Evaluation of Four Methods for Platelet Compatibility Testing
By Janice G. McFarland and Richard H. Aster

Four platelet compatibility assays were performed on serum and platelet or lymphocyte samples from 38 closely HLA-matched donor/recipient pairs involved in 55 single-donor platelet transfusions. The 22 patients studied were refractory to transfusions of pooled random-donor platelets. Of the four assays (platelet suspension immunofluorescence, PSIFT; 51Cr release; microlymphocytotoxicity; and a monoclonal anti-IgG assay, MAIA), the MAIA was most predictive of platelet transfusion outcome (predictability, 74% for one-hour posttransfusion platelet recovery and 76% for 24-hour recovery). The only other assay to reach statistical significance was the PSIFT (63% predictability for one-hour posttransfusion recovery). The degree of HLA compatibility between donor and recipient (exact matches vs those utilizing cross-reactive associations) was unrelated to the ability of the MAIA to predict transfusion results. The MAIA may be capable of differentiating HLA antibodies, ABO antibodies, and platelet-specific antibodies responsible for failures of HLA-matched and selectively mismatched single-donor platelet transfusions.

EFFECTORINESS to platelet transfusions continues to complicate the care of patients with hematologic malignancies and aplastic anemia. When patients respond poorly to transfusions of random-donor platelets, many can be supported with transfusions of HLA-matched, single-donor platelets. As many as one third of the most closely HLA-matched platelet transfusions fail to achieve a satisfactory result, however.

Platelet compatibility testing offers a potential solution to this problem. Methods used to date with varying degrees of success include indirect platelet immunofluorescence, staphylococcal protein A binding, enzyme-linked immunosorbent assays (ELISA), and radioactive antiglobulin binding using labeled polyspecific antiglobulin or radiolabeled monoclonal anti-IgG.

In the present study, we compared four assays to determine which was most useful in predicting responses to closely HLA-matched single-donor platelet transfusions of patients refractory to random-donor platelets.

MATERIALS AND METHODS

Patient selection. Twenty-two patients with bone marrow hypoplasia secondary to chemotherapy, marrow infiltration, or aplastic anemia received HLA-matched and selectively mismatched platelet transfusions. All patients studied had documented poor responses to transfusions of random-donor platelet concentrates (mean one-hour posttransfusion platelet recovery, 8.5% ± 9.9% as determined by a recovery formula using an estimate of 7.5 × 109 platelets per random-donor concentrate to calculate the random platelet dose).

Serial serum samples were collected on these patients throughout the course of platelet transfusion support and stored at −80°C until used. Transfusion events were selected for study if (1) adequate documentation was available to define the patient’s clinical condition at the time of the transfusion, (2) a serum sample was obtained within 3 weeks prior to or (in several instances) shortly after platelet transfusion, and (3) the original donors were willing to return to the blood center to donate a unit or a sample of whole blood from which platelets and lymphocytes were isolated to use in the platelet compatibility tests. Patient records were examined to determine the presence or absence of fever (oral temperature greater than 38°C), sepsis, disseminated intravascular coagulation (DIC), bleeding, and the one- and 24-hour posttransfusion platelet count recovery. Transfused platelet recovery was calculated based on the following formula: Percent recovery = Body weight (kg) × 70 mL/kg × (posttransfusion platelet count – pretreatment platelet count)/μL) × 100/No. of platelets transfused × 0.67. The number of platelets transfused was calculated from the platelet count and volume of the single-donor concentrates. The factor 0.67 was used to correct for anticipated pooling of one third of the transfused cells in the spleen.

One-hour posttransfusion recoveries of 65% or more and 24-hour recoveries of 57% or more were considered successful responses.

Platelet preparation. EDTA (0.6 mL, 5% EDTA/10 mL) was added to platelet-rich plasma (PRP) from units of citrate-phosphate-dextrose-A (CPD-A) whole blood. The PRP was used as a fresh platelet source, or the platelets were isolated and frozen in 5% dimethyl sulfoxide (DMSO) at −80°C. Alternatively, 30 mL of blood in EDTA (0.3 mL, 5% EDTA/10 mL) was drawn from donors who were not eligible to donate a unit of whole blood. The PRP was separated (300 g for ten minutes), the platelets pellets (1,000 g for seven minutes), and the platelet-poor plasma removed. The platelet button was resuspended in buffer for the monoclonal anti-IgG assay (MAIA) (platelet suspension buffer, PSB) or the indirect immunofluorescence test (polyspecific antiglobulin test; sodium azide). Platelets were also frozen in 5% DMSO (−80°C) for use in the 51Cr release assay and in the MAIA.

