Leukocyte Transfusions: Function of Transfused Granulocytes from Donors with Chronic Myelocytic Leukemia

By Harmon J. Eyre, Ira M. Goldstein, Seymour Perry and Robert G. Graw, Jr.

The in vivo function of chronic myelocytic leukemia (CML) leukocytes transfused into infected patients with severe neutropenia was evaluated and the results in four representative patients are reported. The intravascular survival, extravascular migration, and phagocytic capacity of these cells appeared normal in two patients without preformed leukoagglutinins. In two other patients who had only small transient increments in circulating granulocytes and severe transfusion reactions, preformed leukoagglutinins were found. The poor granulocyte recoveries in these patients with antibody could probably be explained by splenic sequestration of the transfused cells. These studies provide evidence supporting the use of CML leukocyte transfusions in patients without preformed leukocyte antibodies.

COMPONENT TRANFUSIONS for the correction of anemia and thrombocytopenia in patients with acute leukemia are generally accepted as useful modes of therapy. Although granulocyte replacement transfusions are theoretically of value they have been regularly used only in a few centers. The limiting factor in their general application for the treatment of patients with granulocytopenia has been the procurement of large quantities of leukocytes from single normal donors. Because of this obstacle, it has been necessary to utilize patients with chronic myelocytic leukemia (CML) for granulocyte donations. Previous reports have cited beneficial results using CML leukocyte transfusions for the treatment of patients with septicemia. Unfortunately, these results were based on the response of patients in clinical settings in which there were usually many uncontrolled variables. Though seemingly useful, based on these studies, leukocyte transfusions for the support of leukopenic patients have not been widely accepted.

From the Human Tumor Cell Biology Branch and the Medicine Branch, National Cancer Institute, National Institutes of Health, Bethesda, Md.

Submitted March 25, 1970; revised May 1, 1970; accepted May 8, 1970.

Harmon J. Eyre, M.D.: Department of Medicine, University of Utah School of Medicine, Salt Lake City, Utah; formerly Clinical Associate, Human Tumor Cell Biology Branch, National Cancer Institute, National Institutes of Health, Bethesda, Md. Ira M. Goldstein, M.D.: Clinical Associate, Medicine Branch, National Cancer Institute, National Institutes of Health, Bethesda, Md. Seymour Perry, M.D.: Associate Scientific Director for Clinical Trials, National Cancer Institute, National Institutes of Health, Bethesda, Md. Robert G. Graw, Jr., M.D.: Senior Investigator, Human Tumor Cell Biology Branch, National Cancer Institute, National Institutes of Health, Bethesda, Md.

Address for reprints: Robert G. Graw, Jr., M.D., Building 10, Room 2B51, National Cancer Institute, Bethesda, Md. 20014.
This study was initiated to obtain objective data concerning the in vivo function of CML leukocytes when transfused into severely neutropenic, infected recipients in order to assess better this mode of therapy. The intravascular survival, circulation, extravascular migration and phagocytic capacity of the transfused CML cells were measured in 10 patients.

The results of studies in four representative patients demonstrating variable transfusion responses are reported. The recipients’ sera were examined for the presence of preformed leukocyte antibodies against donor cells and the results correlated with in vivo leukocyte function.

**Materials and Methods**

**Patients Studied**

Each patient’s clinical history, chemotherapy and transfusion history is shown in Table 1. All recipients had acute leukemia in relapse with severe neutropenia. In each, a serious infection was present which was not improving, despite antibiotic therapy.

**Leukocyte Procurement and Transfusion**

Peripheral blood granulocytes were collected from patients with chronic myelocytic leukemia (CML), using a conventional single unit leukapheresis technique or the continuous flow blood cell separator. Acid citrate dextrose (ACD) was used for anticoagulation.

The leukocytes were rapidly transfused intravenously through a platelet recipient set (Fenwal HB 182). Complete blood counts were performed on the recipient prior to, at 1 hour, 18 hours and daily following each leukocyte transfusion. Post-transfusion increments for total leukocytes and granulocytes were determined for each time interval post-transfusion by subtracting the pretransfusion count from the interval count. Increments in total peripheral granulocyte counts were standardized in relation to an ideal patient with 1 sq. M. of body surface area receiving a transfusion of 1011 granulocytes. Per cent recovery of the transfused granulocytes was determined by multiplying the 1 hour post-transfusion granulocyte increment per cubic millimeter by the estimated blood volume (2500 ml./sq. M.) and dividing by the number of granulocytes infused.

