Selection of Donor Platelets for Alloimmunized Patients Using a Platelet-Associated IgG Assay

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A quantitative immunofluorescence platelet-associated immunoglobulin-G (PA-IgG) assay was used to detect alloimmunity to platelets in 8/12 multitransfused patients and to perform platelet crossmatching in the 8 alloimmunized patients. The correct separation of multitransfused patients into alloimmune and nonalloimmune groups was substantiated with chromium-51-labeled platelet survival studies. For 5 alloimmunized patients, compatible and incompatible donor platelets were demonstrated by PA-IgG crossmatching and were confirmed by platelet survival studies. With the other 3 alloimmunized patients, only PA-IgG incompatible donor platelets were found. Survival studies with 5 of these incompatible donor platelets showed markedly reduced survival times on 4 occasions.

RePEATED BLOOD or platelet transfusions produce alloimmunization of the recipients and unresponsiveness to further random donor platelet transfusions.\(^1\)\(^2\) HLA-compatible donor platelets from related and unrelated individuals initially produce an adequate platelet transfusion response in most of these alloimmunized recipients.\(^5\)\(^10\) Therefore, it has been suggested that HLA typing of a large section of the population would provide a sufficient number of compatible platelet donors to circumvent the problem of alloimmunization and resistance to platelet transfusions. However, three major reasons are against this ambitious plan: (1) HLA typing is very expensive, time-consuming, and complex; (2) it would require a very large file of HLA-typed prospective donors to satisfy the increasing demand for platelets; and (3) with continued use of HLA-matched platelets, some 30%-35% of transfusions ultimately fail to produce a satisfactory response.\(^11\)\(^19\)\(^23\) This unexpected unresponsiveness has been variously attributed to white cell contamination\(^11\) or to antibodies against platelet-specific antigens or currently unidentified HLA antigens.\(^15\)\(^17\)\(^19\)\(^23\) In spite of much progress, the problem of alloimmunization and resistance to platelet transfusions is still with us. It, therefore, seems logical that a predictive platelet crossmatching technique to select the best compatible donor platelets for alloimmunized recipients would be of paramount importance. It could be used as an adjunct to HLA typing or, if effective enough, it could possibly replace the need for HLA typing.

Various platelet crossmatching assays have been proposed, including platelet aggregation,\(^18\)\(^19\)\(^20\)\(^24\) platelet serotonin release,\(^25\) platelet factor 3 release,\(^26\) platelet complement fixation,\(^27\) platelet migration inhibition,\(^13\) and lymphocytotoxic antibody screening.\(^16\)\(^28\) The multiplicity of these assays attests to the difficulty in finding an ideal technique. In addition, further evaluation of these assays has often shown their insensitivity, inconsistency of results, and at times, unusual technical complexities.\(^13\)\(^15\)\(^20\)\(^23\)\(^29\)\(^32\) False negative and false positive crossmatch predictions have also been especially troublesome with these methods.\(^15\)\(^29\)\(^30\)\(^32\)

An immunofluorescence test measuring platelet surface IgG was introduced a few years ago\(^15\)\(^33\) based on the qualitative measurement of fluorescence. When combined with lymphocytotoxicity testing, it correctly predicted 93% of platelet transfusion responses in alloimmunized patients as judged by posttransfusion platelet increments.\(^15\) However, one knows by experience that platelet survival as measured by circulating platelet increments is often subject to error unless the increments are of a significant magnitude or are followed repeatedly for at least 24 hr.

We recently developed a quantitative immunofluorescence platelet-associated IgG (PA-IgG) assay that provided a sensitive method to detect an immune IgG antibody on the platelet surface and in the serum of patients with immune thrombocytopenic purpura (ITP).\(^34\) In the present study, this new PA-IgG assay was used first to detect alloimmunity to platelets in multitransfused patients and then to perform platelet crossmatching of donor platelets for these patients.
Survival of $^{51}$Cr-labeled donor platelets in the sensitized recipients was used as proof of the predictive results.

