Alloimmunization Prevents the Migration of Transfused Indium-111-Labeled Granulocytes to Sites of Infection

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１１１Indium-labeled granulocytes were used to study the effects of histocompatibility factors on the migration of transfused granulocytes to infected sites. Fourteen alloimmunized and 20 nonalloimmunized patients received approximately 10⁸ "In-labeled granulocytes from ABO-compatible, non-HLA-matched donors, and scans were performed over known infected sites. All 14 alloimmunized patients had lymphocytotoxic antibody (LCTAb) and required HLA-matched platelet transfusions. Of the nonalloimmunized patients, 20/20 had positive scans at sites of infection. None of the 20 had ICTAb. 0/17 had a positive lymphocytotoxic crossmatch (LCTXM) and 8/11 had a positive LAXM. Labeled granulocytes failed to reach sites of infection in 11/14 (78%) alloimmunized patients, demonstrating that histocompatibility factors can be of major importance in affecting the outcome of granulocyte transfusions. Granulocytes from random donors are unlikely to be effective in alloimmunized patients. The lack of an adequate crossmatching technique is a major problem limiting the ability to provide granulocyte transfusions for alloimmunized patients.

There are animal data to suggest that alloimmunization adversely affects response to random donor granulocyte transfusions.1-3 Applebaum et al.1 demonstrated low posttransfusion count increments and poor migration of transfused granulocytes to skin chambers in sensitized granulocytopenic dogs. Epstein et al.2 using a septic granulocytopenic dog model, have shown markedly poorer posttransfusion count increments and survival among previously sensitized septic dogs transfused with granulocytes, compared to septic dogs that were not alloimmunized. In a subsequent investigation, Chow et al.3 produced Candida albicans meningitis in a granulocytopenic dog model to study the effects of granulocyte transfusions. Transfused granulocytes could be demonstrated in the cerebrospinal fluid (CSF), and the prolongation of survival was dependent on the count increment achieved peripherally and in the CSF. In transfused alloimmunized dogs, there was no granulocyte migration into the CSF, and these animals died more rapidly than the dogs having granulocytes in the CSF.

Data in humans, evaluating the effects of histocompatibility factors on granulocyte transfusions, are not readily available, in part because of the difficulty in assessing the posttransfusion response. Unlike the situation in animals, granulocyte transfusions from normal donors rarely produce measurable posttransfusion count increments, in part because the transfusions are given slowly over several hours and because there is rapid margination and migration of the granulocytes to sites of infection. In addition, the dose of granulocytes obtained is often relatively small. The peripheral counts involved are also very low (<500 WBC/μl), making the interpretation of any count change or differential white blood cell count extremely difficult and often inaccurate. Thus, posttransfusion count increments cannot be reliably used to measure transfusion success. Clinical response is therefore often used as evidence of benefit, but there are too many variables in any clinical situation that requires granulocyte transfusions to isolate the effect of this single therapeutic maneuver. Recently, Dahlke et al.4 have suggested that alloimmunization has an adverse effect on clinical response to granulocyte transfusions. A poorer outcome was seen among patients having antigranulocyte antibody demonstrated by granulocyte indirect immunofluorescence test (GIIFT). However, only a very few patients with antibody were studied and each had a different infectious illness, making it difficult to interpret the effect of a single variable, such as granulocyte transfusion. However, in a subsequent study, Dahlke et al.5 looked at the outcome of patients with white blood cell antibodies who received granulocyte...
transfusions for gram-negative sepsis. None received compatible granulocytes and all died of sepsis despite broad spectrum antibiotics and granulocyte transfusions. Again other variables must enter into such a study, but the data are suggestive that mismatched granulocyte transfusions may be of little benefit.