MAIA. Monoclonal antihuman IgG was purified from ascites fluid (BRL 9474SA, Bethesda Research Laboratories, Gaithersburg, MD) by methods previously reported.

A method adapted from that described by Lo Buglio et al was used to quantify platelet-bound IgG. Platelets separated from EDTA whole blood or thawed in pooled AB plasma in 5% DMSO were washed three times in phosphate-buffered saline–EDTA (4 mmol/L) containing 1% bovine serum albumin (PSB) at pH 6.8 and the platelet count adjusted to 25 × 10^6 platelets in 0.05 mL buffer. A quantity of 0.250 mL serum (0.6 mL, 5% EDTA/10 mL) was added to the platelet suspension, and the mixture was incubated for 30 minutes at room temperature. The platelets were washed free of serum (1,000 g for seven minutes for three washes) and suspended in 0.3 mL of PSB, and 2 μg of labeled monoclonal antibody in PSB (0.2 mL) was added. In preliminary studies, this amount of mono-
clonal antibody was found to be capable of saturating IgG binding sites on platelets maximally sensitized with HLA or platelet-specific alloantibodies. After 30 minutes at room temperature, 0.1-mL aliquots containing 5 x 10^6 platelets were placed in triplicate in 0.4 mL Starstedt tubes over 0.2 mL 30% Percoll layers if fresh donor platelets were used. The concentration of Percoll was reduced to 20% if frozen platelets were used. The tubes were then centrifuged at 7,500 g for four minutes in a Beckman microfuge (Beckman Instruments, Inc, Fullerton, CA). The platelets migrated to the tube tips and left unbound 125I-labeled monoclonal antibody above the Percoll cushion. The Percoll cushion was then carefully aspirated from the top down by using a Pasteur pipette while leaving the undisturbed platelet button at the tube tip. The tube tips were separated and placed in gamma scintillation counting tubes, and the radioactivity was determined in a gamma counter. The number of molecules of IgG bound per platelet was determined by the following formula developed by LoBuglio and colleagues. Molecules IgG/platelet = 4.02 x 10^6 cpm in sample tube/specific activity x 5 x 10^6, where 4.02 x 10^6 is the number of molecules of monoclonal antibody/ng and specific activity is expressed as cpm per nanogram of labeled protein. The monoclonal anti-Fc antibody binds to all subclasses of IgG at a ratio of 1:1, 123

For each donor platelet preparation studied, an autologous control tube using donor platelets with autologous plasma was tested in addition to a positive control using a known anti-HLA-A2 or anti-PiA11 antisera and an HLA-A2-positive, PiA11-positive platelet preparation. The mean number of molecules IgG per platelet for the autologous controls was determined, and 2 SD above the mean was chosen as the cutoff for a positive result in this assay (2,720 molecules per platelet). The mean coefficient of variation (CV) of the MAIA was 14.5% ± 6.3%.

Platelet suspension immunofluorescence test. The platelet suspension immunofluorescence test (PSIFT) used was adapted from von dem Borne et al. Modifications included omission of the paraformaldehyde fixation step and reduction in the serum:cell ratio to 0.25 mL serum to 10^7 platelets. A fluorescein-conjugated polyvalent goat antihuman immune globulin was used to detect platelet-associated immunoglobulin (Meloy Laboratories, Inc, Springfield, VA). Results were graded 0 to 4+ based on the amount of fluorescence seen under the fluorescent microscope.

Chromium release test. This assay was performed on DMSO-frozen, papain-treated platelets. Patient sera were heat inactivated and added to 51Cr-labeled donor platelets. Complement in the form of normal human plasma anticoagulated with EDTA and supplemented with MgCl2 was added and lysis measured by detection of the amount of released 51Cr after two hours at 37°C. Normal human serum and a known anti-PiA1 antisera were used in each assay as negative and positive controls.

Microlymphocytotoxic crossmatch. Microlymphocytotoxicity testing was done by using standard methodology with patient serum and donor lymphocytes. Patient serum was used at dilutions of 1:2, 1:4, 1:8, and 1:16. Cell death was graded on a scale of 1 to 8. The scores for all dilutions were combined to arrive at a lymphocytotoxicity score. A score greater than 5 was considered a positive result.

