A Randomized Trial of Leukocyte-Depleted Platelet Transfusion to Modify Alloimmunization in Patients With Leukemia

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In an effort to determine whether the use of leukocyte (WBC) depleted platelets could modify the development of alloimmunization, 98 adult patients with acute nonlymphocytic leukemia receiving initial induction therapy were randomized to receive standard pooled platelet concentrates (PC) or WBC-depleted PC. WBC depletion was produced by an additional centrifugation of pooled PC, with removal of 81% of WBC and an associated platelet loss of 27%. Lymphocytotoxic antibody (LCTAb) levels were monitored as a serologic marker of alloimmunization. Overall, 5 of 25 evaluable patients receiving WBC-depleted PC developed LCTAb, compared to 13/31 receiving standard PC ($p = 0.071$). There was no significant difference in alloimmunization rate in the subgroup of patients who had no previous exposure to histocompatibility antigens by pregnancy or prior transfusions (4/15 alloimmunized receiving WBC depleted versus 4/12 receiving standard PC). There was no difference in the number of patients in each group who required HLA-matched platelets during induction therapy. In view of the significant loss of platelets with WBC depletion, the expense and difficulty of providing WBC-poor RBC, the absence of impact on the need for HLA-matched platelets during induction, and the small potential benefit from this approach, WBC-depleted platelets should not be utilized to prevent alloimmunization in patients with leukemia.

The management of alloimmunized patients with leukemia represents a difficult and expensive challenge, and any means by which the rate of alloimmunization can be reduced will be welcomed. There are studies in animals suggesting that leukocytes are more antigenic than platelets and that the administration of platelets depleted of leukocytes fails to evoke an immune response against HLA antigens. Welsh et al. have demonstrated that rats do not mount a primary antibody response against major histocompatibility complex (MHC) antigens when immunized with pure platelet preparations. Rats immunized with small numbers of lymphoid cells, however, have a brisk primary antibody response. These sensitized animals can then mount a secondary antibody response when exposed to a pure platelet preparation. Furthermore, repeated injection of allogeneic platelets alone induces a state of tolerance when injected into nonprimed rats.

These investigators initially postulated that poor responsiveness to platelets alone was related to the absence of the Ia antigen on platelets, which may have been required for initial recognition and processing of other antigens in the MHC. Further experiments, however, suggested that the presence of the Ia antigen alone was insufficient to invoke the primary antibody response. It was also necessary that the antigen be presented on intact, viable lymphocytes, in that the administration of lymphocyte membrane preparations did not result in antibody production. Additional observations in mice by Claas et al. produced similar results and indicated that a primary antibody response to platelets was induced only when allogeneic platelets were contaminated with at least $10^5$ allogeneic leukocytes. Repeated injections of as many as $10^9$ platelets alone did not produce an antibody response, although it was also noted that platelets contaminated with leukocytes tended to produce a stronger response than transusions of comparable numbers of leukocytes alone. Early experience in human transplantation experiments produced analogous results. Dausset and Rappaport demonstrated that highly purified preparations of allogeneic platelets injected intradermally failed to produce sensitization to subsequent skin grafts from the same donors, whereas much lower doses of leukocytes reliably produced sensitization, with subsequent rejection of skin grafts.

Recently, Dutch investigators in Leiden reported that the incidence of alloimmunization was decreased in patients receiving platelet concentrates partially depleted of leukocytes compared to a historical control group. Ninety-three percent of 28 patients treated from 1972 to 1974 became refractory to random donor platelets compared to 24% of 68 patients treated since 1974 with leukocyte-poor platelets. Based on this retrospective study, leukocyte-depleted platelets are now used widely in Europe. Leukocyte depletion is not a straightforward blood banking procedure, however, and results in the loss of approximately 30% of the platelets. In addition, leukocyte-poor red blood cells must also be administered. There were also consider-
able differences between the historical control group and the patients receiving leukocyte-depleted platelets in terms of disease type, type of chemotherapy received, and history of prior exposure to histocompatibility antigens. Lastly, the rate of alloimmunization in the historical control group was considerably higher than observed in our institution\(^9,10\) or by others\(^11,12\) in patients with leukemia. We therefore conducted a prospectively randomized study to determine whether leukocyte depletion could reduce the rate of alloimmunization.

