The Expression of IgG Allotypes on Platelets and Immunization to IgG Allotypes in Multitransfused Thrombocytopenic Patients

By Thomas S. Kickler, Paul M. Ness, Hayden G. Braine, Lisa Richardson, and Mary Farkosh

We investigated whether the platelet-membrane surface carries IgG allotypic antigens and whether these determinants may be important in platelet transfusion therapy. Using a hemagglutination inhibition assay, we showed that the G1m IgG allotypes (a, x, f) and K1m and K3m light-chain allotypes are expressed on the surface of platelets, whereas G3m allotype determinants were not detectable. In 146 multitransfused thrombocytopenic patients, 35 (24%) patients were found to have antiallootypic antibodies. To study the effect of antiallootypic antibodies on platelet transfusion outcome, patients received platelet transfusions from donors, either positive or negative for the IgG allotype to which patients were immunized. Of the 19 antigen-positive and 19 antigen-negative platelet transfusions given, respectively, the mean platelet count increments at 1 hour were 8,402 ± 6,721 (1 SD) and 9,799 ± 5,559 (1 SD) P < .2. Transfusion reactions were not more common when antigen-positive platelet transfusions were given. Despite the presence of IgG alloantic determinants on platelets, alloantic antibodies do not decrease platelet transfusion recovery. Furthermore, passive administration of plasma containing IgG alloantibodies to patients with antiallootypic antibodies does not lead to innocent bystander-mediated platelet destruction.

© 1990 by The American Society of Hematology.

The platelet membrane normally carries IgG, estimated at a few hundred molecules. This value may be even higher after platelets are collected and stored for transfusion. Since polymorphisms of IgG (allotypes) exist, platelet-associated IgG could possibly serve as an antigenic target in patients immunized to IgG alloantigens. In transfused thrombocytopenic patients with antiallootypic antibodies, shortened survival of transfused platelets could be a potential result.

Besides platelet-associated IgG being present on transfused platelets, the plasma in which they are suspended contains IgG. If transfusion recipients are alloimmunized to the allootypic determinants of the transfused IgG, antigen-antibody complexes (ie, anti-IgG-IgG) may form. The resulting immune complexes could bind to transfused platelets, leading to shortened platelet-transfusion survival.

Inherited polymorphisms on human immunoglobulins are called allotypes and have been found on IgG heavy chains (Gm markers), IgA heavy chains (Am markers), and light chains (Km markers). The large number of polymorphisms constituting Gm allotypes rivals that of the HLA System. This has contributed to their usefulness in genetic linkage studies and for disease-association analysis.

The well-described polymorphisms are listed in Table 1. These allotype determinants are found on the constant regions of the heavy chain. Many of these polymorphisms are genetically linked. Therefore, certain haplotypes, such as czax and cst, commonly occur. Each allotype is associated with a particular subclass so that in the nomenclature G1m or G2m, the numerical designation refers to the IgG subclass. There are no well-defined allotypes on IgG 4 and IgA subclasses.

In this report the expression of IgG and light-chain allotype determinants on platelets is shown. We also describe a prospective study of multitransfused thrombocytopenic oncology patients receiving platelet transfusions to determine the incidence of alloimmunization to IgG allotypes and to determine if these antibodies had any effect on platelet transfusion outcome.

MATERIALS AND METHODS

Seum IgG allotyping. Allotyping of serum IgG was done using a hemagglutination inhibition technique in microtitr plates and alloantigens prepared by Allotype Genetic Systems, Inc (Atlanta, GA). The allotype specificities used included the following: -G1m (a), -G1m (f), -G1m (x), -G3m (b), -G3m (g), -Km (i), -Km (3), G1/3m (g5), -G3m (c3, e5), -G3m (c5), -G3m (s), -G3m (t). The coating antigen specificities included anti-G1m (a, x), -G1m (f), G3m (b), G3m (g), Km (i), Km (3), G3m (c3, e5), and G3m (s, t).

In this assay, allotype-typing antiserum is incubated with patient serum and red cells coated with IgG of the allotype specificity to which the typing serum is directed. Failure of the antiserum to agglutinate IgG-coated red cells indicates that the patient's serum contains the allotype in question. Coating of the red blood cells with different allotype specificities is done using anti-D.