**MATERIALS AND METHODS**

**Human Subjects**

Twelve multitransfused patients ranging in age from 16 to 75 yr were studied (Fig. 1, Table 1). Four patients had acute myelocytic leukemia, four refractory anemia, and one each Fanconi’s anemia, sideroblastic anemia, non-Hodgkin’s lymphoma, and double heterozygosity for $\beta$-thalassemia and a high oxygen affinity hemoglobin variant. The patients received 0-137 U of platelet and 4-26 1 U of red cell transfusions over 1 mo to 9 yr. Only 5 patients (nos. 1, 5, 6, 10, 12) had previously received more than 10 U of transfused platelets. Three of these patients (nos. 1, 5, and 6) had demonstrated refractoriness to random donor platelets as indicated by $I^+$ and 24-hr posttransfusion platelet increments less than 10% of the predicted value on 2 or more occasions. No patient had splenomegaly, infection, or disorders associated with accelerated platelet consumption at the time of the study. Normal volunteers ranging in age from 23 to 62 yr were obtained from the medical and laboratory personnel of the Division of Hematologic Research or from the family members of the patients. Informed consent was obtained from all patients and volunteers according to the principles of the Declaration of Helsinki.

**Platelet-Associated IgG (PA-IgG) Assay**

The direct and indirect PA-IgG tests were performed as previously described with a modification of the indirect test. The direct test measures the amount of IgG on the platelet surface, while the indirect test determines the quantity of PA-IgG on the platelet surface after preincubation of platelets with test serum. The quantity of PA-IgG in the test serum, termed the indirect PA-IgG level, was calculated as the difference between the PA-IgG value in the indirect test minus that in the direct test. In the present study for the indirect test, washed platelets were suspended in phosphate-buffered saline containing 15% acid citrate dextrose (pH 5.2) to prevent platelet clumping. With this modified indirect PA-IgG test, a new normal range was established for the indirect (serum) PA-IgG level using 63 normal volunteers: 0.45 fg/platelet (mean $\pm$ 2 SD, 0.14 $\pm$ 0.30 fg/platelet). Reproducibility of the indirect test was assessed by performing 12 assays with a single normal serum sample. The results showed a mean $\pm$ 2 SD of 0.21 $\pm$ 0.08 fg/platelet. All tests were performed in duplicate. In each experiment, control assays using known positive and negative sera were tested.

**Platelet Aggregation**

Detection of platelet alloantibodies in test sera using platelet aggregation was performed by the method of Wu et al. Blood was obtained from patients and normal controls who had stopped for at least 1 wk all medications known to affect platelet function. After incubation of platelet-rich plasma with $^{14}$C-5-hydroxytryptamine creatine sulfate ($^{14}$C-serotonin, Amersham Corp., Arlington Heights, Ill.) for 45 min at 37°C, Impramine (Sigma Chemical Co., St. Louis, Mo.) at a final concentration of 2 $\mu$M was added to prevent platelet serotonin reuptake. The control range of serotonin release determined from 24 normal sera was 12.4% $\pm$ 6.7% (mean $\pm$ 2 SD). A positive serotonin release was defined as a test result greater than the upper limit of the control range (i.e., $>$19%).

**Abo and Rh Typing**

ABO and Rh typing were performed by standard agglutination methods.

**Lymphocyte HLA Typing and Lymphocytotoxic Antibody Testing**

The standard NIH lymphocyte microcytotoxicity technique modified from Terasaki’s method was used to determine HLA haplotypes and to perform lymphocytotoxicity antibody testing. The following HLA specificities were identified: A1, A2, A3, A9, A10, A11, A23, A24, A25, A26, A28, A29, A30, A31, A32, A33, and A36 at the A locus and B4, B5, B6, B7, B8, B12, B13, B14, B15, B16, B17, B18, B21, B22, B27, B35, B37, B38, B39, B40, B41, B42, B44, B45, B47, B49, B50, B51, B52, and B53 at the B locus. HLA matching was defined according to Duquesnoy et al. In brief, A represents all four antigens identical in the donor and recipient; B1U, only three antigens detected in donor and all present in recipient; B1X, three donor antigens identical to recipient and fourth cross-reactive; B2UX, three antigens determined in donor, two identical with recipient, third cross-reactive; B2X, two donor antigens identical to recipient, third and fourth cross-reactive; C and D, one and two or more antigens of donor not present in recipient and not cross-reactive. Lymphocytotoxicity was graded as follows: 0, no detectable antibody; 1+, antibody against $\leq$10% of the cells; 2+, 10%-49%; 3+, 50%-74%; and 4+, 75%-100%. Reactions of 2-4+ were considered positive.

