Long-Term Follow-up of Patients With Leukemia Receiving Platelet Transfusions: Identification of a Large Group of Patients Who do not Become Alloimmunized

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

Alloimmunization is the major complication of platelet transfusion therapy in patients with acute leukemia. To evaluate whether alloimmunization continues to be a long-term problem in patients surviving induction therapy, 114 patients with acute nonlymphocytic leukemia (ANLL) who survived more than 6 mo and who received multiple courses of chemotherapy and abundant platelet transfusions were studied. Clinical response to random donor platelets and lymphocytotoxic antibody (LCTAb) were measured pretreatment and serially throughout the study period. Fourteen patients (12%) were alloimmunized upon admission. 34 (30%) patients became alloimmunized during remission induction therapy, and 66 (58%) patients did not become alloimmunized during that period. Sixty-one of these 66 patients (92%) never became alloimmunized and responded to random donor platelets during their subsequent course despite the fact that they received multiple further platelet transfusions, whereas the alloimmunized patients tended to remain alloimmunized for their entire clinical course. There was no difference in age or sex between groups, and prognostic factors predicting alloimmunization could not be detected. In greater than 90% of patients not alloimmunized at admission, the presence or absence of LCTAb after induction predicts later alloantibody production. This information can be used to plan the type of platelet transfusions (HLA-matched or random donor) needed for subsequent maintenance and induction therapy. It may also help to identify a group of patients to whom more aggressive maintenance chemotherapy may be more safely administered.

Alloimmunization represents the major complication of platelet transfusion therapy for patients with acute leukemia. Alloimmunized patients have a poor response to random donor platelets and require HLA-matched platelets to achieve posttransfusion count increments and hemostasis. Because current intensive remission induction therapy of adult acute leukemia produces relatively short aplastic periods of 2-4 wk, and alloantibody formation can be delayed in patients receiving chemotherapy, alloimmunization is frequently not a major factor in platelet transfusion support during the induction therapy period. However, patients achieving complete remission at our institution and elsewhere are usually treated with intensive maintenance therapy, thus requiring repeated platelet transfusions during remission. In addition, patients not achieving complete remission and those who relapse are likely to receive additional chemotherapy and therefore continue to require platelet transfusions. It is in these groups of patients receiving postinduction therapy that the need for histocompatible platelets can be of particular importance.

There are relatively few data available on the long-term platelet transfusion responsiveness of patients with leukemia receiving periodic chemotherapy and continued platelet transfusions. In the present study, we describe long-term follow-up of a large group of patients with acute nonlymphocytic leukemia (ANLL) studied both serologically and clinically. Of particular interest was a substantial group of patients who did not become alloimmunized during remission induction therapy and subsequently never made alloantibody despite further multiple platelet transfusions during maintenance and relapse therapy. The recognition of this group of nonimmunized patients can be helpful clinically and can assist in the rational planning of platelet transfusion requirements.

MATERIALS AND METHODS

Patients

Patients with newly diagnosed acute nonlymphocytic leukemia (ANLL), treated at the Baltimore Cancer Research Center between 1972 and 1979, were reviewed. All patients received induction chemotherapy with an anthracycline and cytosine arabinoside. All patients received red blood cell transfusions as needed and pooled random donor platelets prophylactically for platelet counts of 15-20,000/cu mm and therapeutically for bleeding. A transfusion averaged 6-8 U (1 U = 0.7 x 10^11 platelets).

Patients achieving complete remission (CR) received relatively intensive maintenance chemotherapy on a regular basis, requiring frequent platelet transfusions. Patients who failed to achieve CR or who relapsed were usually treated further with investigational agents that produced aplasia, requiring multiple red blood cell and platelet transfusions. Patients selected for evaluation were those with ANLL who survived longer than 6 mo, who received multiple subsequent random donor platelet transfusions, and for whom adequate serial lymphocytotoxic antibody data were available. This time period was chosen to allow an evaluation of the long-term clinical patterns of transfusion response and to allow time for alloantibody formation. Patients who received only autologous...
frozen platelets\(^{10}\) following complete remission were excluded from long-term evaluation because the documentation of their long-term response to random donor platelets was not available. Therefore, the group of patients evaluable is comparable to patient populations in other centers.