Allotyping of platelet associated IgG. The detection of IgG allotypes on platelets was also performed using a hemagglutination inhibition assay. Platelets were first prepared by centrifugation of EDTA or citrated anticoagulated whole blood at 1,500g. After preparation of the platelet-rich plasma (PRP), the platelets were isolated by centrifugation at 3,000g for 5 minutes and washed three times in Tyrode's buffer. The count was adjusted to 10^6 platelets per microliter. The supernatant of the last wash was saved to use as a control to determine if all plasma IgG had been removed from the platelets by washing. Fifty microliters of the platelet suspension was incubated with 50 µl of antiallootypic antibodies of the specificities listed above. The dilutions of the typing serum varied from 1:8 to 1:32, depending upon the reactivity of the typing sera. A negative control of phosphate-buffered solution (PBS)-bovine serum albumin (BSA) (1%) and a positive control of pooled sera were used for each assay.

Identification of antiallootypic antibodies. Screening for antiallootypic antibodies was done using a direct agglutination technique in

From Johns Hopkins University School of Medicine, Pathology Department; and the Johns Hopkins Oncology Center, Baltimore, MD.

Submitted September 21, 1990; accepted April 20, 1990.

Supported by a grant from the American Association of Blood Bank Foundation.

Address reprint requests to Thomas Kickler, MD, Johns Hopkins Hospital, Blood Bank, 600 N. Wolfe St, Baltimore, MD 21205.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. section 1734 solely to indicate this fact.

© 1990 by The American Society of Hematology.

0006-4971/90/7604-0009$3.00/0


849
which patient sera was incubated with group-O red cells coated with IgG of the allotypes listed above. Uncoated red cells served as a control to ensure that the patients’ sera did not contain antibodies to red blood cell antigens.

Quantitation of platelet-associated IgG. Platelet-associated IgG was quantitated on platelets collected by plateletpheresis after 24 hours storage at room temperature. The method of LoBuglio et al was used with a murine monoclonal antibody (MoAb) specific for the Fc portion of IgG (Southern Biotechnology Associates, Birmingham, AL).

Patient population. We prospectively screened 146 platelet-transfusion recipients for antiallootypic antibodies after chemotherapy for leukemia, bone marrow transplantation, or chemotherapy for solid tumors. Samples for antiallootypic antibodies were collected 4 to 5 weeks into the patients’ hospitalization. The average numbers of red blood cell and platelet-transfusion donor exposures before screening the sera were eight (range, 6 to 25) and 64 (range, 22 to 78), respectively.

Twelve patients with antiallootypic antibodies received single-donor platelet transfusions from 19 donors positive and 19 donors negative for the particular allotype to which the transfusion recipient was immunized. Platelet transfusions were given when the patients’ platelet counts were less than 10,000 to 15,000/μL. The patients studied in the transfusion experiments were not clinically refractory to platelet transfusions and were not alloimmunized to HLA antigens by lymphocytotoxicity testing. Refractoriness was defined as failure to achieve a 1-hour post-transfusion corrected count increment of greater than 5,000 on more than two occasions when transfused with ABO-compatible random-donor platelets less than 48 hours old. Permission was obtained from the Johns Hopkins School of Medicine Investigational Review Board.

Platelet count increments. Post-transfusion platelet-count increments were expressed as corrected count increments to normalize for number of platelets given and the body surface area of the patient. The corrected count increment was calculated with the following formula: 

\[ \text{CCI} = \left( \frac{\text{post-transfusion platelet count} - \text{pretransfusion platelet count}}{\text{surface area in m}^2/\text{number of platelets} \times 10^{11}} \right) \times \text{corrected count increment of greater than 5,000 1 hour after transfusion was considered a satisfactory outcome.} \]

Statistical methods. The standard Student’s t test was used to test the difference of the means of the antigen-positive and antigen-negative platelet transfusion outcomes. To determine whether there is an association between the presence of antiallootypic antibodies and transfusion outcome, a χ² test was done.

