Alloimmunization Following Prophylactic Granulocyte Transfusion

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Nineteen noninfected adults receiving initial induction chemotherapy for acute nonlymphocytic leukemia (ANLL) were randomized to receive either prophylactic granulocyte transfusion or platelet transfusion alone on an alternate-day schedule. An average of 11 granulocyte transfusions (range 3–19) were administered/patient with a mean dose of $11.5 \times 10^8$ granulocytes/transfusion. The groups were identical with respect to age, sex, number of days on study, granulocytopenic days, percent of days receiving systemic antibiotics, febrile days, complete remission rate, and incidence of minor infection. Significant transfusion reactions were much increased in the granulocyte transfusion group (7/10 versus 1/9 in controls) and were associated with the development of lymphocytotoxic antibodies (7/10 versus 4/9 controls), refractoriness to platelet transfusion, repeated fevers, and a pulmonary infiltrate in one patient. Alloimmunization to granulocytes occurred as early as the second week in some patients complicating platelet support during induction and maintenance. No severe infections occurred in the granulocyte transfusion group while three fungal infections occurred in the controls. The high rate of alloimmunization suggests that histocompatibility considerations indicate that prophylactic granulocyte transfusion should not be routine therapy and should be studied only in investigational settings.

Despite recent advances in infection prevention, infections of variable severity develop in approximately 60%–70% of patients undergoing induction therapy for acute nonlymphocytic leukemia (ANLL) at the Baltimore Cancer Research Program. In contrast, the widespread use of prophylactic platelet transfusions has almost completely eliminated hemorrhage as a cause of death in patients receiving induction therapy for ANLL. Presently, granulocyte transfusions are utilized almost exclusively in patients with serious systemic infections rather than as a means of preventing infection. Granulocyte transfusions are of proven efficacy in the former situation, and two recent prospectively randomized studies have demonstrated significant decreases in acquisition of serious infections in granulocytopenic patients receiving granulocyte transfusions on a daily schedule.

Provision of granulocyte transfusions on a daily schedule would be difficult for most specialized transfusion services, and we therefore attempted to approach the question of whether transfusions administered on an alternate-day basis could decrease infection acquisition in a prospectively randomized study. This schedule would conform approximately to the platelet transfusion requirements of most patients with leukemia and, because granulocytes can be collected simultaneously...
with platelets using differential centrifugation machines, such an approach, if successful, would represent minimal additional effort to what is becoming a common approach to platelet transfusion and collection. Because of these considerations and the published “successful” results, there has been a growing interest in many centers to provide prophylactic granulocyte transfusions as “routine” therapy. During the course of our study, disturbingly high rates of transfusion reactions and recipient alloimmunization were noted in the group of patients receiving granulocyte transfusions, resulting in an early decision to terminate the study. The intent of this article is to emphasize that granulocyte transfusions are still not “routine” or innocuous and to suggest that histocompatibility considerations may preclude widespread utilization of prophylactic granulocyte transfusion therapy.

**MATERIALS AND METHODS**

**Study Design**

This investigation was designed to measure the rate of acquisition of infection in adult patients with ANLL receiving alternate-day granulocyte and platelet transfusions compared to patients receiving a similar program of prophylactic platelet transfusion alone. Patients were randomly allocated to receive either combined granulocyte-platelet transfusions on a 4 of 7 day schedule or platelet transfusions alone on a 3–4 of 7 day schedule. All previously untreated patients receiving initial induction chemotherapy for ANLL, who had no evidence of bacterial infection at the time of admission, were eligible for randomization. Infected patients, patients receiving antibiotics, patients known to be alloimmunized, and patients in whom the ANLL was possibly related to treatment for another malignancy were excluded from the study. All patients were treated in regular two-bed hospital rooms and received a standardized program of infection prevention consisting of aggressive oral and cutaneous hygiene under the supervision of an infection control nurse, and alimentary tract microbial flora suppression with oral nonabsorbable antibiotics as previously described. Induction chemotherapy consisted of a 7-day continuous infusion of cytosine arabinoside (100 mg/sq m/day) in combination with a 3-day course of either daunorubicin (45 mg/sq m/day i.v.) or adriamycin (30 mg/sq m/day i.v.).