Criteria for Alloimmunization

Lymphocytotoxic antibody (LCTAb) was measured on admission and serially (at least monthly) in all patients using the microlymphocytotoxicity technique\(^{11}\) by Dr. P. Terasaki, Los Angeles. Lymphocytotoxic antibody was chosen because increasing levels of this antibody have been shown to correlate well with refractoriness to random donor platelets.\(^{12}\) Alloimmunization was defined as cytotoxicity against greater than 20% of a panel of lymphocytes.\(^{13}\) In addition, the patients were monitored clinically for response to random donor platelet transfusions and for the apparent need for HLA-matched platelet transfusions. The decision to give HLA-matched transfusions was based on failure to achieve count increments 1 hr after transfusion as previously described.\(^{12}\)

HLA Typing

Lympocytes were HLA typed using standard methods for A and B loci, and HLA frequencies were compared between alloimmunized and nonalloimmunized patients.\(^{14}\)

Statistics

Statistical analysis of HLA antigen frequency between patient groups was done by the Fisher exact test. Wilcoxon analysis was used for evaluation of complete remission duration and survival.

RESULTS

A total of 114 patients were evaluable and were divided into three groups based on their LCTAb production during induction. One-hundred patients had no LCTAb on admission, and 14 patients were either alloimmunized on admission or developed an anamnestic response to their initial platelet transfusion with LCTAb production within 1 wk of their initial exposure to platelet transfusion. The majority of patients were followed until death and the median follow-up of those patients still living is 50 mo. Table 1 provides a schema of the long-term results of these patient groups.

Groups A and A\(_1\)

Sixty-six patients (58%) who had no lymphocytotoxic antibody on admission did not make LCTAb during induction or within 6–8 wk of the initiation of chemotherapy. Sixty-one (Group A) of these 66 patients (92%) subsequently never made LCTAb and continued to respond well to random donor platelets for their entire clinical course. This group received a median of 65 U of platelets (range 12–192 U) during induction. Eight of the 61 patients received a few HLA-matched platelet transfusions late in their course when they seemed to be responding poorly to random platelets. This invariably occurred at a time when the patients were either very ill, with fever or bleeding shortening platelet survival, or when their leukemia was progressing and organomegaly was causing platelet sequestration. There was no difference in posttransfusion count increments between random and matched transfusions under these circumstances.

The five remaining patients (group A\(_1\)) also had no LCTAb during induction or early maintenance courses and continued to respond to random donor platelets. An average of 20 mo (range 10–36 mo) after induction, however, these 5 patients developed LCTAb and began to require HLA-matched platelets. Although very small, this group of five patients was interesting in that these patients had a long median duration of remission (31.5 mo) and survival (33.6 mo).

Group B

Thirty-four of the 114 patients (30%) who had no LCTAb on admission became alloimmunized during the induction period. These patients received a median of 49 U of platelets (range 10–206 U) during induction. These patients, for the most part, continued to demonstrate LCTAb when later challenged with random donor platelets, and most required HLA-matched platelets for subsequent transfusions. Interestingly, however, 6 patients in this group appeared to lose their ability to make LCTAb during a time when they were very ill, and they again responded to multiple random donor platelet transfusions. One additional patient temporarily lost alloantibody while in remission, presumably because subsequent platelet transfusions were cryopreserved autologous platelets\(^{9,10}\) and thus repeated HLA antigenic stimulation did not occur.

### Table 1. Alloimmunization: Long-Term Follow-up

<table>
<thead>
<tr>
<th>Induction</th>
<th>Maintenance, Relapse (&gt;6 mo)</th>
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<tbody>
<tr>
<td>114 patients</td>
<td>66 (58%) 34 (30%) 14 (12%)</td>
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<tr>
<td><em>LCT Ab</em></td>
<td><em>LCT Ab</em></td>
</tr>
<tr>
<td>61 (92%)</td>
<td>5 (8%)</td>
</tr>
<tr>
<td>40 (83%)</td>
<td>8 (17%)</td>
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\(\text{e} \) Refers to absence of lymphocytotoxic antibody; \(\text{e-e} \) Refers to development (>20%) of lymphocytotoxic antibody.
**Group C**

Fourteen patients (12%) who met the study criteria demonstrated either alloimmunization on admission with an initial poor response to the first random donor platelet transfusion or an anamnestic antibody response with LCTAb production within 1 wk of transfusion. All of these patients had LCTAb present when measured during the first week of treatment. This group received a median of 83 U of platelets (range 50–178 U) during the induction period, a mixture of random and HLA-matched cells. These patients required HLA-matched platelets very early in their treatment course and continued to require them during subsequent treatments. However, two patients later lost their ability to make LCTAb and again responded to random donor platelets. As in group B, this occurred at a time when they were in relapse and very ill.

Other characteristics of the three major patient groups are presented in Table 2. Group A includes only the 61 patients who were negative for LCTAb on admission and who subsequently never made antibody. The data would not change, however, if the other five patients were included. There were no significant differences in age or sex among the groups, and ABH blood group distribution was the same among the three patient groups. Because the study criteria included a minimum of 6 mo survival, the overwhelming majority, 100 patients (88%), had achieved complete remission. Group B received fewer platelets during induction than the other two patient groups, but all groups received substantial numbers of transfusions. As noted in a previous study, however, there is no dose–response relationship between the development of alloimmunization and the number of units of platelets given during induction.