RESULTS

We tested 146 patients’ sera for antiallootypic antibodies. Their diagnoses included acute leukemia (109), chronic myelogenous leukemia (20), lymphoma (12), and malignant tumors (9). The average number of units of red blood cell and platelet transfusions given were 18 and 64, respectively. Thirty-five patients (24%) were found to have one or more antiallootypic antibody specificity (18 patients had one specificity, eight had two specificities, and nine patients had three specificities). The specificities of the antibodies were: anti-G1m (f), 14; anti-G1m (czax), 10; anti-G3m (cs), 8; anti-G3m (c5), 12; anti-G3m (g), 4; and anti-K1m, 13 (Table 2). In the 146 study patients, only two patients with antiallootypic antibodies experienced allergic or febrile transfusion reactions compared with 11 patients without antiallootypic antibodies having transfusion reactions.

Platelet aliquots were obtained from p hềesis donor bags that had been stored for 24 hours to study the expression of IgG allotypes on the platelets. The total number of IgG molecules per platelet was 558 molecules ± 113 (1 SD; minimum, 321 molecules; maximum, 842 molecules), n = 42. All 10 blood donors with K1m or K3m allotype expressed these antigens on their platelets. Similarly, 10 of 10 G1m (a) positive donors, 8 of 10 positive G1m (x), and 8 of 10 G1m (f) positive donors expressed these polymorphisms on their platelets. When we tested 10 donors positive for G3m (b0), G3m (g5), G3m (t), and G3m (c3/5), none expressed these phenotypes on their platelets. The platelets of 40 control patients whose sera were negative for a given IgG allotype were tested and found to be negative for the corresponding IgG allotype. This control ensured that the hemagglutination inhibition assay used in detecting IgG allotypes on platelets was not producing false-positive results.

By testing washed platelets, it is possible that residual free serum could account for the positive results. This possibility is unlikely, since the buffer from the last washing of the platelets tested negative.

Twelve patients with antiallootypic antibodies were given platelet transfusions from donors either positive or negative for the IgG allotype to which the patient was alloimmunized. The specificity for the patient’s antiallootypic antibodies were anti-K1m, czax, and anti-f. Nineteen antigen-positive donors and 19 antigen-negative donors were transfused. The mean platelet count increment at 1 hour was 8402 ± 6712 (1 SD) for the allotype-positive transfusions and 9799 ± 5595 (1 SD) for the allotype-negative transfusions (P > 2) (Fig 1). Since the means of these two groups did not differ signifi-

<table>
<thead>
<tr>
<th>Table 1. Allotypes of Human Immunoglobulins</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Heavy Chains</strong></td>
</tr>
<tr>
<td>-------------------</td>
</tr>
<tr>
<td>G1m (IgG1)</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>G2m (IgG2)</td>
</tr>
<tr>
<td>G3m IgG3</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>A2m (IgA2)</td>
</tr>
<tr>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Table 2. Specificities of Antiallootypic Antibodies</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Specificity</strong></td>
</tr>
<tr>
<td>-----------------</td>
</tr>
<tr>
<td>Anti-G1m (f)</td>
</tr>
<tr>
<td>Anti-G1m (czax)</td>
</tr>
<tr>
<td>Anti-G3m (g)</td>
</tr>
<tr>
<td>Anti-G3m (c5)</td>
</tr>
<tr>
<td>Anti-G3m (c3/5)</td>
</tr>
<tr>
<td>Anti-K1m</td>
</tr>
</tbody>
</table>
IgG ALLOTPIC EXPRESSION ON PLATELETS

851

Fig 1. Platelet transfusion outcome in patients with anti-
alotypic antibodies. Open circles show the corrected count incre-
ments for platelet transfusions from donors negative for the IgG
alotype, against which the patient is immunized. Closed circles,
the transfusion outcome from donors positive for this IgG alotype.
The formula for the corrected count increment was as follows:
CCI = (change in platelet count/no. platelets x 10^11) x body
surface area (in m^2).

Fig 1. Platelet transfusion outcome in patients with anti-
alotypic antibodies. Open circles show the corrected count incre-
ments for platelet transfusions from donors negative for the IgG
alotype, against which the patient is immunized. Closed circles,
the transfusion outcome from donors positive for this IgG alotype.


tically using the Student's t test, it does not appear that
antibodies to IgG alotypic determinants found on platelets
affect platelet-transfusion outcome.