MATERIALS AND METHODS

Patient Population and Transfusion Policy

All newly diagnosed patients with acute nonlymphocytic leukemia were eligible for randomization on the study. No patient had received prior chemotherapy, and patients who had received prior immunosuppressive therapy (i.e., corticosteroids) or who had “secondary” leukemia following treatment for another cancer were not included. All patients were treated with a continuous 7-day infusion of cytosine arabinoside (100 mg/sq m/day) and either daunorubicin (45 mg/sq m/i.v. \(\times\) 3 days) or daunorubicin (30 mg/sq m/i.v. \(\times\) 3 days).

Platelet transfusions were administered for the therapy of bleeding or prophylactically at platelet counts of 15,000–20,000/\(\mu\)l, as previously described.\(^7\) These transfusions consisted of pooled platelet concentrates prepared from multiple random donors and stored at 22\(^\circ\)C for less than 48 hr. Whole blood was obtained and centrifuged at 2,400 rpm (1,490 g) for 3 min. The platelet-rich plasma was separated and centrifuged at 4,300 rpm (4,554 g) for 6 min, and the platelet concentrates were resuspended in 50–60 ml of plasma. From 4 to 10 U of platelet concentrate was pooled and administered per transfusion. The control group received standard platelet concentrates, while the study group received platelet concentrates that had been subjected to an additional centrifugation after pooling (see below). The randomization was stratified according to sex and prior exposure to histocompatibility antigens by other blood transfusions or pregnancy. Patients received frozen, deglycerolized, or occasionally washed red blood cells so that the platelet preparations provided the major antigenic exposure.\(^13\)

Study Duration

Patients were studied only during their initial induction therapy. Previous data from our laboratory have demonstrated that alloimmunization with lymphocytotoxic (anti-HLA) antibody formation tends to occur within 4–8 wk of initial exposure to platelet transfusions.\(^10\) The rate of subsequent alloimmunization, despite multiple additional transfusions, seems to be very low in patients with leukemia receiving further maintenance chemotherapy.\(^18\) Lymphocytotoxic antibody determinations were done on admission and weekly for approximately 2–3 mo. Standard lymphocytotoxicity techniques\(^19\) were utilized (assay performed by Dr. P. Terasaki, UCLA). Sera were tested against 80–100 lymphocytes, with a “positive” result representing cytotoxicity against at least 20% of the cell panel. The endpoints of the study were: (1) the development of lymphocytotoxic antibody, which was measured because it correlates quite well with refractoriness to platelet transfusion;\(^16,18\) and/or (2) the development of poor increments following random donor platelet transfusions requiring HLA-matched platelets. All patients who had poor day-to-day increments had 1-hr posttransfusion increments done following the administration of fresh platelets as previously described.\(^17\) Patients who had poor 1-hr increments in the absence of clinical findings, such as splenomegaly, disseminated intravascular coagulation, etc., that could account for poor increments, then received an HLA-matched platelet transfusion if available.\(^17\)

Statistical comparisons were done using the Fisher exact and Student’s \(t\) tests.

RESULTS

Platelet Preparation

Initial experiments were conducted to determine the best means of leukocyte depletion of the pooled platelet concentrates. A variety of relatively slow centrifugations of differing durations were compared (Table 1). Similar leukocyte depletion and platelet losses were noted over a fairly wide range of centrifugation speeds and time. A final centrifugation of 1,000 rpm (183 g in a Sorvall RC3 Centrifuge) for 10 min was chosen. Consistent results were obtained throughout the course of the study, with an overall mean leukocyte depletion of 81% and an accompanying platelet loss of 27%. The mean final white blood cell count, which consisted almost entirely of lymphocytes, was \(0.12 \times 10^9\)/U in the leukocyte-depleted preparations and \(0.65 \times 10^9\)/U in the control group.

Patient Population

A total of 98 patients were randomized in the study, 48 in the control group and 50 in the leukocyte-depleted group. As listed in Table 2, a number of these patients were excluded from evaluation. A total of 7 patients did not survive the minimum study period of 28 days. It was felt that this minimal period of time was necessary to determine whether an antibody response had developed. Fifteen patients received white blood cell transfusion from non-HLA-matched donors because of progressive infection and were therefore not included. Two patients who received white blood cell transfusions from HLA-identical siblings were included in the analysis. Many patients required platelet transfusion either at admission or shortly after.