**Radioisotope Labeling Techniques**

Diisopropylfluorophosphate (DF32P) and sodium chromate (51Cr) were employed in the leukocyte kinetic studies utilizing modifications of previously described procedures.

Labeled leukocytes were infused within a 10–20 minute period and serial venous blood samples were collected at 15, 30 minutes, 1, 2, 4, 8, 18 and 24 hours for specific activity determinations using a modification of the dextran sedimentation and hypotonic lysis technique and liquid scintillation counting as described by Duvall and Perry. The intravascular leukocyte survival curves were expressed as the per cent recovery of labeled transfused cells versus time interval post-transfusion.

**Body Surface Scanning**

Simultaneous external body counts were obtained with each venous blood samples using a 2-inch collimated, thallium-activated, sodium iodide crystal and a gamma spectrometer (Model 132A, Nuclear-Chicago Corp., Des Plaines, Ill.). Areas over the spleen, liver, heart and upper right lung were monitored in all patients and, in three of the patients, known localized infections were also scanned (axillary adenitis, perineal cellulitis and lobar pneumonia). In the fourth patient, an area over a previously unsuspected lobar pneumonia was monitored while counting over the right lung.
Table 1.—Summary of Clinical Case Histories

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age and Sex</th>
<th>Diagnosis †</th>
<th>Duration of Disease (Months)</th>
<th>Chemotherapy</th>
<th>Prior Transfusion History*</th>
<th>Infection</th>
<th>Antibiotic Therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td>L.W.</td>
<td>37</td>
<td>F</td>
<td>AML</td>
<td>9</td>
<td>Ara-C † Vincristine Prednisone Methotrexate 6-mercaptopurine</td>
<td>42 14</td>
<td>Axillary hidradenitis (<em>Pseudomonas</em>) Polymyxin B</td>
</tr>
<tr>
<td>C.P.</td>
<td>27</td>
<td>M</td>
<td>AML</td>
<td>1</td>
<td>Vincristine Prednisone Methotrexate 6-mercaptopurine</td>
<td>17 113 3</td>
<td>Perineal cellulitis (<em>Pseudomonas</em>) Polymyxin B Kanamycin</td>
</tr>
<tr>
<td>C.S.</td>
<td>36</td>
<td>M</td>
<td>AML</td>
<td>5</td>
<td>Ara-C</td>
<td>30 184 2 8</td>
<td>Pneumonia (<em>Pseudomonas</em>) Carbenicillin</td>
</tr>
<tr>
<td>N.W.</td>
<td>33</td>
<td>M</td>
<td>ALL</td>
<td>3</td>
<td>Vincristine Prednisone Methotrexate 6-mercaptopurine</td>
<td>15 85 4</td>
<td>Pneumonia sepsis (<em>Pseudomonas</em>) Carbenicillin</td>
</tr>
</tbody>
</table>

* RBC, packed red blood cells; WB, whole blood; WBC, white blood cells.
† AML, acute myelocytic leukemia, ALL, acute lymphocytic leukemia.
†† Ara-C, cytarabine hydrochloride (Cytosar).
At selected times following the transfusion, gamma camera scintiphotos were obtained and linear profile scanning was performed in an attempt to determine the relative body distribution of labeled cells.

**Radioautography**

In patient L.W., exudates were collected before and serially following the transfusion of labeled leukocytes for determination of specific activity and preparation of radioautographs using the technique described by Ronai. The percentage of labeled cells in the exudate was determined.

**Phagocytic Function and Hexose Monophosphate Shunt (HMS) Activity**

HMS activity in response to phagocytosis of polystyrene latex particles (Dow Chemical Co., Midland, Mich.) was measured utilizing both heparinized venous blood and leukocyte suspensions in Hank's balanced salt solution according to previously described methods. Both homologous normal AB+ serum and serum obtained from the transfusion recipients were used in the reaction mixtures. Increments in $^{14}$CO$_2$ release ($\Delta$ CPM), as measured by the difference between the counts per minute of the dissolved CO$_2$ from the reaction flasks with and without latex, were used as the measure of HMS activity in response to phagocytosis.

