Alloimmunization Following Platelet Transfusion:
The Absence of a Dose–Response Relationship

By Janice P. Dutcher, Charles A. Schiffer, Joseph Aisner, and Peter H. Wiernik

A major concern about the use of prophylactic platelet transfusions is the development of alloimmunization. To determine whether the rate of alloimmunization is related to the number of platelet transfusions, we measured the development of lymphocytotoxic antibody in the first 2 mo of induction therapy in patients with acute nonlymphocytic leukemia. All patients received prophylactic random donor platelets and packed red blood cells during induction. No patient had lymphocytotoxic antibody present at admission. One hundred and six patients received an average of 9.3 platelet transfusions (range 2–34) containing an average of 61 U (range 9–236). The rate of alloimmunization was 38% overall and correlated with refractoriness to platelet transfusions. Ten of 19 patients receiving <4 transfusions became immunized, compared with 30/87 patients receiving >4 transfusions. There was no relationship between the number of platelet transfusions given and the rate of severity of alloimmunization, suggesting prophylactic platelets need not be withheld expressly to prevent alloimmunization.

The current standard remission induction therapy for acute leukemia involves intensive chemotherapy to marrow aplasia, which is followed by an acellular period lasting from 2 to 6 wk or more depending on response and intercurrent complications. These patients require specialized supportive care, including frequent platelet transfusions to prevent hemorrhage and to enable them to survive the induction period. Several treatment centers, including our own, advocate the use of prophylactic platelet transfusions in such patients facing prolonged thrombocytopenia to prevent rather than “catch up to” hemorrhage. This remains a controversial issue however.

In general, a prophylactic platelet transfusion approach requires that more platelets be given, which is more expensive and increases the risk of transfusion-related hepatitis. More critical however, is the potential risk of alloimmunization following multiple platelet transfusions. It is possible that an overly liberal transfusion approach will increase the rate and rapidity of the onset of alloimmunization, causing subsequent difficulty during times of hemorrhage with a requirement for HLA-matched platelet transfusions.

Alloimmunization can be demonstrated by assaying for lymphocytotoxic antibody directed toward HLA antigens which correlates well with decreased responsiveness to random donor platelets. The rate of alloimmunization among patients receiving frequent platelet transfusions is quite variable. Reports range from 30%-50% to 100%, probably owing to differences in patient population. In addition, it is unclear whether the lymphocytotoxic antibody first detected several weeks after the onset of transfusion has developed in response to cumulative platelet antigenic stimuli (from many transfusions), or whether one or just a few platelet transfusions will suffice to cause immunization. Thus, it is possible that the additional antigenic exposures occurring between the first transfusion and subsequent formation of antibody are of no importance in adding to the immunization rate. To study this question, we analyzed a group of patients with leukemia followed with serial lymphocytotoxic antibody measurements to determine the rate of alloimmunization and the effect of different “doses” of transfusions.

MATERIALS AND METHODS

Patients

Adults with acute nonlymphocytic leukemia (ANLL) treated between 1972 and 1979 with an anthracycline and cytosine arabinoside during their first attempt at remission induction were studied. Only patients who lived for at least 8–12 wk following induction and for whom adequate lymphocytotoxic antibody data were available on admission and after 8–12 wk of treatment were considered evaluable. This time span allowed adequate time for potential alloantibody formation. All patients evaluated had no lymphocytotoxic antibody detectable at the start of therapy. Patients who demonstrated lymphocytotoxic antibody formation within 1 wk of their first platelet transfusion, suggesting an anamnestic response, were eliminated from study. Since this early alloimmunization is a measure of secondary antibody response, these patients therefore will demonstrate alloimmunization unrelated to the number of platelets given. None of the patients received corticosteroids as part of their chemotherapy.

Serum samples were tested (Dr. P. Terasaki, Los Angeles, Calif.) by the microlymphocytotoxic technique. Alloimmunization was defined as cytotoxicity against greater than 20% of a panel of 80–100 lymphocytes. Multiple serial samples were available from most patients.

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RESULTS

One hundred and six patients, 56 male and 50 female, median age 50 yr (range 16–76) were evaluable. During the induction period, these patients received an average of 9.3 platelet transfusions (range 2–34) containing an average of 61 U of platelets (range 9–236). The overall rate of alloimmunization was 38%. Nineteen males (34%) and 21 females (42%) developed lymphocytotoxic antibody. As shown in Fig. 1, there was no relationship between the number of units of platelets transfused and the percent of patients who became immunized. Similarly, there was no relationship when the alloimmunization rate was plotted against the number of transfusions (Fig. 2). Nineteen patients (10 female and 9 male) received ≤4 platelet transfusions. Ten of 19 (53%) became alloimmunized within the study period (6 female, 4 male). Eighty-seven patients (40 female and 47 male) received more than 4 transfusions. Thirty (34%) became alloimmunized (15 female, 15 male). This demonstrates similar rates of alloimmunization at “low” and “high” numbers of platelet transfusions (p > 0.1). In addition, cytotoxicity against greater than 50% of the lymphocyte panel developed in most patients and was independent of the number of transfusions received (Fig. 2).