The frequency of the 28 most common HLA-A and B loci was analyzed statistically to determine if any association exists between antibody formation or the lack of LCTAb formation and a specific HLA antigen. There was no increased frequency of a particular HLA antigen in any patient group when compared with HLA antigen distribution among normals.14

There was no statistically significant difference in survival between the groups (Table 2) with median survivals of 12 mo (group A), 13.9 mo (group B), and 16 mo (group C). Of 61 patients in group A, 55 have died. In group B, 23 of 34 are dead, and in group C, 13 of 14 have died. Of 53 patients in group A who achieved complete remission, 49 have relapsed. In group B, 25 of 30 patients with complete remissions have relapsed, and in group C, 12 of 12 have relapsed. There was a difference in complete remission duration (group B versus group A, p = 0.03) with Wilcoxon analysis, but this difference was due to prolonged remissions in four patients in group B and may be related to many other factors. The clinical significance of this difference is questionable, however, when one examines the median values of remission duration (group A 6 mo, group B 8 mo, group C 7 mo) and considers the number of relapses.

**DISCUSSION**

Because alloimmunization makes transfusion supportive care more difficult and challenging, it is important to note its frequency and to attempt to define groups of patients at higher or lower risk if possible. Among 114 patients who lived longer than 6 mo, 46% at some point became alloimmunized and required HLA-matched platelets. The important clinical lesson of this study, however, is that approximately 50% of patients with acute leukemia who survive the induction therapy period and who do not develop LCTAb during induction will never make LCTAb. In addition, one can predict the long-term alloimmunization status of approximately 90% of patients with leukemia depending on their status following induction. Therefore, certain conclusions regarding transfusion practices in these patients can be made. Patients who become alloimmunized following induction will continue to require HLA-matched platelets. However, patients who do not become immunized during induction can be easily and repeatedly transfused with random donor platelets and can therefore be given subsequent prophylactic transfusions liberally without concern for the induction of alloimmunization. These data may also allow prospective identification of a group of patients who are more easily supportable and...
who may be able to receive more intensive maintenance therapy designed to prolong remission duration with relative safety.

Additionally, knowing in advance which patient is more likely to be alloimmunized and which is not allows for more effective management of our program of autologous platelet cryopreservation. In our program, alloimmunized patients have become an important source of platelets for themselves. By recognizing that nonalloimmunized patients are extremely unlikely to become refractory to random donor platelets, we are able to establish priorities regarding which patients should have their platelets cryopreserved.

Recently, there has been considerable interest in developing methods for the prevention of alloimmunization, with particular interest in the use of single donor platelets to modify the pattern and frequency of alloimmunization. Although there are few data in humans to substantiate this approach, the use of single donor platelets has become a common practice in many blood centers. The present study indicates that less than 50% of patients with ANLL (since a significant number of patients still die during induction therapy) would stand to benefit from this approach, even if it proved to be effective. In addition, leukocyte-poor red blood cells would be necessary in any program that attempted to limit the number of platelet donors per patient. Given the increased donor risk (albeit small), increased expense, the possibility of immunization to additional non-HLA antigens, and the relatively small population of patients to be benefited, the use of single donor platelets for patients with leukemia should be restricted to alloimmunized patients except in investigational settings.  

If one could distinguish among the three groups of patients prospectively, attempts to reduce alloimmunization could be directed more efficiently toward the patients at highest risk. Unfortunately, there are no obvious clinical characteristics that distinguish the groups or seem to prevent or to be associated with alloimmunization. Other studies of alloimmunization to platelets have also been unable to identify additional causative factors. There is no association of lymphocytotoxic antibody formation with HLA antigens at either the A or B locus, but this does not exclude an association with the DR locus, which has not been investigated. Previous studies could not demonstrate an association between LCTAb formation and serious infection or an association with timing of chemotherapy with respect to the first platelet transfusion during induction.

The lack of antibody production in response to an antigenic stimulus reflects a complex interaction between the host and the antigen. A certain small percentage of normals fails to produce antibody to a variety of antigens including histocompatibility antigens. More importantly, it is known that in animals and humans treated with chemotherapy, there is a decreased antibody response when compared with normals. The behavior of group A, however, suggests some form of immunologic tolerance occurring in this population where there is never a LCTAb response, despite repeated antigenic exposure. Tolerance may be totally host-related, due to the leukemic process itself, or related to the timing of the initial antigenic exposure and the chemotherapy. Whether this represents specific tolerance to histocompatibility antigens or general humoral immune hyporesponsiveness is unclear. Studies in which patients are exposed to new antigens in a more systematic fashion than was possible in this study could help to approach this question.

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