concentrates were reseminated when a given donor-recipient pair failed to produce a compatible transfusion response initially or when a recipient who initially demonstrated a compatible transfusion increment failed to maintain this response with time.

Platelet counts were performed by phase-contrast microscopy14 on the recipients on the morning of transfusion (precount), and 1 and 20 hr after the completion of the transfusion (postcount). To facilitate comparison among recipients of different sizes receiving varying doses of platelets, the platelet increments measured at 1 and 20 hr post-transfusion were corrected according to the formula:

$$\text{Corrected Increment} = \frac{\text{Observed platelet increment}}{\text{Number of units transfused}} \times \text{B.S.A.} \times \frac{\text{B.S.A.}^*}{\text{B.S.A.}^*}$$

A compatible transfusion response is defined as a corrected 1-hr post-transfusion increment of ≥7500 platelets per cu mm × sq m/U (≥50% of the expected observed response of 15,000 platelets per cu mm × sq m/U)16 or a corrected 20-hr post-transfusion increment of ≥4500 platelets per cu mm × sq m/U (≥50% of the expected observed response of 9000 platelets per cu mm × sq m/U).16 In a preliminary examination of the transfusion response, it was found upon analysis of 200 platelet transfusions which resulted in a compatible response at 1 hr (≥7500 platelets per cu mm × sq m/U) that 36% (72 of 200) failed to have a compatible response measured at 20 hr (≥4500 platelets per cu mm × sq m/U). It was felt, therefore, that a more severe and significant test of transfusion response was the increment measured at 20 hr post-transfusion.

*Where the observed platelet increment is the difference between the post- and pretransfusion platelet counts; the B.S.A. is body surface area (square meters) as estimated from the nomogram of Sendroy and Cecchini.15
Alloimmunization was determined by failure to obtain satisfactory post-transfusion increments following multiple unrelated histoincompatible transfusions. Serums from all recipients were assayed serially for antibodies to HL-A antigens (lymphocytotoxicity assay) against a panel of lymphocytes from 100 HL-A-typed individuals, representing all known HL-A specificities.

For selected donor-recipient pairs, recipients' serums were analyzed serially for leukoagglutinating antibodies and complement-dependent lymphocytotoxicity and granulocytotoxicity against donor cells.

Statistical analysis was performed using the Fisher Exact Test or the large sample approximation. The Mantel-Haenszel Life Table Chi Square Test was used for analysis of the duration response.

RESULTS

The response to transfusion of platelet concentrates prepared using the standard technique was analyzed in 125 donor-recipient pairs (142 transfusions for 17 recipients). Fifteen of the 17 recipients had four HL-A antigens defined, and the remainder had three antigens defined. There were five genotypic MLC nonreactive sibling pairs out of 25 A-matched pairs. The match grades of the remaining 100 pairs were: 41 B-1 matches, 34 B-2 matches, and 25 C matches. The transfusion response employing the resedimentation technique was analyzed in 47 donor-recipient pairs (63 transfusions for 17 recipients).

As can be seen in Fig. 1, the initial transfusion response using standard platelet concentrates (the double cross-hatched portion of the bars) produced a compatible response in only 44%-72% of the pairs studied, depending on HL-A match grade. When the platelet concentrates from the same donor were further
Table 1. Effect of Resedimentation on the Cellular Components of Platelet Concentrates

<table>
<thead>
<tr>
<th>Method of Procurement</th>
<th>Number of Platelets (x 10^1)</th>
<th>Number of White Blood Cells (x 10^8)</th>
<th>Per Cent Granulocytes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard</td>
<td>0.880*</td>
<td>8.36*</td>
<td>3%</td>
</tr>
<tr>
<td></td>
<td>(0.437-1.678)</td>
<td>(4.80-22.50)</td>
<td>(0.42)</td>
</tr>
<tr>
<td>Leukocyte-poor</td>
<td>0.664</td>
<td>0.29</td>
<td>0%</td>
</tr>
<tr>
<td></td>
<td>(0.277-1.444)</td>
<td>(0.01-1.84)</td>
<td>(80.1-99.9)</td>
</tr>
<tr>
<td>Per cent reduction</td>
<td>21.4%</td>
<td>95.7%</td>
<td>100%</td>
</tr>
<tr>
<td></td>
<td>(1.3-48.6)</td>
<td>(80.1-99.9)</td>
<td></td>
</tr>
</tbody>
</table>