An additional problem in assessing histocompatibility factors is the lack of an adequate serologic test to predict clinical response prior to transfusion. Human granulocytes have a variety of antigens on their surface with each presumably capable of stimulating an antibody response. The relative strength of various antigens and the clinical importance of antibodies directed against each continues to be a matter of debate. The presence of antigranulocyte antibodies of different types can be demonstrated by a number of in vitro tests. Leukoagglutinating antibodies and granulocytotoxic antibodies presumably detect granulocyte-specific antigens, while lymphocytotoxic antibodies are directed against HLA antigens. McCullough et al. have shown that the presence of granulocytotoxic antibodies has no effect on the migration of transfused granulocytes to sites of infection. There are insufficient data about lymphocytotoxic or leukoagglutinating antibody, however. A relatively new test, the granulocyte indirect immunofluorescence test (GIIFT), appears to be a sensitive test for detecting antigranulocyte antibody, but it is nonspecific and detects only the binding of antigranulocyte antibody. It does not differentiate between HLA antibodies or granulocyte-specific antibodies binding to the test granulocytes. However, if the lymphocytotoxic crossmatch is negative, then a positive GIIFT reflects granulocyte-specific antibodies. As will be demonstrated in this study, heavily transfused patients frequently have both types of antibodies.

Indium-111 oxine is a new radioisotope complex that has demonstrated usefulness in blood cell labeling. The advantages of this isotope include tight cytoplasmic binding with minimal elution, preservation of viability and function of labeled granulocytes, and energy peaks in a range that can be scanned. Indium-111-labeled granulocytes have been used successfully to localize occult abscesses in nongranulocytopenic patients. Very rapid migration to even small sites of infection by In-labeled granulocytes in granulocytopenic patients has been demonstrated. This technique provides a sensitive and specific tool for investigating granulocyte migration. In this study, the effect of histocompatibility factors on granulocyte transfusion was investigated by performing scans with In-labeled granulocytes in both alloimmunized and nonalloimmunized patients. Evidence of migration of the labeled granulocytes to sites of infection was felt to represent the potential effectiveness of granulocyte transfusions in these patients.

**MATERIALS AND METHODS**

**Patient Selection**

Alloimmunized and nonalloimmunized granulocytopenic patients with easily identifiable sites of infection were studied using allogeneic In-labeled granulocytes. Patients with infections in the upper abdomen were not studied because of the substantial hepatic and splenic uptake that occurs routinely with indium scans. Patients were designated as alloimmunized based on the following clinical criteria: (1) poor 1-hr posttransfusion count increments following transfusion of random donor platelets and a requirement for HLA-matched platelets and (2) serologic confirmation by lymphocytotoxic antibody against greater than 20% of a panel of lymphocytes. Recent work by our group has demonstrated a direct correlation between low corrected count increments and lymphocytotoxic antibody activity of greater than 20%. The alloimmunized patients selected for these studies were well known to be refractory to random donor platelets and to have high levels of multispecific lymphocytotoxic antibody. Leukoglutinin crossmatches were not done prospectively, but only in conjunction with each scan.

**Leukocyte Labeling**

Leukocytes were labeled using a modification of the method reported by Thakur, as previously described. Fifty milliliters of whole blood was obtained from an ABO-matched, non-HLA-matched normal donor and anticoagulated with acid citrate dextrose. The red blood cells were sedimented with a final concentration of 3% hydroxyethyl starch in upright syringes. The leukocyte-rich and platelet-rich plasma was expressed, diluted to a total volume of 50 ml with saline, and centrifuged at 180 g for 3 min at 22°C to obtain platelet-rich plasma, which was discarded. The leukocyte pellet was washed once in normal saline with 1% human serum albumin, and the cells were resuspended in 5 ml of normal saline.

The leukocyte suspension was then incubated with 1 mCi of In-oxine (Mediphysics, Bloomfield, NJ) at room temperature without agitation for 30 min. The suspension was centrifuged at 180 g for 5 min, the supernatant was counted and discarded, and the labeled cell preparation was resuspended in 10 ml of normal saline. An aliquot of the cell preparation was assayed for radioactivity to allow calculation of the dose administered.

**Scanning**

The In-labeled granulocytes were administered rapidly with a syringe through an intravenous line. Scanning was performed with an Anger-type large field of view gamma-scintillation camera and counts were accumulated with the Medical Data System A-squared computer system. At 30 min, the area of the known infection was surveyed for activity and scanned for 10 min. Follow-up scans of the site of infection were obtained at 24 hr in all patients and at 4 hr in 12 patients. Scans were read by one of the authors (G.S.), without prior knowledge of alloimmunization status, using standard criteria for areas of increased activity.