**$^{51}$Cr-Sodium Chromate Platelet Survival Studies**

Platelet chromium-51 survival studies were performed according to Morrison and Baldini with minor modifications. Whole blood (450 ml) was collected in phlebotomy bags containing 67.5 ml of citrate phosphate dextrose solution (Fenwal Labs, Deerfield, Ill.). Contaminating erythrocytes and leukocytes were eliminated from the platelet-rich plasma by centrifugation at 370 g for 2 min. $^{51}$Cr-sodium chromate (E.R. Squibb & Sons, Inc., Princeton, N.J.) was incubated for 30 min with the platelets. The platelets were then washed once with autologous plasma (200-230 ml), resuspended in 20 ml plasma, and infused into the patient. Blood samples for counting were taken 5 min, 30 min, 2 hr, and 4 hr postinfusion on the first day and daily thereafter for up to 10 days. The normal range of allogeneic platelet survival was determined from 12 studies performed in nonthrombocytopenic, nonalloimmune volunteers. The normal ranges of maximum recovery and survival time were 39%–71% (mean $\pm$ SD, 55% $\pm$ 16%) and 7.2-9.8 days (8.5 $\pm$ 1.3 days), respectively.

**Alloimmunity and Crossmatch Testing Data Analysis**

An elevated indirect PA-IgG level (>$0.45$ fg/platelet) or a positive platelet aggregation (>$5%$), serotonin release ($>$19%), or lymphocytotoxicity test indicated platelet alloimmunity and an
incompatible crossmatch result. Normal results for these assays represented a compatible crossmatch test. Alloimmunization was substantiated by a reduced platelet survival study without another cause for the reduction. The prediction of a crossmatch test was judged against platelet survival studies. A correct prediction was defined as (1) a negative crossmatch test associated with a normal platelet survival or (2) a positive crossmatch test associated with a reduced platelet survival. An incorrect prediction was (1) a negative crossmatch test and a reduced platelet survival ("false negative" prediction) or (2) a positive crossmatch test and a normal platelet survival ("false positive" prediction). An equivocal prediction included a negative crossmatch test and mildly reduced platelet survival with an explanation for the reduction other than alloimmmunity.

RESULTS

Demonstration of Alloimmunity

Sera from the 12 patients were tested against a donor panel of 8 normal volunteers and family members of individual patients using the indirect platelet-associated IgG (PA-IgG), platelet aggregation, and platelet serotonin release assays. Patients 1–8 showed elevated indirect PA-IgG levels in 2–10 tests (Fig. 1), indicating alloimmunity to the donor platelets. The direct PA-IgG levels were normal for all

Table 1. Relationship Between Platelet Survival, Platelet Crossmatching Tests, and HLA Matching

<table>
<thead>
<tr>
<th>Patient</th>
<th>Diagnosis*</th>
<th>Transfusions</th>
<th>Platelet Count†</th>
<th>Platelet Survival Max.</th>
<th>Recovery‡</th>
<th>Days‡</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Group I</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>AML</td>
<td>137 15 &lt;1</td>
<td>D 140 0.35 0 0  +</td>
<td>71 8.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>RA</td>
<td>10 24 &lt;48</td>
<td>C 110 0.21 0 0  -</td>
<td>52 8.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>RA</td>
<td>0 26 D</td>
<td>150 0.11 0 0  -</td>
<td>60 6.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>FA</td>
<td>3 37 &lt;48 B2UX</td>
<td>110 0.28 0 0  +</td>
<td>34 8.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>AML</td>
<td>127 3 BIX</td>
<td>30 0.26 0 0  -</td>
<td>57 3.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6§</td>
<td>AML</td>
<td>75 9 C</td>
<td>165 0.47 0 0  +</td>
<td>65 0.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7§</td>
<td>RA</td>
<td>0 36 A</td>
<td>210 0.74 0 0  +</td>
<td>20 0.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8§</td>
<td>β-thal</td>
<td>0 261 BUI</td>
<td>220 2.10 53 65  +</td>
<td>69 7.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group II</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>SA</td>
<td>0 57 D</td>
<td>300 0.33 0 0  -</td>
<td>47 9.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>AML</td>
<td>54 18 8 D</td>
<td>182 0.31 0 0  -</td>
<td>64 8.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td></td>
<td></td>
<td></td>
<td>&lt;0.45 &lt;5 &lt;19</td>
<td>39–71 7.2–9.8</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: AML, acute myeloid leukemia; RA, refractory anemia; FA, Fanconi’s anemia; β-thal, double heterozygosity for beta-thalassemia and high-affinity oxygen hemoglobin; SA, sideroblastic anemia.

†At time of platelet survival study.
‡The total platelet survival was the time at which 10% of the maximum recovery value was reached. The maximum platelet recovery value was determined at 5 min after infusion, which may explain the apparently "high" incremental recovery values compared with reported 30-min recoveries for alloimmunity.
§No compatible donor platelets by PA-IgG testing.
| Patients 11 and 12 died before platelet survival studies could be performed.
| Donors were family members as follows: B2UX, mother; D, father; C, daughter; all other donors were from the panel of volunteers.

