Morphologic Evidence for the In Vivo Activity of Transfused Chronic Myelogenous Leukemia Cells in A Case of Massive Staphylococcal Septicemia

By Stephen B. Shohet

Granulocyte transfusions from donors with chronic myelogenous leukemia have been used with increasing frequency since this source of cells was first suggested by Morse in 1961.1,2,3,4 Prior to that time, all efforts in white cell transfusion had been ineffective because of the inadequate numbers of white cells available from normal donors, although the feasibility of such therapy had been shown by Brecher and Cronkite in dogs over fifteen years before.5,6 Chronic myelogenous leukemia white cell transfusions have been given in the past to patients with leukemia, lymphoma, epithelioma, choriocarcinoma, or other neoplastic diseases. With the unsuccessful exception of one case of chloramphenicol toxicity,2 all of these cases had been treated with large doses of immunosuppressive chemotherapeutic drugs or steroids prior to white blood cell transfusion. Therefore it might seem that prior reduction of immunologic capacity might be necessary for a successful granulocyte transfusion, although it can be argued that this is more important for subsequent transfusions or for the establishment of a marrow homograft. In addition, to our knowledge, no morphologic evidence of biologic activity of the transfused leukocytes has been demonstrated. These considerations and the influence of granulocyte sequestration on the control of infection, together with the potential hazards of granulocyte transfusions, are explored in the following report.

Materials and Methods

The chronic myelogenous leukemia cells were obtained from a 58 year old female patient (P.B.B.H.-V.M.) with chronic myelogenous leukemia. She had anemia, hepatosplenomegaly, a white blood cell count of $1.4 \times 10^5$ per cu. mm., and differential blood film showing 66 per cent polymorphonuclear leukocytes, 12 per cent myelocytes, 11 per cent promyelocytes, 3 per cent myeloblasts, 5 per cent eosinophils, and 5 per cent basophils. Her marrow was consistent with the diagnosis of chronic myelogenous leukemia, and eight of eleven metaphase plates from peripheral blood were positive for the Philadelphia 1 chromosome by the method of Moorhead.7 Further characterization of these white blood cells showed an alkaline phosphatase of only 7 on a scale with a maximum of 400/100 cells and a “drumstick” count of 27/500 cells.8

White blood cells were obtained by collection in a plastic blood collection bag with
ACD-A anticoagulant.* The bags were filled maximally (approx. 650 cc.), and then blood was allowed to settle for two hours at room temperature in an upright position. A unit of packed cells was returned to the patient and a second bag of blood was obtained. Following sedimentation, an initial fraction of cell-poor plasma was removed (100 to 150 cc.), and the white blood cells and remaining platelet-rich plasma were steriley transferred into empty bags with a standard separation press and weighed and counted. Smears for differential counts were prepared and then the remaining cells were immediately transfused without a filter. The time lapse from the completion of venesection to transfusion was 2 hours and 40 min. The differential smears for both transfusions were similar to that of the donor's whole blood with the exception that less than 2 per cent red blood cells were present: (transfusion 1-62 per cent polymorphonuclear leukocytes, 12 per cent myelocytes, 13 per cent metamyelocytes, 5 per cent promyelocytes, 5 per cent basophils, 3 per cent blasts; transfusion 2-60 per cent polymorphonuclear leukocytes, 20 per cent myelocytes, 8 per cent metamyelocytes, 10 per cent promyelocytes, 4 per cent blasts).

Peripheral white blood cell counts were performed in duplicate on a Coulter Model A Counter and verified daily by manual chamber counts. "Drumstick" counts were obtained by counting five hundred consecutive polymorphonuclear leukocytes in Giemsa-stained preparation.

CASE REPORT

The 24 year old male patient (P.B.B.H. - J.R.) was admitted to the Peter Bent Brigham Hospital with a chief complaint of shortness of breath and pain in the chest of twelve hours duration. The history disclosed that the patient had been well until three days prior to admission when he developed a sore throat and fever of approximately 101 F. At the time the patient lived in an endemic area of influenza. He was immediately started on oral tetracycline therapy and later penicillin and ampicillin by his local physician. His symptoms, however, were not improved, and twelve hours prior to admission the patient was awakened from sleep with pleuritic low anterior chest and epigastric pain. In addition he had marked shortness of breath. At this time the patient was hospitalized at another hospital and a chest film disclosed bilateral infiltrates in both upper lobes and lingula. A white blood cell count was 144/cu.mm. with 33 per cent myelocytes, 30 per cent band forms, and 35 per cent polymorphonuclear cells. The patient was sent to the Peter Bent Brigham Hospital, where he was found to have marked bronchial breathing over the right and left upper lung fields, reduced breath sounds and rales over the left lower chest, and a palpable friction rub in the left axilla. Examination of the heart revealed a loud pleuropericardial rub at the apex. A repeat white blood cell count was 1200, but at this time the differential showed no mature granulocytes with 10 per cent myelocytes, 80 per cent lymphocytes, 2 per cent plasmacytes, and 8 per cent atypical lymphocytes. A bone marrow aspiration revealed small foci of active marrow, but these were very rare and most of the marrow smear was aplastic. Smears and cultures of nasal and tracheal aspirates and of the throat and blood disclosed a penicillin-resistant, coagulase-positive, Staphylococcus aureus. Blood gases at the time of admission, while the patient was on 100 per cent oxygen by face mask, revealed a PO2 of 100 mm. of mercury, a pH of 7.44, a PCO2 of 26.5 mm. of mercury, and a CO2 content of 18.5 m.Eq./L.