**Crossmatching**

Serologic crossmatches were performed in all donor–patient pairs by both lymphocytotoxicity and leukoagglutination assays. Lymphocytotoxic crossmatches (LCTXM) detecting anti-HLA antibody
were done by Dr. P. Terasaki (UCLA, Los Angeles) using standard microlymphocytotoxicity methods. Leukogglutination crossmatches (LAXM), which are felt to detect granulocyte-specific antibodies, were done with a slide technique using a 2-hr incubation with appropriate positive and negative controls for each study. Leukogglutination reactions were graded on a scale of 1-4, based on the degree of agglutination, the number of clumps, and the number of cells involved, as previously described.

**Informed Consent**
This study was approved by the University of Maryland Human Volunteers Research Committee. All participants gave informed consent. All donors of granulocytes met the criteria of the American Association of Blood Banks for ordinary blood donation and were HBS antigen negative.

**RESULTS**

**Cell Preparation**
Approximately $10^8$ granulocytes (range $0.63 \times 10^8 - 1.5 \times 10^8$), labeled with an average of 390 $\mu$Ci of $^{111}$In (range 360-560 $\mu$Ci), were administered to each patient. Leukocyte differential counts in the administered cell preparations averaged 95% granulocytes (range 88%-100%). The total number of platelets was approximately $10^9$, and there were approximately 5 red blood cells per granulocyte in the final cell preparations. Red blood cells have been shown to bind <5% of $^{111}$In in a mixed cell suspension. The labeled granulocytes were tested for viability with trypan blue dye exclusion, with a mean viability of 95% (range 88%-100% viable). Evaluation of the final cell preparation with the phase microscope always showed a free suspension of granulocytes without clumping.

To demonstrate that our scans represented granulocyte migration, and not migration of contaminating platelets, two control studies using only platelets prepared by the same labeling method described above were done. Only the top layer of platelet-rich plasma was utilized to ensure that no WBCs were present for labeling. This preparation method, utilizing only saline for platelet resuspension, produced significant morphological changes, such as spiculation, rounding of platelets, and occasional clumping as evaluated by phase microscopy. The scans in these two nonalloimmunized patients with obvious sites of infection showed localization only in the spleen. There was no uptake at sites of infection at 30 min or at 24 hr. It is therefore unlikely that platelets prepared and labeled in this fashion contributed significantly to the results of the scans at the sites of infection.

**Patient Studies**
Granulocytes labeled with $^{111}$In were given to 32 patients (15 males and 17 females), with a median age of 41 yr (range 19-72). Patient diagnoses included acute nonlymphocytic leukemia (23 patients), acute lymphocytic leukemia (6 patients), lymphoma, breast cancer, and chronic myelocytic leukemia in blast crisis (1 patient each). All patients were granulocytopenic (<100 granulocytes/µl) at the time of the study and all received allogeneic, ABO-compatible, random donor granulocytes. Only one patient was also receiving therapeutic granulocyte transfusions at the time of the study.

All patients had obvious infections, with half occurring in the oropharynx. Other sites included rectum, lung, and extremities. An example of the sensitivity of this technique is demonstrated in Fig. 1A, which shows the clinical appearance of a paronychia of the middle finger of a granulocytopenic patient with acute nonlymphocytic leukemia. This is a minor infection in a normal host, but produced a fever to 103°F and required hospitalization and treatment with intravenous broad spectrum antibiotics in this patient. Figure 1B shows the patient's indium scan at 30 min following injection of labeled granulocytes. The intense uptake in the distal phalanx of the infected finger is easily seen on the right, as well as a lymphangitic streak extending along the entire finger.

**Nonalloimmunized Patients**
Table 1 presents the scan results seen in 20 clinically nonalloimmunized patients. No patient in this group had a positive lymphocytotoxic antibody screen, and all responded well to random donor platelets. All scans were positive at known sites of infection. There were no negative scans. Scans were positive at both 30 min and 24 hr postinfusion, demonstrating the rapid migration of labeled granulocytes to sites of infection, as previously described. Crossmatch results were correlated with scan results. None of 17 lymphocytotoxic crossmatches done were incompatible. However, 3 of 18 leukoagglutinin crossmatches done showed incompatibility. Nonetheless, the scans all showed localization of labeled granulocytes to sites of infection.