Fig. 1. Indirect platelet-associated IgG (PA-IgG) levels for the 12 patients testing their sera with the panel of donor platelets. Normal range <0.45 fg/plt shown by stippled area. HLA matching grades are indicated as follows: A, closed triangle; B, open circles; C and D, closed circle.
patients, thus excluding an autoantibody in their sera as the cause of the elevated indirect PA-IgG levels. All the indirect PA-IgG levels were normal with sera from patients 9–12 (Fig. 1). Therefore, the 12 patients were clearly separated into alloimmune (group I) and nonalloimmune (group II) groups based on the PA-IgG assay.

When tested against the donor panel, the sera of only 3 alloimmune patients (nos. 1, 3, 8) produced positive platelet aggregation results, implying alloimmunity in these patients. Similarly, a positive serotonin release was observed with the sera of just 2 patients (nos. 6 and 8). A positive lymphocytotoxicity antibody test, indicating alloimmunity, was found with the sera from 5 of 8 alloimmune patients (Table I).

**ABO, Rh, and HLA Matching**

ABO and Rh mismatches did not influence the results of the indirect PA-IgG levels or platelet survival studies, similar to previous findings. For the PA-IgG crossmatches shown in Fig. 1, all donor–recipient pairs were HLA-C and D matches except for 6 pairs, of which 5 were used for survival studies (see Table 1).

**Relationship Between Platelet-Crossmatch Testing, 51Cr-Platelet Survival Studies, and HLA Typing**

Both compatible and incompatible PA-IgG crossmatch tests were found for patients 1–5 (Fig. 1). Two platelet survival studies using PA-IgG compatible and incompatible donor platelets were then performed for each of these 5 patients. Nine of the 10 survival studies confirmed the PA-IgG crossmatch predictions (Table 1). For these 9 studies, PA-IgG compatible platelets survived normal times of 6.5–8.7 days, while incompatible donor platelets demonstrated reduced survival times of 0.1–2.4 days. The survival that did not confirm that the PA-IgG crossmatch was equivocal. The survival time was 3.5 days using PA-IgG compatible donor platelets in a severely thrombocytopenic (30,000/µl) patient (no. 5) during relapse of acute leukemia. This study was considered equivocal since a reduced survival time may be seen with very severe thrombocytopenia. For patients 6–8, only incompatible PA-IgG platelet crossmatches were found with the donor panel (Fig. 1). With these 3 patients, 5 survival studies were then performed using donor platelets with the lowest indirect PA-IgG levels or with HLA-A or B matches (Table 1). Four of the 5 studies showed markedly reduced survival times of 0.1–0.9 days. The only incorrect PA-IgG prediction was a false positive crossmatch with patient 8 (Table 1), a result we cannot currently explain. As illustrated in Fig. 2, an elevated indirect PA-IgG level indicated a reduced survival time of 0.1–2.4 days on 9/10 occasions, whereas a normal PA-IgG level was associated with a platelet survival of 3.5–8.7 days.

Platelet aggregation and platelet serotonin release crossmatch tests were performed for the 8 alloimmu-
Table 2. Predictability of Platelet Crossmatching Tests Compared With $^{51}$Cr-Platelet Survival Results

<table>
<thead>
<tr>
<th></th>
<th>Indirect PA-IgG</th>
<th>Platelet Aggregation</th>
<th>Serotonin Release</th>
<th>Lymphocytotoxicity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of results</td>
<td>15</td>
<td>8</td>
<td>7</td>
<td>10</td>
</tr>
<tr>
<td>Number of negatives</td>
<td>0</td>
<td>8</td>
<td>9</td>
<td>5</td>
</tr>
<tr>
<td>Number of positives</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Equivocal results</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Seventeen tests performed.

of the I
confirmed by normal platelet survival times (Table I, crossmatch result for patient 8. showed compatible crossmatches with all donor plate-
tin release, and lymphocytotoxicity antibody tests
pairs showing the most elevated indirect PA-IgG

An additional positive crossmatch for each assay was a
survival studies (Table I). These crossmatch
assays were negative for all but 3 and 2, respectively,
of the 15 studies performed. The positive crossmatches
predicted incompatible donor platelets with shortened
survival times on only 2 and 1 occasions, respectively.
An additional positive crossmatch for each assay was a
false positive result for patient 8, similar to the predic-
tion with the PA-IgG assay. The positive crossmatches
with these assays were found with the donor–recipient
pairs showing the most elevated indirect PA-IgG
levels.