*Median range in parentheses.

processed to remove contaminating leukocytes, the initial compatible transfusion responses improved to 72%–96% of the pairs (p < 0.05).

Table 1 illustrates the loss of platelets and white blood cells incurred as a result of the resedimentation technique. The leukocyte-poor platelet concentrates have 95% of the white blood cells removed, with preservation of 79% of the original platelets collected. The loss of platelets incurred was not considered in the calculation of the increments. Rather, the actual number of units collected was used in the formula above, even though each unit contained approximately 20% fewer platelets.

Although resedimentation of platelet concentrates significantly increases the per cent of the donor-recipient pairs resulting in a compatible transfusion response (Fig. 1), following continued transfusion exposure a reduction in response is seen for both the standard and leukocyte-poor platelet concentrates.

The duration of compatible transfusion responses for the donor-recipient pairs, using both techniques, is depicted in Fig. 2. Donors were plateletheperesed
at a frequency of two to four times monthly. The median duration of compatible responses using standard platelet concentrates for all donor-recipient pairs (without regard for HL-A type) was 3.5 mo. The median duration for the leukocyte-poor platelet concentrates has not yet been reached but will be in excess of 6 mo, which is statistically different \( p < 0.02 \) compared to the standard platelet concentrates. When the longevity of response is analyzed by HL-A match grade, the A and B-I matches, combined, have a median duration of 4.5 mo (standard platelet concentrates), and the B-2 and C matches, combined, have a median duration of 1.5 mo, \( p < 0.01 \). When this duration of response to leukocyte-poor concentrates is analyzed by match grade, neither A and B-I (combined) nor B-2 and C (combined) have reached a median, and there is no statistical difference.

Analysis of recipient serums for HL-A antibody revealed that only one patient failed to make HL-A antibodies. Of the remaining 16, the level of antibody changed during the period of study. On “initial” serum samples, the median level of cytotoxicity against the panel of 100 donors was 44\% (0\%-80\%) and on the “final” specimens, 80\% (17\%-100\%). In an attempt to identify other antibodies in the recipients’ serums which would correlate with poor response to HL-A-matched platelet concentrates, 38 serially obtained serums from four recipients were studied in assays of leukoagglutination, lymphocytotoxicity, and granulocytotoxicity using cells obtained from 12 donors. In each instance, leukoagglutinating antibody in the recipients’ serums correlated with poor transfusion increments observed with the standard platelet concentrates. Two of the 12 donor-recipient pairs tested, showing positive correlation with poor transfusion response and leukoagglutinating antibody, were genotypic, HL-A-identical siblings. Demonstrable lymphocytotoxicity, using serums from three of the recipients, was present against seven nonfamily donors at a time when the standard platelet concentrates still resulted in compatible transfusion responses. Figure 3 depicts the clinical transfusion response history for a representative patient who was serially transfused with platelets processed by both techniques from the same unrelated HL-A-matched (B-I) donor. The correlation of poor platelet response with the development of leukoagglutinating antibody as well as the restoration of compatible increments with the use of leukocyte-poor platelet concentrates can be seen in Fig. 3.

A final observation is that in 21 of 24 (87\%) instances where standard collections from the same donor produced urticaria, fever, and/or chills, recipients of leukocyte-poor platelet concentrates failed to have transfusion reactions, even when poor transfusion increments were obtained.