Transfusions were initiated when the patient required platelet transfusion either prophylactically (platelet count <20,000/µl) or because of hemorrhage and/or when the granulocyte count fell below 500/µl. Frozen red blood cell transfusions were utilized to limit as best as possible the antigenic exposure to the platelet and granulocyte donors.

The study end-point for individual patients was defined as: (1) the acquisition of a microbiologically or clinically documented severe infection, (2) marrow recovery with a rise in granulocyte count to 500/µl with an elimination of platelet transfusion requirement, (3) 60 days on study, or (4) the development of alloimmunization as evidenced by transfusion reactions or refractoriness to platelet transfusion. If HLA-compatible donors were available such patients were continued on study. Systemic antibiotic therapy with broad spectrum antibiotics was begun empirically in patients with a fever of >101°F and granulocytes <500/µl if there was any suspicion of an infectious etiology. Antibiotics were continued a minimum of 48 hr and discontinued if no definite infection could be documented. The decision to administer antibiotics was made by the patient’s primary physicians.

**Donors**

All donors were ABO-compatible with the recipients, satisfied standard American Association of Blood Banks health criteria, and donated either platelets or platelets and granulocytes for an individual patient once a week. No attempt was made to HLA match donors and recipients, because, despite the availability of family members and an HLA typed pool of approximately 1000 donors, it would have been difficult to obtain sufficient numbers of HLA-matched donors for most of the patients.

**Collection and Transfusion Methodology**

Patients receiving platelets alone received platelets from 3–4 different donors collected by a manual technique of 4-U platelethpheresis. The Haemonetics Model 30 (Haemonetics Corporation, Braintree, Mass.) was used for the simultaneous collection of granulocytes and platelets as previously described.
Hydroxyethyl starch was utilized as a rouleauxing agent, and premedication with dexamethasone (10 mg p.o. 4 hr prior to donation or 10 mg i.v. immediately predonation) was employed to maximize granulocyte yields. Transfusions were administered within 2 hr of collection over a 1½-2-hr period of time. Recipients were not premedicated in the absence of a history of transfusion reactions. Granulocyte and platelet yields were routinely measured, and 1-hr and 18-hr posttransfusion blood counts were obtained. Corrected increments were expressed as: Absolute increment x BSA (sq m)/Number of cells administered x 10⁶ for platelets; x10⁶ for granulocytes.

Lymphocytotoxic antibody screens were performed weekly on each patient (Dr. P. Terasaki, Los Angeles, Calif.). Serial measurement of leukogglutinins against a panel of 4-6 donors was done retrospectively and was not utilized as a means of donor selection. Leukogglutination was done by the slide agglutination method with a 2-hr incubation using granulocytes collected by gravity sedimentation.

Informed written consent was obtained from each patient and donor prior to their participation.

RESULTS

Ten patients were randomized to the control group of platelet transfusion alone, while 12 patients were initially allocated to the granulocyte group. One control group patient manifested evidence of alloimmunization after her first platelet transfusion and was not continued on the study, while two patients developed infections requiring antibiotic therapy prior to receiving their first granulocyte transfusion and were also disqualified. A third patient refused further granulocyte transfusions after two severe transfusion reactions and was considered evaluable as an example of alloimmunization and not in terms of possible protection against infection. As shown in Table 1, the nine evaluable patients in each group were comparable in terms of age, sex, degree of granulocytopenia, and time on study. All but one of the female patients had prior pregnancies or miscarriages, and most patients in both groups had received red blood cell transfusions prior to referral to our institution. Two patients in the control arm had acute progranulocytic leukemia and developed disseminated intravascular coagulation requiring heparin therapy. These patients, one of whom died of an intracranial hemorrhage, and another patient in the control group who developed disseminated infection with Torulopsis glabrata were the only patients in whom clinically significant hemorrhage occurred.

Infections and Clinical Outcome

The incidence and severity of infection are summarized in Table 2. A total of three severe infections, all of which were fungal in etiology, occurred in the control
group. Two patients developed pulmonary aspergillosis (*Aspergillus flavus*), which resulted in death in one patient from dissemination and intracerebral hemorrhage despite the administration of Amphotericin B and therapeutic granulocyte transfusions and remission of her leukemia. The third patient, who also received therapeutic granulocyte transfusions, developed an eventually fatal fungemia with *T. glabrata*. The portal of entry was a gingival infection in an elderly patient with poor dentition who had undergone tooth extraction shortly after randomization on the study. No severe infections occurred in the transfused group, and no patient required therapeutic granulocyte transfusions.