<table>
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<th>rpm (\times)</th>
<th>Time (min)</th>
<th>WBC Loss (%)</th>
<th>Platelet Loss (%)</th>
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<td>27</td>
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* Sorvall RC3 Centrifuge.
† Sum of observations done periodically throughout the study.
The mean platelet count precentrifugation was \(.74 \times 10^9\)/U.
thereafter prior to determination of lymphocytotoxic antibody levels. Fourteen patients were initially randomized, but were found to have lymphocytotoxic antibody present on admission, generally due to prior pregnancies, and were excluded from evaluation. One patient was lost to follow-up, one received only a single platelet transfusion, and there were four protocol violations. These generally consisted of the administration of packed red blood cells (not leukocyte depleted) during times of emergency.

Because of these exclusions, the two groups were not entirely comparable, despite the stratification prior to randomization. The control group had a larger proportion of patients who had received prior transfusions, had prior pregnancies, or had been both transfused and pregnant (Table 2). There was considerable heterogeneity in the transfusion and pregnancy histories both between and within the two groups. Some patients had simply received a few units of red blood cells prior to transfer to our center, while others had received a variable number of red blood cell transfusions in the past for other medical conditions. There was similar variation in the number of pregnancies in the female patients, although the number of pregnancies per female patient was also greater in the control group.

Alloimmunization

Table 3 presents the results in the group of patients who had no prior known exposure to histocompatibility antigens. This group of patients probably represents the “cleanest” test of any approach to modify alloimmunization. The alloimmunization rates (as assessed by the development of lymphocytotoxic antibody) were similar, with 33% (4 of 12) patients in the control group becoming alloimmunized compared to 4 of 15 (27%) in the leukocyte-depleted group. In 5 patients in each group, the only prior exposure to histocompatibil-
patients receiving leukocyte-depleted platelets became alloimmunized. This difference was not statistically significant. Of clinical importance is that only 19% of the control group (6 patients) and 16% of the leukocyte depleted (3 patients) group required HLA-matched donors during induction because of poor count increments following random donor platelet transfusion. This is because antibody production in such patients is frequently delayed and does not develop until the third or fourth week, at a time when patients may be entering remission and producing their own platelets. These other patients with lymphocytotoxic antibody did require histocompatible platelets during subsequent courses of treatment.

**DISCUSSION**

These results indicate that there is no significant reduction of alloimmunization in patients with acute nonlymphocytic leukemia receiving “standard” induction chemotherapy by the administration of leukocyte-depleted random donor platelet concentrates. Any apparent difference between the two groups in patients with previous exposure to histocompatibility antigens is most probably explained by the marked heterogeneity in these patients and the imbalance between the two groups. We therefore elected not to continue the study in this group of patients because of the absence of difference noted in the previously nonexposed patients (Table 3) and the difficulties in obtaining adequate numbers of patients with comparable prior transfusion or pregnancy histories. In addition, in the animal experiments mentioned earlier, it was only possible to eliminate a primary rather than a secondary antibody response by the administration of leukocyte-depleted platelets.

These results differ from those reported by Eernisse and Brand.\(^7\) Although the absence of concurrent controls in the earlier study may account for some of this discrepancy, it should also be noted that the number of transfusions and total leukocytes administered in the present study were different. A smaller number of units of platelets were administered in the Dutch study. However, a previous study from our laboratory suggests that the development of alloimmunization seems to be independent of the number of platelet transfusions administered.\(^18\) A total of \(\sim 5 \times 10^8\) leukocytes were administered per transfusion by Eernisse and Brand compared to 0.12 \(\times 10^9\)/U (or approximately 0.5-1 \(\times 10^9\)/transfusion) in the present study. As demonstrated in Table 1, we were unable to produce leukocyte depletion to the degree reported by Eernisse and Brand. Although longer and “harder” centrifugations could result in lower white blood cell counts, the platelet loss using such such centrifugations would be prohibitive.