Because pregnancy can represent an earlier, “pretransfusion” source of exposure to histocompatibility antigen, the numbers of previously pregnant females were evaluated. Among 21 females with lymphocytotoxic antibody formation, 15 (71%) had prior pregnancies, whereas in 30 females who did not develop lymphocytotoxic antibody, 19 (63%) had been pregnant. Thus, pregnancies did not necessarily facilitate the development of alloimmunization. It should be kept in mind however that most of the patients who were not included in this study because they developed immediate, presumably anamnestic antibody rises were females who had been pregnant previously.

Patients who received fewer transfusions during induction therapy usually presented with higher platelet counts and therefore often completed chemotherapy prior to receiving any platelet transfusions. This situation is in contrast to patients initially thrombocytopenic who received platelets and chemotherapy together, and who generally received a larger number of transfusions (Table 1). There was no significant difference in antibody formation comparing those receiving their initial platelets before chemotherapy with those receiving platelets after completion of chemotherapy (p > 0.2) or simultaneously with chemotherapy (p > 0.2). Thus, the timing of chemotherapy did not seem to play a role in the rate of alloimmunization.

Blood Products

All patients received multiple units of packed red blood cells as required. Leukocyte-poor blood cell preparations were used infrequently.

All platelets were obtained from unmatched random donors. Six to eight units of platelets were given prophylactically for platelet counts less than 15·20,000/cu mm as well as therapeutically for bleeding, as previously described. Platelet bag counts were done throughout the study period and consistently averaged between 0.7 and 0.8 × 10^11 platelets per unit of platelet concentrate. Five patients received platelets prior to referral to our center and these transfusions were taken into consideration.

Patients receiving granulocyte transfusions were excluded from study because of evidence suggesting that the rate of immunization is accelerated and increased following granulocyte transfusion.

Statistics

Statistical comparisons were performed by Fisher's exact analysis.
ALLOIMMUNIZATION BY PLATELETS

DISCUSSION

This study demonstrates an overall rate of alloimmunization of 38% in a group of patients with ANLL during remission induction therapy. These previously untreated patients were managed with random donor platelets, packed red blood cells, and relatively standard intensive chemotherapy and are therefore comparable to patients with ANLL treated at other centers. Patients were eliminated from study who received granulocyte transfusions, prior chemotherapy, or corticosteroid therapy, to minimize the effect of other factors that could modify the rate of lymphocytotoxic antibody formation. The lack of relationship between the rate of alloimmunization and the number of platelets given is of major importance. The percentage of patients developing antibody at low transfusion levels is similar to that at higher numbers of transfusions. The rate of alloimmunization appears to be quite uniform at all “doses” of platelets, even following very low numbers of transfusions.

It should be emphasized that previous studies have demonstrated a direct relationship between the levels of lymphocytotoxic antibody and decreasing response to random donor platelets. Very few patients with antibody cytotoxic to greater than 30%-50% of the lymphocyte panel achieve clinically significant increments 1 hr posttransfusion of random donor platelets. Conversely, recent analysis of more than 200 patients with ANLL treated at our institution showed that refractoriness to random donor platelets is very uncommon (less than 5% of total patients) in the absence of lymphocytotoxic antibodies. Thus, the measurement of anti-HLA antibody by lymphocytotoxicity is a reliable and clinically relevant predictor of responsiveness to unmatched platelet transfusions.

Twenty-one patients receiving greater than 60 U of platelets demonstrated no lymphocytotoxic antibody or loss of responsiveness to random platelets. This lack of antibody production tended to be consistent over time. Most of these patients failed to develop lymphocytotoxic antibody later in their course despite subsequent antigenic stimuli. This suggests decreased immune responsiveness in this group or the possible development of a state of relative immune tolerance to histocompatibility antigens. It has been demonstrated in numerous studies that patients with leukemia and animals and patients receiving chemotherapy have diminished immune responsiveness. Comparison of antibody formation in patients with leukemia to

Table 1. Relationship of Chemotherapy to Initial Platelet Transfusion

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<tr>
<th>Timing of Initial Platelet Transfusion</th>
<th>Patients Transfused</th>
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<tr>
<td></td>
<td>≤4 Transfusions (n=19)</td>
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<tr>
<td>Before start of chemotherapy</td>
<td>Ab+</td>
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<tr>
<td>0-48 hr</td>
<td>0†</td>
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<tr>
<td>&gt;48 hr</td>
<td>1</td>
</tr>
<tr>
<td>During chemotherapy</td>
<td>2</td>
</tr>
<tr>
<td>After completion of chemotherapy</td>
<td>7</td>
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*Lymphocytotoxic antibody positive or negative.
†Entries are numbers of patients transfused.
that of patients with aplastic anemia shows an overall higher lymphocytotoxic antibody and major blood group antibody response in the latter group. Interestingly, studies of HLA antigen immunization of normals show wide variability of antibody response when intradermal leukocytes and skin grafts were utilized as antigens. Thus, certain normals do not make anti-HLA antibody, and this variability must enter into assessment of a leukemic population as well. Chemotherapy also alters immune responsiveness, but in our study and earlier studies, there was no obvious relationship between the timing of chemotherapy and the development or lack of development of lymphocytotoxic antibody.

This study fails to demonstrate a dose–response relationship for the development of alloimmunization.

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