The incidence of minor infections was similar in both groups. Mild mucositis, cellulitis at an area of i.v. infiltration, and concurrent episodes of cellulitis of the hand and mild esophagitis of unknown etiology were documented in three control patients. A small area of labial cellulitis and an episode of *Escherichia coli* sinusitis in a patient with a prior history of chronic sinusitis occurred in the patients receiving granulocytes.

Both groups of patients spent more than 50% of days on study receiving systemic antibiotics. Patients receiving granulocytes received more separate courses of antibiotics because of the initiation of empiric antibiotics following severe transfusion reactions as described below. In both groups temperatures of >101°F were recorded on approximately 30%-40% of days on study. The complete remission rate was 77% overall and was similar in both groups.

**Transfusion Results (Table 3)**

The mean dose of granulocytes administered per transfusion was $11.5 \times 10^9$ (range 3.4-24), with a total dose/sq m varying between 15 and $172 \times 10^9$. The lowest dose of granulocytes was administered to a patient with an unusually short duration of granulocytopenia (5 days <500/µl) who required only 3 transfusions. The mean total number of transfusions administered was similar in both groups, although the mean total dose of platelets was higher in the granulocyte group because of the higher platelet yields achieved with the Haemonetics Model 30. In some patients receiving granulocytes who had concurrently higher platelet counts
Table 3. Transfusion Results

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Granulocyte Transfusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of transfusions</td>
<td>11.8 (platelet) (8-16)</td>
<td>11 (granulocyte) (3-19)</td>
</tr>
<tr>
<td>No. of donors/patient</td>
<td>4.4† (3-6)</td>
<td>4.9 (3-7)</td>
</tr>
<tr>
<td>Total platelet dose x 10^11</td>
<td>38 (24-50)</td>
<td>63 (23-108)</td>
</tr>
<tr>
<td>Platelet dose/sq m</td>
<td>21 (15-26)</td>
<td>37 (16-64)</td>
</tr>
<tr>
<td>Total granulocyte dose x 10^9</td>
<td>—</td>
<td>132 (15-296)</td>
</tr>
<tr>
<td>Granulocyte dose/sq m</td>
<td>—</td>
<td>77 (15-172)</td>
</tr>
<tr>
<td>Transfusion reactions</td>
<td>1/9</td>
<td>7/10</td>
</tr>
<tr>
<td>Lymphocytotoxic antibody</td>
<td>4/9</td>
<td>7/10</td>
</tr>
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Values are expressed as means with ranges in parentheses.

*Four patients received a single random donor platelet transfusion in addition to the platelets administered with the granulocytes.

†Five patients received a total of 13 random donor platelet transfusions in addition to the single donor platelets.

from prior transfusions, the Model 30 collection was centrifuged with most of the platelets removed from the granulocyte preparation.

The rate of clinically important transfusion reactions (temperature rise >2°F with chills) was significantly higher in the patients receiving granulocyte transfusions occurring between 1 and 17 days (mean 9.4, n = 7) after the initiation of transfusion. Although reactions were ameliorated by premedication with antihistamines and acetaminophen, they tended to occur repeatedly in patients who had become alloimmunized and necessitated a switch to HLA-matched donors in 3 patients. HLA-matched granulocytes were not administered to 3 of the other alloimmunized patients because immunization occurred shortly before endogenous marrow recovery and remission of the leukemia. In another patient, who subsequently refused further transfusions, a diffuse pulmonary infiltrate, persisting 2–3 days, developed following his third granulocyte transfusion despite negative RBC and lymphocytotoxic and leukoagglutinin crossmatches with his donor. One week later, lymphocytotoxic antibody became detectable in this patient.