In addition to the metabolic studies, overnight broth cultures of *Pseudomonas* organisms obtained from two of the patients (L.W. and N.W.) were concentrated by centrifugation, washed with normal saline, resuspended in Hank's BSS, and incubated with leukocyte suspensions obtained from their respective leukocyte donors. Slides were made from samples of these reaction mixtures.

**Leukoagglutinins**

Sera obtained from the four recipients prior to transfusion were rendered complement-free by heating to 56°C for 30 minutes and tested for the presence of leukoagglutinins directed against leukocytes obtained from their respective donors utilizing a previously described technique.

**RESULTS**

**Response to Leukocyte Transfusions**

The responses to the leukocyte transfusions are summarized in Table 2. Leukocyte recoveries in patients C.S. and N.W. were very low as determined by absolute counts at one hour post-transfusion. In contrast, recoveries in patients C.P. and L.W. fell within the range of those seen with the infusion of autologous cells in normal subjects or in patients with CML.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Pretransfusion WBC Count (x10$^5$/cu.mm.)</th>
<th>Pretransfusion Absolute Granulocyte Count/cu.mm.</th>
<th>Total No. Granulocytes Transfused (x10$^11$/sq. M.)</th>
<th>Absolute Granulocyte Increment/cu.mm.</th>
<th>1 Hr. Post-transfusion Granulocyte Increment/sq. M./10$^11$</th>
<th>1 Hr. Post-transfusion Granulocyte Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>L.W.</td>
<td>30.1</td>
<td>0</td>
<td>0.27</td>
<td>2082</td>
<td>7,670</td>
<td>19.1</td>
</tr>
<tr>
<td>C.P.</td>
<td>14.7</td>
<td>147</td>
<td>0.18</td>
<td>3558</td>
<td>19,700</td>
<td>49.3</td>
</tr>
<tr>
<td>C.S.</td>
<td>6.3</td>
<td>126</td>
<td>0.34</td>
<td>54</td>
<td>158</td>
<td>0.4</td>
</tr>
<tr>
<td>N.W.</td>
<td>3.1</td>
<td>434</td>
<td>0.13</td>
<td>94</td>
<td>705</td>
<td>1.7</td>
</tr>
<tr>
<td></td>
<td>0.55</td>
<td>0</td>
<td>0.18</td>
<td>120</td>
<td>666</td>
<td>1.7</td>
</tr>
</tbody>
</table>

*Granulocyte increment per sq. M. body surface per 10$^{11}$ granulocytes transfused.
Both patients with low recoveries had transfusion reactions within 1–2 hours following the infusion, characterized by transient chills, fever and malaise. No symptoms were observed in the other two patients.

**Intravascular Survival**

The intravascular survival curves of the transfused labeled CML leukocytes were complex, but there were gross differences that could be used to characterize the different responses. The curves are shown in Fig. 1. The t $\frac{1}{2}$ survival for each was estimated and is shown in Table 3. In patient C.S., only the initial component was used to calculate the t $\frac{1}{2}$ because of the borderline specific activity in the second component. In the other three patients, a line of best fit for the entire curve was utilized. By 4 hours, in patient C.S., and 16 hours in patient N.W., less than 0.1 per cent of the labeled cells were present. In contrast, in patients C.P. and L.W., 18–20 per cent of the cells present 1 hour post-transfusion were circulating at 20 hours.

**Site of Sequestration**

Sites of sequestration as determined by external body counting and scanning are shown in Table 3. In the two patients (N.W. and C.S.) with shortened intravascular leukocyte survivals, there was marked splenic sequestration in the first 4 hours. In both patients, areas of localized pneumonia failed to show increased radioactivity when compared to the contralateral uninvolved lung.
LEUKOCYTE TRANSFUSIONS

Table 3.—Survival and Sequestration of CML Leukocytes as Determined by DF³²P and ⁵¹Cr Labeling

<table>
<thead>
<tr>
<th>Patient</th>
<th>Survival Time (t ½ hr.) *</th>
<th>Scanning; External Body Counts †</th>
</tr>
</thead>
<tbody>
<tr>
<td>L.W.</td>
<td>7</td>
<td>Sequestration in lesion with maximum activity at 16 hours in exudate from adenitis; bone marrow sequestration</td>
</tr>
<tr>
<td>C.P.</td>
<td>5</td>
<td>Sequestration in site of cellulitis; maximum activity at 42 hours; little splenic activity</td>
</tr>
<tr>
<td>C.S.</td>
<td>0.3</td>
<td>Splenic sequestration at 1 hour with no lesion localization</td>
</tr>
<tr>
<td>N.W.</td>
<td>1</td>
<td>Splenic sequestration at 4 hours with no lesion localization</td>
</tr>
</tbody>
</table>

* t ½ determined from DF³²P specific activity curves.
† Determined by scintillation scanning of ⁵¹Cr radioactivity counting and gamma camera scanning.