Of the 19 transfusions to which the recipients were
alloimmunized, 11 resulted in corrected count increments
greater than 5000. Thirteen of the transfusions from donors
negative for IgG allotypes, against which the recipients were
alloimmunized, resulted in increments of greater than 5000.
By χ² analysis, the observed χ² for 1 df is .736, P > .100. This
analysis also does not support an association between platelet-
transfusion outcome and the presence of antialotypic antibod-
ies.

DISCUSSION

The relative proportion of IgG subclasses in serum is IgG 1
(60% to 70%), IgG 2 (14% to 20%), IgG 3 (4% to 8%), and
IgG 4 (2% to 6%). The concentrations of all four subclasses
are genetically controlled with some relationship to the Gm
type. In individuals homozygous for G1m (az), the IgG 1
levels are higher than in heterozygotes. In studying which
allootypes are expressed, we observed that only the allootypes
associated with IgG 1 or the Km allootypes were detectable. If
the IgG on the platelet surface is primarily derived from
passive adsorption from surrounding plasma, one would
expect that the relative proportion of IgG subclasses on the
platelet surface would reflect the plasma IgG concentrations
with IgG1 > IgG 2 > IgG 3 > IgG 4. This may account for our
finding only the IgG allotypes of IgG1 and the Km markers
on platelets.

As many as 20% of HLA-identical platelet transfusions
given to HLA-immunized patients may be failures. This
experience suggests that other antigens expressed on plate-
lets might account for platelet destruction. Since the allo-
types associated with IgG1 and the light chains are expressed
on platelets, these antigens could serve as potential antigenic
targets leading to platelet destruction in alloimmunized
patients. However, we could not demonstrate that antiallo-
typic antibodies significantly lower 1 hour post-transfusion
platelet recovery.

In determining whether immune destruction of a cell
occurs, the antigen density, antibody titer, and immunoglob-
ulin class may all be important. Although we did not
quantitate the number of allotype determinants per cell, we
measured the total number of IgG molecules per platelet to
be less than 1,000. This observation is in contrast to HLA
class I antigens, which number 4,000 to 10,000 molecules per
platelet, or the PL46 antigen, with approximately 20,000 to
40,000 molecules per platelet. Because of the few antigenic
targets per cell available for antialotypic antibody binding,
these antialotypic antibodies may not cause accelerated
destruction of transfused platelets.

It has been reported that circulating immune complexes
may cause platelet-transfusion refractoriness. Since plate-
lets are known to contain Fc and C3 receptors, it is possible
that circulating immune complexes may bind to platelets,
leading to their destruction. The report of Kutti et al6 does
not address the nature of the immune complexes that may be
causing the transfusion refractoriness. However, subsequent
preliminary work by Blumberg et al7 suggests that multitrans-
fused patients may have antibodies to allogeneic plasma
proteins, such as complement components, fibrinogen, or
albumin, that may give rise to immune complexes when these
patients are transfused with blood products. In our patients
with antibodies to IgG, we were not able to confirm the
hypotheses that plasma proteins against which patients are
immunized may bind to transfused platelets leading to
shortened platelet survival. It is possible that the quantity of
plasma that is given with platelet transfusions is insufficient
to yield high levels of anti-IgG-IgG complexes.

In summary, despite the expression of some IgG allotypes
on platelets, no significant effect on platelet transfusion
outcome is observed in patients alloimmunized to these IgG
allotypic markers.

REFERENCES

1. Logublio AF, Court WS, Vinocur L, Maglotti G, Shaw GM: Immune
thrombocytopenia—use of a 125I labeled antihuman IgG
monoclonal antibody to quantify platelet bound IgG. Blood 309:459,
1983

2. Rosse WF, Devine DV, Ware R: Reactions of immunoglobulin
G binding ligands with platelets and platelet associated immunoglob-
ulin G. J Clin Invest 73:489, 1986

3. Koerner TAW, Williams LCJ, Weinfeld HM: The effect of
pretransfusion storage on platelet associated IgG and IgM and
evidence for two pH dependent storage lesions. Transfusion 26:469,
1986

4. van Loghem E: Genetic studies on human immunoglobulins, in

From www.bloodjournal.org by guest on October 23, 2017. For personal use only.
The expression of IgG allotypes on platelets and immunization to IgG allotypes in multitransfused thrombocytopenic patients

TS Kickler, PM Ness, HG Braine, L Richardson and M Farkosh