Lymphocytotoxic antibody was not detectable in any of the patients at the time of entry into the study. Lymphocytotoxic antibody against >10% of cells in an 80–100 cell panel developed during the on-study period in 7 of 10 patients receiving granulocytes compared to 4 of 9 controls. All of the granulocyte transfusion recipients who experienced transfusion reactions developed lymphocytotoxic antibody, usually within a week of the onset of reactions. In both groups, antibody was directed against multiple HLA antigens with cytotoxicity against >50% of panel lymphocytes, despite the relatively limited number of donors to whom they had been exposed. In an additional patient receiving granulocytes, a weakly positive lymphocytotoxic crossmatch, possibly suggesting B-cell antibodies, was detected. No transfusion reactions or other evidence of alloimmunization was noted in this patient or in the patient receiving only 3 granulocyte transfusions. One other patient in the control group developed transient elevations of low titers of antibody
at day 45 following a total of 15 platelet transfusions. The mean number of days from the first transfusion to the detection of lymphocytotoxic antibody was 17 (range 9–33, n = 7) in the granulocyte group and 14 (range 10–20, n = 4) in the platelet group.

Absolute posttransfusion granulocyte count increments were low (maximal increment, 900/μl at 1 hr in one patient, with all others <400/μl and most <150/μl). Although occasional increments of 100–200 granulocytes/μl persisted until 18–24 hr, in most patients the granulocyte count returned to baseline by the next day. In 2 patients, corrected platelet count increments >10,000/μl persisted for a period of time, despite absent granulocyte increments and transfusion reactions. In most patients, however, parallel decrements in platelet and granulocyte count increments occurred.

Leukoagglutination is an inexact test with a considerable potential for false positive results, even when appropriate positive and negative controls are utilized. Therefore, results from sera on a given date were only considered positive if complete (4+) clumping was detected against >50% of the cell panel in duplicate samples. Using these criteria, 5 of the 19 patients (3 controls, 2 granulocyte transfusion group), all of whom had negative lymphocytoxic antibody, had leukoagglutinins on admission before platelet or granulocyte therapy but usually after RBC had been administered prior to referral to our institution. Eighteen of the 19 patients eventually developed leukoagglutinins, and the leukoagglutinins tended to develop 7–14 days before detection of lymphocytotoxins. This was particularly true in the patients receiving granulocyte transfusion. It was difficult to correlate the presence of leukoagglutinins with the low and variable granulocyte count increments or with the occurrence of transfusion reactions. Of note in this regard is that two of the patients who never had reactions to granulocyte transfusion and who failed to develop lymphocytotoxic antibody had positive leukoagglutinins from the day of admission.

A diffuse erythematous and eventually desquamating skin rash, evidence of hepatic dysfunction and high fevers with no documented infectious source, occurred in one patient approximately 16 days following the initiation of granulocyte transfusions. Graft-versus-host reaction was not detected on skin biopsy, and the symptoms resolved as the patient achieved remission.

DISCUSSION

Controlled trials in animals and two trials in humans employing a daily schedule of transfusion have demonstrated the effectiveness of prophylaxis against infection by granulocyte transfusion. Preliminary results of two other studies that utilized an intermittent schedule are available, one of which utilized partially HLA-matched granulocyte donors and demonstrated a trend for decreased infection and the other of which is as yet inconclusive on this point. In the present study, an alternate-day schedule and a relatively modest dose of granulocytes were utilized, the lower dose being related to the discontinuous flow nature of the Haemonetics Model 30 and the failure to obtain adequate yields in some donors despite steroid premedication. Although there was an increased incidence of fungal infections in the control group, these differences are probably not significant. The T. glabrata infection occurred in a patient with poor dentition and a mucosa further damaged by surgery. The two A. flavus infections are a reflection of an increased
incidence of Aspergillus infections at our institution due to an environmental source, and it would be premature to conclude that it was the granulocyte transfusions that prevented Aspergillus infections in the experimental group. Furthermore, although the number of days of antibiotic therapy were the same, in the granulocyte group, antibiotics were frequently begun because of transfusion reactions, whereas in the control group, other clinical signs of infection were often present. Thus, systemic antibiotics could have served as excellent infection prophylaxis in the granulocyte group. The minor infections (cellulitis, mucositis, esophagitis) are those frequently and sometimes unavoidably seen during induction therapy for leukemia and were equally distributed in both groups.