Once splenic sequestration had occurred in these patients, there was no further significant change in radioactivity over the spleen, indicating that the cells did not recirculate. In contrast, patients C.P. and L.W. with longer leukocyte intravascular survival times and better recoveries, showed no significant splenic sequestration. In both of these latter patients, marked localization of radioactivity was detected over local sites of infection. Patient C.P., who had a severe perineal cellulitis, rapidly accumulated radioactivity in the lesion within 24 hours, which reached a maximum by 42 hours, indicating continued migration of labeled leukocytes to the inflammatory site. A representative gamma camera scintiphoto taken 24 hours post-transfusion is shown in Fig. 2.

Migration of the transfused granulocytes into an infected lesion was demonstrated by studying the exudate from the axillary lesions of patient L.W. Touch preparations of the exudate obtained prior to transfusion revealed numerous bacteria and necrotic epithelial cells, but only scant numbers of polymorphonuclear and mononuclear cells containing phagocytic vacuoles. Serial prepara-
tions obtained following the transfusion revealed increasing numbers of granulocytes containing ingested bacteria. By 16 hours post-transfusion, the exudate consisted almost entirely of granulocytes and radioautographs demonstrated that more than 90 per cent of these cells were labeled. Specific activity determinations of the exudate at this time showed a higher activity in the inflammatory lesion than in the peripheral blood.

**Phagocytic Function**

Changes in granulocyte HMS activity in response to phagocytosis were measured using cell suspensions obtained from leukocyte donors and heparinized venous whole blood from respective recipients prior to and serially following transfusion. In this test system, the increments in HMS activity following phagocytosis are directly proportional to the absolute numbers of granulocytes in the reaction mixtures up to a concentration of 5000 granulocytes per cu. mm. The results of two such experiments are presented in Fig. 3, which includes the range of values determined in 10 hematologically normal volunteers. As shown, the pretransfusion values for both recipients are low, reflecting absolute granulocytopenia. Following transfusion in patient L.W., there was approximately a fivefold increase in activity. This increase persisted for 2 hours and then gradually declined. At 20 hours following transfusion, a significant increase above the pretransfusion value persisted. Donor CML cells were found to produce the same increments in HMS activity with both normal homologous and recipient serum.

Results of the leukocyte HMS studies in patient N.W. were in marked contrast to the previous patients. Immediately following transfusion, a slight increment in measurable HMS activity was noted, but additional measurements at 1–20 hours approached pretransfusion levels. When this recipient’s serum (N.W.) was added to the donor’s leukocytes, there was a decrement in HMS activity as compared to that measured using a normal homologous serum.

*Pseudomonas* organisms responsible for the infections in two of the patients were maintained in broth culture. Leukocyte suspensions obtained from their
respective donors were found capable of phagocytizing these organisms in the presence of recipient serum. Bactericidal assays were not performed.

**Leukoagglutinin Tests**

Leukoagglutinins, directed against donor cells were found in pretransfusion serum samples obtained from the patients having the poor transfusion responses (C.S. and N.W.). Serum from patients L.W. and C.P. failed to reveal leukoagglutinins when tested with cells obtained from their respective donors.

**DISCUSSION**

Leukemic patients with granulocytopenia, resulting either from their primary disease or from cytotoxic chemotherapy, frequently respond poorly to severe bacterial infections despite treatment with appropriate antibiotics. Because of this lack of responsiveness, leukocyte replacement transfusions have been utilized in the supportive care of these patients. Since, until recently, it has been impossible to obtain adequate quantities of normal granulocytes for transfusion, leukocytes from patients with CML have been used in most centers. Although there have been many reports concerning the clinical use of white blood cells, there is little information concerning the in vivo function of these transfused cells.