DISCUSSION

Transfusion of allogeneic blood products regularly results in recipient sensitization to histocompatibility antigens.\(^8\) Sensitization to donor leukocytes can be detected by the development of leukoagglutinating or cytotoxic antibodies in the recipients’ serums, usually within 4-8 wk of beginning transfusions.\(^10\) In the presence of such antibodies, transfusion reactions occur following transfusion of whole blood or blood products.\(^21,22\) When platelets and/or leukocytes are
transfused under these circumstances, poor post-transfusion increments are observed.23

During the past few years, it has been observed, in a sequence of clinical experiments, that the HL-A antigen system can be used for the “matching” of platelet donors for patients alloimmunized to random mismatched blood donors.9,10 Most recently, it has been shown that long-term platelet transfusion support for alloimmunized thrombocytopenic recipients is possible if HL-A-compatible unrelated donors are employed.11 Our data confirm the value of HL-A matching for unrelated donor-recipient pairs. It appears from this study that the duration of compatible responses may also be influenced by match grade, for the median duration of response for A and B-1 matches (4.5 mo) is three times as long as that for B-2 and C matches (1.5 mo), p < 0.01. But, more importantly, these results indicate that with continued transfusion exposure we have observed a loss of effectiveness of matched platelet concentrates, regardless of HL-A match grade. This may, in part, be due to the fact that we are unable to identify all HL-A specificities for each donor and recipient. Thus, the sensitization of some recipients to undetected or as yet undefined HL-A specificities (even when thought to be phenotypically identical) is a possibility. The fact that “full house” (four antigens defined) typings have been available on 71% of the A and B-1 matched pairs diminishes the likelihood of this interpretation in most instances. However, recent findings suggest a possible third HL-A locus25,26 which may account for unresponsiveness due to HL-A antigen sensitization as also measured by the increased level of HL-A antibody against the panel of random donors (44%–80%). The improved response from platelets
further processed to remove contaminating leukocytes (granulocytes, lymphocytes, and monocytes) suggests that antibody, directed against cellular elements found to contaminate the platelet concentrate, but not required for platelet effectiveness, can interfere with the intravascular circulation of “compatible” platelets. These observations have been substantiated in four of the patients with demonstrable leukoagglutinating antibody against donor leukocytes in situations where only leukocyte-poor platelet concentrates have produced effective post-transfusion increments.

It is of interest that compatible platelet transfusion response can occur in the presence of complement-dependent lymphocytotoxic antibodies as demonstrated by a positive lymphocyte cross-match. This may occur because the antibodies are directed at donor non-HL-A leukocyte antigens and may therefore be specific non-HL-A alloantigens, or, alternatively, they may be HL-A or non-HL-A antigens which are not expressed on the surface of the platelets, such as have been described for HL-A 12.21.29

In rare instances we observed a failure of recipients to produce antibody directed against HL-A antigen specificities present on donor cells. One donor included in this study differed from the recipient by having HL-A 18, to which the recipient failed to produce anti-HL-A 18, despite twice monthly exposure for 2 yr. Failure to produce cytotoxic antibody despite continued transfusion exposure has been described by Opelz in renal transplant recipients.3 Unlike Opelz’s data, where only 50% of recipients produced antilymphocyte antibody, 94% (16 of 17) of our recipients made lymphocytotoxic antibody during their transfusion course.

Thus, the poor transfusion responses obtained with standard platelet concentrates may not always be due to a direct effect on donor platelets. Rather, we postulate that a leukoagglutinin antigen-antibody reaction occurs with the platelets being trapped as a so-called “innocent bystander,” with subsequent removal by the reticuloendothelial system.

The few transfusion reactions in recipients of leukocyte-poor platelet concentrates suggests that reactions associated with standard platelet concentrates are related to contaminating leukocytes rather than to the platelets per se. With this in mind, it seems logical to test all potential new platelet donors using leukocyte-poor platelet concentrates, thereby sparing the recipient the discomfort of transfusion reactions and the risk of post-transfusion granulocytopenic episodes as have been described with incompatible platelet transfusions.24

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