**Alloimmunized Patients**
In contrast, among clinically alloimmunized patients (Table 1), only 3 of 14 scans were positive. This is significantly different ($p = 0.00001$) compared to the number of positive scans in nonalloimmunized patients. Thus, clinical alloimmunization status was highly predictive of granulocyte localization to sites of infection. Crossmatch results in patients with negative scans showed that 8 of 11 patients received granulocytes incompatible by leukoagglutination, and 10 of 11 patients received granulocytes incompatible by lymphocytotoxicity.

Of the alloimmunized patients with positive scans, only one of three received granulocytes incompatible...
by lymphocytotoxicity. In the two that were compatible, the donor and recipient were inadvertently partially HLA-matched.

Table 2 presents an evaluation of crossmatch compatibility status correlated with scan results. In positive scans, 1 of 20 LCTXM and 5 of 21 LAXM were incompatible. With comparable infections, but negative scans, 10 of 11 LCTXM and 8 of 11 LAXM were incompatible. Crossmatches were repeated to confirm the results in alloimmunized patients with positive scans using the same donor and patient sera frozen the day of the scan. All leukoagglutinin crossmatches noted as positive were strongly positive (3+-4+), and this result was correlated with scan results. Thus, both false positive and false negative crossmatches were seen with this leukoagglutinin assay. There was no
correlation between strength of LCTAb or grade of crossmatch and scan results, since all of the alloimmunized patients studied had high levels of antibody. In these frequently transfused patients, lymphocytotoxic and leukoagglutinating antibodies usually occurred together, and it was therefore difficult to separate the effects of the two types of antibody.

**DISCUSSION**

The use of $^{111}$In-labeled granulocytes provides a visual demonstration of the localization of infused granulocytes to sites of infection and a means of assessing the potential effectiveness of granulocyte transfusions in the individual patient being evaluated. In these studies, alloimmunization to random donor platelets correlated ($p = 0.00001$) with the absence of localization of labeled granulocytes to sites of infection. This strongly suggests that random donor granulocytes would be ineffective in controlling infection in alloimmunized patients because the granulocytes do not reach the sites of infections. This result is identical to the previously cited data obtained in dog models. $^1$-$^3$ Thus, histocompatibility factors appear to be of major importance in granulocyte transfusions, and random donor granulocytes should not be given unless an evaluation of alloimmunization status is made. Alloimmunized patients with a poor likelihood of response to random donor granulocytes can be identified by assessing response to random donor platelets or the need for HLA-matched platelets and the presence of lymphocytotoxic antibody.

Obviously, it is critical to try to determine which patients are more likely to benefit from granulocyte transfusions. Granulocyte collection is expensive, and there is always some risk to the donor with apheresis procedures. Apheresis should not be undertaken unless there is a reasonable expectation of benefit to the granulocyte transfusion recipient, and it would seem inappropriate to use a donor if there is a substantial chance that the granulocytes will not be helpful. There is also risk to the alloimmunized recipient from the use of incompatible granulocytes, with the possibility of transfusion reactions $^{26-31}$ that may range in severity from fever to pulmonary infiltrates and respiratory compromise. $^{29-31}$

Older studies using granulocyte transfusions from donors with chronic myelocytic leukemia $^{29,32}$ suggest that decreased posttransfusion count increments result following random donor transfusions to alloimmunized patients. Attempts have also been made to sort out the relative clinical importance of HLA versus granulocyte-specific histocompatibility factors. However, these studies have used peripheral count increments to assess the effect of preformed antibody or positive crossmatches on transfusion effectiveness. As previously mentioned, peripheral counts are a poor measure of response in granulocyte transfusions. Higby et al. $^{33}$ in 1974, reported an increased posttransfusion increment following transfusion of HLA-matched granulocytes when compared with unmatched granulocytes, but there was no information about granulocyte-specific antibodies. This becomes important because we have noted that in highly alloimmunized patients, lymphocytotoxic and leukoagglutinating antibodies are almost always found concurrently in the same patients. This has also been observed by Goldstein et al. $^{29}$ Thus, an assessment of the effect of HLA antibodies on granulocyte migration also requires an assessment of the presence or absence of granulocyte-specific antibodies. Ungerleider et al. $^{33}$ were unable to determine any statistically significant relationship between posttransfusion granulocyte recovery and the presence of positive lymphocytotoxic, granulocytotoxic, or leukoagglutinating crossmatches, although those patients with preformed antibody in general had smaller posttransfusion increments. There was a wide spread in the data, and the absolute increments were quite low, making any difference between groups also very small and difficult to interpret.