The first successful series of leukocyte transfusions was accomplished in 1953 by Brecher et al. in irradiated dogs. These authors were able to show leukocyte circulation and migration to sites of infection.

Kauder and his associates demonstrated circulation and lesion localization in normal human subjects receiving labeled leukocytes transfused in whole blood. A population of previously untransfused patients was identified, in whom normal homologous leukocytes had a markedly shortened intravascular survival. These authors suggested that either circulating or cellular antileukocyte antibodies might be responsible for the observed rapid disappearance. Galbraith determined the intravascular survival of transfused CML leukocytes in recipients with nonhematological malignancies and found that the CML cells disappeared in a complex fashion, with shortened intravascular half lives, when compared to autologous cells. In one patient, in vivo phagocytosis by transfused CML cells has been demonstrated.

Granulocyte function following transfusion must be documented for correct interpretation of the clinical responses observed. In this study an attempt was made to objectively quantitate the in vivo function of the transfused cells. This permits a more refined evaluation of granulocyte transfusions by decreasing the number of unknown variables inherent in reporting patient response.

Two patients presented in this study had good responses to granulocyte transfusions with relatively normal granulocyte recoveries, intravascular survival, lesion localization and increments in phagocytic capacity as measured by HMS activity. The demonstration in patient L.W. of donor phagocyte migration into an infected lesion with subsequent ingestion of bacteria provides unequivocal evidence that transfused leukocytes can retain this function in their new host. There was improvement in both of these patients with
clearance of the lesion and defervescence in one patient and improvement in the second patient. However, whether these responses were the result of the leukocyte transfusions cannot be determined from these studies.

Two other patients are presented who demonstrated poor responses to granulocyte transfusions and in whom the transfusions were followed by chills, fever and malaise. Granulocyte recoveries were poor, intravascular survival was markedly shortened and there was evidence of splenic sequestration. No increase in phagocytic capacity of the blood was observed in either patient and granulocyte migration to sites of infection did not occur. The clinical courses of these patients were unchanged by the transfusions.

Retrospective testing of recipient sera against donor leukocytes in the four patients presented revealed the presence of leukoagglutinins in the sera of patients with poor transfusion responses and their absence in the patients with good responses. It seems likely that leukoagglutinins were responsible for the poor recoveries, lack of granulocyte function and for the transfusion reactions as well. Of the remaining six patients, tests for leukoagglutinins were performed in five but were positive in only one. In these six patients, studies similar to those in the four patients reported in this paper gave comparable results.

All patients had been transfused with many units of blood components prior to the granulocyte transfusions but no relationship was found between the number of units received by any individual and the presence of leukoagglutinins. Although leukoagglutinins were found in the two patients with prior WBC transfusions described in detail in this paper, they were not detected in the remaining patients studied who also received prior WBC transfusions. The reason for this is not apparent but immunosuppressive therapy may have played a role.

Previous studies have shown that the leukocyte recovery following transfusion is influenced by several other factors including the pretransfusion granulocyte count, preceding therapy, and clinical status of the patient. The subjects in the present study were matched as closely as possible to minimize the influence of these factors.

This study serves to define some of the parameters involved in evaluating leukocyte transfusions and demonstrates the need for a better understanding of the role of immune mechanisms. Dausset, in a recent review of the status of leukocyte transfusion immunology, pointed out the frequency of antileukocyte isoantibodies in transfused subjects and suggested that leukocyte alloantigen determinations could lead to a better choice of an appropriate donor. Lymphocytes from all donors and recipients at this institution are currently typed for antigens of the HL-A system, and studies are in progress to determine the function of leukocytes transfused from donors with identical and random HL-A phenotypes. In addition, all recipients are tested for the presence of preformed circulating leukocyte antibodies against donor leukocytes in an attempt to eliminate severe transfusion reactions, and enhance the survival of the transfused cells.
LEUKOCYTE TRANSFUSIONS

ACKNOWLEDGMENT

The authors wish to express their appreciation to Dr. Edward S. Henderson for allowing them to study his patients and to Dr. Louis S. Zieger for obtaining the gamma camera scintiphotos.

REFERENCES


Leukocyte Transfusions: Function of Transfused Granulocytes from Donors with Chronic Myelocytic Leukemia

HARMON J. EYRE, IRA M. GOLDSTEIN, SEYMOUR PERRY and ROBERT G. GRAW, JR.