Lymphocytotoxicity testing showed only positive or
negative crossmatch results for each donor pair tested
(Table 1). This assay also provided a false positive
crossmatch result for patient 8.

Indirect PA-IgG, platelet aggregation, platelet sero-
tonin release, and lymphocytotoxicity antibody tests
showed compatible crossmatches with all donor plate-
lets for the 4 nonalloimmunized patients (nos. 9–12,
Fig. 1, Table 1). These crossmatch predictions were
confirmed by normal platelet survival times (Table 1,
Fig. 2).

Table 2 summarizes the overall crossmatch predic-
tion results of the 4 assays for the 12 patients. The
PA-IgG assay correctly indicated the platelet survival
results on 15/17 occasions (88%), platelet aggregation
8/17 (47%), serotonin release 7/17 (41%), and
lymphocytotoxicity 10/17 (59%). False negative
results were seen in 29%–53% of the studies with the
latter 3 assays. Also, these 3 assays did not discrimi-
nate compatible from incompatible donor platelets in
patients 1–5, since only positive or negative cross-
matches were seen with these tests for each donor
pair.

Finally, the PA-IgG assay predicted the survival of
donor platelets in the 8 alloimmunized patients inde-
pendent of HLA typing. HLA-C and D matched
platelets, which were PA-IgG compatible, survived
normally in the alloimmunized recipients (Table I).
Recipient HLA-A2 antigen status did not influence
the response to these C- and D-matched platelets, as
has been shown to be possible.9 HLA-A- and
B-matched platelets showed two normal, one interme-
diate, and two markedly reduced platelet survivals
(Table 1). The PA-IgG assay correctly predicted 4 of
these 5 survival outcomes, including the two reduced
survivals.

DISCUSSION

Our quantitative immunofluorescence PA-IgG
assay provided a rapid, sensitive, and reproducible
method to detect platelet alloimmunity and to cross-
match donor platelets for alloimmunized patients. In
this study, the correct separation of 12 multitransfused
patients into alloimmunized and nonalloimmunized
groups based on PA-IgG testing was confirmed for all
patients by $^{51}$Cr-labeled platelet survival studies.
Moreover, when used to crossmatch donor platelets for
alloimmunized patients, the PA-IgG assay correctly
predicted the in vivo survival of these platelets on
15/17 occasions (88%) with one additional equivocal
result. As predicted by PA-IgG crossmatching, plate-
let incompatibility was associated with survival times
of less than 2.5 days, whereas in vitro compatible
platelets had survival times of 6.5–8.7 days. The
reduced platelet survival seen in one case with an
equivocal PA-IgG crossmatch may be explained by
the patient’s very severe thrombocytopenia, a situation
reported to produce a modest reduction in platelet
survival.34,41 Importantly, the PA-IgG assay could
differentiate compatible and incompatible donor
platelets for individual alloimmunized patients and
could indicate patients showing incompatibility to all
donor platelets tested, even HLA-A- and B-matched
platelets.

This indirect PA-IgG assay provided a quantitative
measurement of platelet-associated IgG, thus avoiding
the subjective interpretation necessary with previously
reported qualitative immunofluorescence assays.15,13
These qualitative PA-IgG assays detected platelet
alloimmunity in 13/16 multitransfused patients33 and
correctly predicted platelet crossmatches in 76/82
cases (93%) when combined with lymphocytotoxicity
testing.15 However, assessment of crossmatch predic-
tions in the latter study15 was based only on postrans-
fusion platelet increments and the alloimmune status
of the patients in the former report33 was not substan-
tiated. The present study combines a quantitative
PA-IgG assay with platelet survival measurements to
confirm these previous findings and to provide more
solid substantiation of the correctness of PA-IgG crossmatching. Also, this study indicates that platelet crossmatching for alloimmunized patients is feasible using a PA-IgG technique.

The control measurements with platelet aggregation, platelet serotonin release, and lymphocytotoxicity testing in this study found these assays to be inadequate crossmatching techniques, as previously reported by others. The authors wish to thank Jean Bishop and Susan Berry for technical assistance and Vicki Shackelford for secretarial assistance.

REFERENCES

37. Daly PA, Schiffer CA, Aisner J, Wiernich PH: Platelet transfusion therapy. One-hour post-transfusion increments are valuable in predicting the need for HLA-matched preparations. JAMA 243:435, 1980
Selection of donor platelets for alloimmunized patients using a platelet-associated IgG assay

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