McCullough et al. $^{12}$ have also utilized $^{111}$In-labeled granulocytes as a method of studying the effects of histocompatibility factors. They compared the results of $^{111}$In scans with crossmatches by granulocytotoxicity, leukoagglutination, and lymphocytotoxicity. In this study, the presence of granulocytotoxic antibodies clearly did not adversely affect survival, recovery, or migration of labeled granulocytes. The patient population evaluated was quite heterogeneous, composed of some granulocytopenic patients and some patients with

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<th>Crossmatch</th>
<th>Scan Positive</th>
<th>Scan Negative</th>
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<td>Lymphocytotoxic</td>
<td>1/20*</td>
<td>10/11</td>
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<tr>
<td>Leukoagglutinin</td>
<td>5/21</td>
<td>8/11</td>
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*Positive crossmatch/crossmatches done.
normal granulocyte counts. In addition, the patients studied had variable prior transfusion histories with different degrees of exposure to HLA and granulocyte antigens. From their studies in this heterogeneous group of patients, McCullough et al. concluded that measurement of leukoagglutinating antibodies was the most accurate method of predicting the outcome of indium scans and that only a positive leukoagglutinin crossmatch correlated with poor granulocyte migration. This statement, however, is based on the results of only two infected patients who had positive leukoagglutinin crossmatches and false negative scans, so that further studies are necessary to verify this conclusion.

In contrast, five patients in our study had positive leukoagglutinin crossmatch and positive scans. This may reflect differences in the methods of performing the leukoagglutinin assays. In addition, McCullough showed positive scans in six patients with positive lymphocytotoxic crossmatch, and our study also showed one such patient. These data suggest that a positive lymphocytotoxic crossmatch may not represent a barrier to effective granulocyte transfusion in some patients.

Our study differs from McCullough's in several important aspects. First, we have investigated a homogeneous, heavily transfused granulocytopenic patient population (the group that frequently is considered for therapeutic granulocyte transfusions). The alloimmunized patients in this group were strongly alloimmunized and required HLA-matched platelets. Therefore, it is not surprising that the majority had leukoagglutinating antibody in addition to high levels of lymphocytotoxic antibody. Both types of crossmatches were positive, demonstrating incompatibility, in most of these alloimmunized patients. We therefore have so far been unable to separate the effects of each antibody on granulocyte migration in our patient population. It is also possible in some patients that the presence of antibodies of both types may exert an additive or synergistic effect. Studies currently in progress at our institution are attempting to sort out the effects of lymphocytotoxic and leukoagglutinating antibody on granulocyte migration. Granulocytes from platelet donors known to be HLA compatible and effective in these patients are being labeled with In an attempt to isolate the influence of granulocyte-specific antibodies.

The data presented here suggest the ineffectiveness of mismatched granulocytes, in that there was no localization of labeled granulocytes to sites of infection in alloimmunized patients. The clinical data presented by Dahlke et al. suggest confirmation of this, showing poor survival of septic patients who received mismatched granulocytes despite broad spectrum antibiotics and granulocyte transfusions. Although a number of variables may enter into such a clinical study, granulocyte incompatibility is very likely to affect the potential for benefit from granulocyte transfusions.

In summary, histocompatibility factors play an important role in granulocyte transfusions, and the presence of alloimmunization represents a relative contraindication to the use of random donor granulocyte transfusions, since specific crossmatching of granulocyte transfusions remains difficult. Conversely, our data indicate that histocompatibility testing is not necessary for granulocyte transfusions in nonalloimmunized patients. Although numerous methods of detecting antigranulocyte antibodies exist, the relative clinical usefulness of each is unclear, except for the previously noted lack of clinical correlation of lymphocytotoxic antibodies. Similarly, a number of crossmatching methods are available, but none has been adequately tested with good clinical endpoints. In addition, none of the methods can provide the result in a reasonable period of time in order to allow prepheresis testing the day of transfusion. Until a better method is developed, the use of granulocytes from HLA-matched donors who have been good platelet donors in the past, and whose transfusions have produced no reactions, may be the safest approach in alloimmunized patients. In a sense, these donors have already provided a "biologic" crossmatch. Each transfusion must be monitored, however, and the donors changed or transfusions discontinued if severe transfusion reactions occur.

ACKNOWLEDGMENT

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