The difference in leukocyte depletion seems to result from the very different fashion in which platelets for transfusion are prepared in some centers in The Netherlands.\(^19\) Briefly, whole blood is centrifugated at high speed and as much as possible of the plasma is removed. Auffy coat containing red blood cells, white blood cells, and platelets is then removed and stored in a small bag at 4°C. When platelets are needed, multiple buffy coats are pooled in a larger bag to which either saline or saline mixed with hydroxyethyl starch is added. This bag is subjected to a slow centrifugation, which results in a layer of platelet-rich plasma above a red blood cell layer. The platelet-rich plasma is then recentrifuged for further leukocyte depletion. This procedure is obviously markedly different from and totally incompatible with present blood banking practice in the United States. In addition, platelets in the buffy coat must be stored at 4°C with the known marked decline in posttransfusion platelet survival associated with storage at this temperature.\(^20,21\)

One should also consider a number of other factors when contemplating any method of decreasing the rate of alloimmunization. Any such approach would require the administration of leukocyte-poor red blood cells, which markedly increases the cost and burden on blood banks. Presently, frozen red blood cells cost approximately 1.5-3 times as much as packed red blood cells. It is also important to calculate the number of patients who could potentially benefit from such an approach (Table 5). If one assumes that one starts with 100 patients with acute nonlymphocytic leukemia, then approximately 10%-15% of patients will be alloimmunized on admission (Table 2) and not benefit from any approach to modify alloimmunization. The complete remission rate with current therapy is 60%-70%,\(^22,24\) with an initial mortality rate of 10%-20%, resulting in a further decrease to approximately 75 patients. The alloimmunization rate in this study and in 200–300 patients previously studied at our institution is only 40%-50%,\(^10,16\) resulting in a further decrease to approximately 35 patients. It is unlikely

| Table 5. Potential Benefit of Strategies to Modify Alloimmunization |
|----------------------|-----------------|-----------------|
|                      | Approximate Frequency | Remaining Patients |
| Initial total        | 100              |                 |
| Alloimmunized on admission | 10%            | 90              |
| Mortality during induction | 10%-20%       | 75              |
| Alloimmunization rate | 40%-50%         | 35              |
| Possible effectiveness | 50%-70%        | 25              |
| Granulocyte transfusions | 15%            | 21              |
| Intensive subsequent therapy | 75%            | 16              |
that any approach to reduce alloimmunization would be totally effective. If one (perhaps optimistically) assumes the 70% decrease in alloimmunization rate reported in the Leiden study, the number of patients is decreased to 25. A variable number of patients (15% in the present study) will require granulocyte transfusions, which are not routinely HLA matched, for unresponsive infections, further decreasing the number of patients. Not all patients are candidates for intensive subsequent therapy when alloimmunization can represent a major problem. If approximately 75% of such patients would be candidates for such therapy, then a maximum of only about 15% of the initial group of patients would stand to derive long-term benefit from an effective strategy to reduce alloimmunization, if indeed such a strategy existed. Short-term benefit is considerably lower, because only 20% of patients required HLA-matched platelets on clinical grounds during induction. The majority of alloimmunized patients can be supported with present technology of donor selection by HLA typing or with autologous frozen platelets.25 Unfortunately, there are no characteristics that reliably predict which patients are more likely to become alloimmunized9,10 so as to allow more efficient application of any program of prevention.

Thus, although it may be possible with dramatic changes in the means of platelet concentrate production to restudy the issue of more profound leukocyte depletion, it is unlikely, given the above calculation, that this will be a cost-effective approach in the management of patients with acute leukemia, particularly because of the associated platelet loss. Furthermore, HLA antigens can be detected in plasma,26 and a recent report27 suggests that lymphocytotoxic antibodies can develop following infusion of cell-free plasma to patients with renal insufficiency. Although this observation requires further confirmation, it suggests that some patients could potentially become immunized due solely or predominantly to the plasma in which the platelets are suspended, regardless of the leukocyte “contamination.” It should also be noted that there are no data demonstrating that any other approach to modify alloimmunization, such as the use of single donor platelets or partially HLA-matched single donor platelets, is effective.28 Both of these approaches are of theoretical interest and may be worthy of study in patients with aplastic anemia who are not receiving concurrent cytotoxic therapy and hence have a higher rate of alloimmunization. The “breakdown” presented in Table 5, however, indicates that these approaches are unlikely to have substantial impact on the treatment of patients with acute leukemia. We therefore recommend that patients with acute leukemia receive standard pooled platelet concentrates from random donors with packed red blood cells as initial transfusion therapy, with single donor histocompatible platelets reserved for the minority of patients who become alloimmunized.

REFERENCES

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