Statistical analysis. Two-by-two chi-square comparisons of antibody test results with one- and 24-hour posttransfusion platelet recoveries were performed for all tests by using the cutoff values for successful transfusion responses defined before. For purposes of this study, the following definitions of sensitivity, specificity, and predictability were used: Sensitivity = true positives/(true positives + false negatives). Specificity = true negatives/(true negatives + false positives). Predictability = (true positives + true negatives)/all test results. True positive refers to those donor-recipient pairs with positive antibody test results and poor transfusion responses, true negative to negative test results with satisfactory transfusion responses, false-positive to positive test results and satisfactory responses, and false-negative to negative test results and poor transfusion responses.

RESULTS

In all, 55 transfusions were studied. Thirty-five transfusions from 25 donors administered to 17 patients were documented to occur in the absence of fever, bleeding, splenomegaly, sepsis, or DIC (group 1). One-hour posttransfusion platelet recoveries were available on all 35 transfusions in group 1; 24-hour recoveries were available in 34. Twenty transfusions from 17 donors were given to 14 patients in whom one or more of the clinical factors listed before were present (group 2). One-hour recovery data were available on all 20 of the group 2 patients, and in 19, 24-hour data were available. Nine patients received transfusions classified in groups 1 and 2 because of changes in their clinical status. Serum samples from the patients were collected a mean of 3.5 ± 8.1 days prior to transfusion. Forty-six samples were collected pretransfusion (zero to 22 days), and nine were collected posttransfusion (one to 15 days).

Results of all four assays were correlated with the transfusion responses for group 1 transfusions. Sensitivity, specificity, and predictability at one and 24 hours were calculated for each assay (Table 1). Of the four assays, the MAIA was most predictive of transfusion outcome (Fig 1). At 24 hours, its specificity was 100%, ie, no false-positive reactions were obtained. The only other assay to reach statistical significance in predicting recovery was the PSIFT. However, PSIFT had only 40% sensitivity at one hour. When group 1 and group 2 transfusions were combined, the ability of the MAIA test to predict transfusion responses at one and 24 hours was reduced (Fig 2). Even so, specificity at 24 hours was 86%.

Donor-recipient matches utilizing cross-reactive HLA associations (B1X, B2X, B3X, B2UX) (Table 2) were compared with those that were more completely matched (A, B1U, B2U). At one hour, the response to the cross-reactive matches was predicted less well by the MAIA (Table 3) than

<table>
<thead>
<tr>
<th>Table 1. Prediction of Transfusion Responses</th>
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<td>Sensitivity</td>
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<td>Specificity</td>
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<td>LCT</td>
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<td>Sensitivity</td>
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<td>Predictability</td>
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<td>HCr release</td>
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<td>Specificity</td>
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<td>Predictability</td>
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Abbreviations: LCT, lymphocytotoxicity test; NS, not significant.
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The response to fully matched transfusions (Fig 3B). This difference was less apparent at 24 hours (Fig 4A and 4B).

When the mean of the positive reactions for the cross-reactive group of transfusions was compared with that of the fully matched group at 24 hours, we found that positive reactions in the latter group were characterized by levels of platelet-associated IgG about twice as great (7,310 molecules per platelet) (Fig 5).

ABO antibodies have been implicated as a cause of refractory platelet responses in some patients. The ABO blood groups of both recipients and donors were analyzed for cross-reactive and fully matched transfusions. In evaluating the 24-hour data, four of nine positive reactions noted in the fully matched group were found to be associated with transfusion of platelets from an A or AB donor to a group O patient. Only one of the positive reactions in the cross-reactive group represented this type of incompatibility (Fig 4). These numbers were too small to allow meaningful statistical analysis.

One group O patient with a previously demonstrated high-titer anti-A in his serum (agglutinating and hemolytic) was included in the study. Three transfusions from group A donors (HLA match grades A, B2U, B1X) and two transfusions from group O donors (HLA match grade B2UX) were

![Crossmatch results using the MAIA. One-hour (A) and 24-hour (B) platelet recoveries (abscissa) and number of molecules of IgG measured per platelet in the MAIA crossmatch (ordinate) are shown for group 1 transfusions. A total of 2,720 molecules per platelet or greater was considered a positive result based on the mean number of molecules per platelet plus 2 SD in the normal autologous controls (n = 34). A successful one-hour recovery was considered to be 65% and a successful 24-hour recovery, 57%. $\chi^2 = 8.11, P < .01$ for one hour recovery; $\chi^2 = 11.77, P < .001$ for 24-hour recovery ($2 \times 2$ chi-squared test of homogeneity).](image1)