The overall outcome in terms of complete response rate and survival were excellent and similar in both groups. The Seattle study in bone marrow transplant recipients and the French experience in patients with leukemia receiving more intensive chemotherapy than was used in our study similarly failed to demonstrate improved survival or response rate, attesting to the value of empiric antibiotic therapy and therapeutic granulocyte transfusion. Thus, because initially noninfected patients with leukemia have an excellent overall response rate (72% in our previous induction chemotherapy study), it would appear that even daily prophylactic granulocyte transfusion might not add major benefit in terms of survival to this group. In addition, infections tend to develop late in the induction course of such patients, frequently occurring shortly before marrow recovery and usually not requiring therapeutic granulocyte transfusions. Indeed, because of the higher remission rates and shorter periods of aplasia following modern aggressive induction therapy of ANLL, this phase of the disease may no longer be the best “arena” in which to study the effect of prophylactic granulocytes on patient survival.

Whether prophylactic granulocytes would be effective in reducing infectious morbidity in elderly patients at higher risk, in patients with leukemia in relapse, or for situations with longer durations of aplasia remains to be investigated and proven. Prior to initiating such trials, however, the problems with alloimmunization that were noted in our study should be taken into consideration. The incidence of moderately severe transfusion reactions was markedly increased in the patients receiving granulocyte transfusions, despite the fact that only a small number of donors were utilized/patient. In our past experience, transfusion reactions were virtually nonexistent in nonalloimmunized recipients of granulocytes prepared by differential centrifugation. Furthermore, because the transfusion reactions occurred in the patients who developed lymphocytotoxic antibody, they were almost certainly immunologically mediated.

Similarly, lymphocytotoxic antibodies developed in more patients than in the control group. Although it can be argued that the higher platelet dose in the granulocyte group contributed to the antibody formation, it should be noted that the transfusion reactions tended to occur early in the transfusion course at a time when smaller doses of platelets had been administered. In addition, prior studies in patients with leukemia did not demonstrate that lymphocytotoxic antibody formation was necessarily a function of the platelet dose. This study also confirms the sometimes confusing information provided by slide leukoagglutinin testing and indicates that more precise techniques are needed.

In addition to patient anxiety and discomfort, recurrent fevers and the confusing
clinical picture caused by the transfusion reactions, the development of antigranulocyte antibodies makes further donor selection quite difficult, given the present confused state of the art of granulocyte crossmatching. Further platelet support during induction and maintenance therapy was also made more difficult by the development of alloimmunization. Mannoni et al. also noted a high rate of alloimmunization (approximately 70%) utilizing nonmatched donors,7 while Thompson et al.22 have described the development of a variety of granulocyte-specific antibodies in 74% of patients receiving therapeutic granulocyte transfusion. In the study of Clift et al., alloimmunization was not a critical factor because HLA, MLC-identical marrow donors were used for most transfusions.6 Because available evidence in humans and animals strongly indicates that histoincompatible granulocytes are ineffective and can be harmful to alloimmunized recipients,23-25 one may actually be depriving some patients of the potential benefit of therapeutic granulocytes by prophylactic transfusion. Although one may reduce this problem by using “matched” transfusions from the onset, it is presently possible to reliably match only for HLA and not for other, probably clinically important, granulocyte-specific antigens. In addition, although relatively few donors were utilized for each patient, the lymphocytotoxic antibodies that developed were expressed against large numbers of HLA antigens, making further donor selection more difficult. The broad serologic cross-reactivity of the HLA antigens is the probable explanation for this phenomenon. Very few centers have large numbers of HLA-typed donors available, as evidenced by the fact that regional blood centers, the largest suppliers of granulocyte transfusion in the U.S., presently almost exclusively utilize “random” donors for granulocyte transfusion.26 It may therefore be most prudent to keep HLA-matched family and nonrelated donors “in reserve” for the early administration of therapeutic transfusions rather than risking sensitization to these donors in the prophylactic setting.

Thus, although the scientific point about prevention of infection with daily prophylactic granulocyte transfusion seems to have been proved,6,7 it appears that histocompatibility considerations would mandate that this technology is not suitable for routine therapy and should not be supplied by blood centers except for usage in investigational settings. Research dealing with granulocyte histocompatibility has been hampered by an absence of reproducible, quantifiable posttransfusion parameters. Recently 111Indium has been shown to be a firmly bound granulocyte label allowing effective scanning over infected areas that may allow correlation of in vivo results with crossmatching tests.27 Until further data are available, however, the problem of histocompatibility testing will continue to present a roadblock to the rational clinical use of prophylactic and, indeed, even therapeutic granulocyte transfusions.

REFERENCES


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CA Schiffer, J Aisner, PA Daly, SC Schimpff and PH Wiernik