![Crossmatch results using the MAIA for all transfusions in groups 1 and 2 at one hour (A) and 24 hours (B). Units are as in Fig 1. $\chi^2 = 6.24, P < .05$ for one-hour recovery, $\chi^2 = 10.25, P < .01$ for 24-hour recovery ($2 \times 2$ chi-squared test of homogeneity).](image2)

**Table 2. Classification of Donor/Recipient Pairs on the Basis of HLA Match**

<table>
<thead>
<tr>
<th>HLA Match</th>
<th>Description</th>
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<tr>
<td>A</td>
<td>All 4 antigens in donor identical to those of recipient</td>
</tr>
<tr>
<td>B1U</td>
<td>Only 3 antigens detected in donor, all present in recipient</td>
</tr>
<tr>
<td>B2U</td>
<td>Only 2 antigens detected in donor, both present in recipient</td>
</tr>
<tr>
<td>B1X</td>
<td>Three donor antigens identical to recipient, fourth antigen cross-reactive with recipient</td>
</tr>
<tr>
<td>B2UX</td>
<td>Only 3 antigens detected in donor: 2 identical with recipient, third cross-reactive</td>
</tr>
<tr>
<td>B2X</td>
<td>Two donor antigens identical to recipient, third and fourth antigens cross-reactive with recipient</td>
</tr>
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From Duquesnoy et al.³
analyzed. The mean MAIA result for group A transfusions was 9,595 ± 1,473 molecules IgG per platelet (Fig 5), and that of the group O platelets was 689 ± 420 molecules IgG per platelet. These MAIA results correlated with the transfusion responses: group A transfusions, 12%, 19%, 0% recovery at one hour; and group O transfusions, 128%, 49% recovery at one hour.

**DISCUSSION**

Of the four methods studied, the MAIA test was most predictive of transfusion response at one and 24 hours. The majority of the MAIA assays were performed on frozen donor cells, which suggests that frozen platelets can be used to select donors for refractory patients. In contrast, the PSIFT lacked sensitivity in our hands. Others have found this assay to be more sensitive. Two possible explanations are (1) the lower serum:cell ratio used in our studies and (2) the fact that all transfusions studied by us were matched or only slightly mismatched for class I HLA antigens. It may be that more extreme HLA incompatibilities are necessary to demonstrate positive reactions in the PSIFT. The lymphocytotoxicity test was not predictive of the transfusion response. This is probably due to the fact that closely HLA-matched donors were used for these patients. Antiglobulin enhancement of the microcytotoxicity test, not used in this study, might have improved the sensitivity of the lymphocyte crossmatch. The failure of the ^51^Cr release method to predict platelet transfusion responses probably reflects the requirement of this assay for complement-fixing antibodies.
Although the MAIA provided the best prediction of transfusion response, occasional false-positive and, more common, false-negative reactions were noted, particularly when recoveries one hour after the transfusion were evaluated. The false-positive reactions for one-hour recovery predictions may reflect the high sensitivity of the MAIA for detection of platelet-bound IgG, i.e., it may be capable of detecting amounts of IgG insufficient to cause poor one-hour recoveries but sufficient to cause platelet destruction in 24 hours. False-negative reactions might be explained by the inability of our monoclonal antibody to detect immunoglobulins other than IgG. Alternatively, nonimmune factors causing platelet consumption that were not apparent clinically may have affected recoveries in some patients.

It is evident that the MAIA predicted responses to transfusions that were apparently matched for all class I HLA-A and -B antigens as well as those that were slightly mismatched (cross-reactive) (Fig 4). The positive crossmatches seen with platelets of match grades A and BU could be caused by antibodies reactive with platelet-specific antigens or with A or B blood group substances. Subtle Class I HLA antigen differences not characterized by available serological reagents might also explain the positive reactions seen with these well-matched but unrelated donor/recipient pairs. The larger number of ABO-incompatible transfusions in the fully matched group with positive crossmatches supports the possibility that ABO antibodies are important in some of these positive reactions. Deposition of extremely high levels of IgG by one patient’s serum (G.M.) onto donor cells (Fig 5) suggests the presence of one or more antibodies reacting with platelet-specific antigens that are expressed more densely on the platelet surface than HLA antigens.  

These preliminary results showing differences in the frequency of positive crossmatches and the strength of individual reactions with HLA-cross-reactive, HLA-fully matched, and ABO-incompatible donor-recipient pairs suggest that the MAIA may be useful in distinguishing HLA from non-HLA seroreactivity as the cause of platelet refractoriness in alloimmunized patients. Larger numbers of transfusions must be analyzed to investigate this possibility.

ACKNOWLEDGMENT

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