A diagnosis of overwhelming staphylococcus pneumonia and septicemia was made. The marked leukopenia was believed in part due to the sequestration of white blood cells in the lungs. The possibility that an antecedent viral infection had depressed the marrow was also considered to be likely. The patient was treated with cephalothin (1 Gm. Q. 6 H., I.V.) and oxygen. Eighteen hours after admission, following the positive blood-culture report, he received a white blood cell transfusion of 1.2 x 10^11 granulocytes concentrated in approximately 300 cc. of plasma derived from the patient with chronic myelogenous leukemia. However, his clinical state deteriorated rapidly, and he required a tracheostomy twenty-four

*Bag # PC 210 containing 50 cc. 4 per cent Na citrate anticoagulant (A); Fenwall Laboratories, Morton Grove, Illinois.
Fig. 1.—White blood cell counts during hospital course after transfusions of chronic myelogenous leukemia cells. The percentage of polymorphonuclear leukocytes were as follows: 1 hour—56; 13 hour—58; 16 hour—78; 20 hour—72; 24 hour—56; 32 hour—74; 36 hour—72.

hours after admission when hypoxia and hypercapnia rapidly developed. On the morning of the second hospital day, the patient had an acute hypoxic episode and a blood CO₂ of 29 mm. of mercury and FCO₂ of 57 mm. of mercury. Following vigorous artificial respiration, his PO₂ was raised to approximately 60 mm. of mercury and he was transferred to the hyperbaric chamber at the Children's Hospital Medical Center. There he was curarized and maintained at an ambient pressure of approximately 1.6 atmospheres of air. Initially his blood PO₂ was stable at approximately 60 mm. of mercury and his blood oxygen saturation was approximately 90 per cent. During his stay in the hyperbaric chamber, Kanamycin (200 mg. Q. 6 H., I.V.), and decadron (5 mg. Q. 6 H., I.V.) were added to his regimen. In addition, he received a second concentrated white cell transfusion of $1.8 \times 10^{11}$ granulocytes in 320 cc. of plasma from the same donor. Although a transient defervescence of fever occurred following this white blood cell transfusion, the patient became progressively lethargic and eventually hypoxic. Approximately eight hours prior to death, he developed bilateral hydrothoraces and pulmonary edema. Although this complication was transiently controlled with digitalis and drainage of the transudates, he eventually developed cardiac arrhythmias and died.

Postmortem examination disclosed a massive staphylococcal pneumonia with necrosis of vessels and interstitial tissue in the lungs. No true abscesses were present in the lungs or other tissues because of the dearth of mature polymorphonuclear leukocytes. However, some young granulocyte precursors were observed in microscopic sections of the lungs and marrow spaces. No Barr bodies could be seen in the hematoxylin and eosin sections of lung, liver, or spleen, but very few mature polymorphonuclear leukocytes were present in these tissues. The marrow was generally aplastic with rare foci of erythropoietic activity and there was no evidence for leukemia or other neoplastic disease. Many of the young myelocytes seen had ingested Staphylococci. Large colonial masses of free Staphylococci were also present.
Table 1.—Number of Drum Sticks in 500 Consecutively Examined Polymorphonuclear WBC

<table>
<thead>
<tr>
<th>Sample</th>
<th>Patient 2 Hours Before Trans. (A)</th>
<th>Patient 2nd Trans. (A)</th>
<th>Patient 4 Hours After Trans. (B)</th>
<th>Patient 16 Hours After Trans.</th>
<th>Patient 28 Hours After Trans.</th>
<th>Patient 34 Hours After Trans. (C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number Barr Bodies</td>
<td>27</td>
<td>0</td>
<td>36</td>
<td>24</td>
<td>30</td>
<td>23</td>
</tr>
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(A) 27 cells available for counting.
(B) Buffy coat preparation.
(C) Samples taken 10 min. post mortem with some loss of morphologic detail.

Fig. 2.—Polymorphonuclear leukocyte from patient’s blood 22 hours after chronic myelogenous leukemia transfusion. Ingested Staphylococci are abundant.

RESULTS OF SPECIAL STUDIES

The results of the studies which could be performed during this emergency are summarized in Figure 1. As can be seen there was a definite over-all rise in the patient’s white blood cell count following the white cell transfusion. The first transfusion had only a minimal and transient effect, but the second transfusion had a marked and sustained effect. After both transfusions there was an immediate rise in white blood cell count followed by a fall to a lower but still elevated post-transfusion plateau. A drop in temperature followed the second transfusion until fever returned preterminally and the white blood cell count rose precipitously. At this time, the differential count showed 72 per cent polymorphonuclear leukocytes, 12 per cent metamyelocytes, 6 per cent myelocytes, 4 per cent promyelocytes, 4 per cent lymphocytes, and 2 per cent blasts.

Evidence supporting the presumption that the white cells circulating in the recipient were those of the donor is found in Table 1. In this table, it is shown...
MORPHOLOGIC EVIDENCE

Fig. 3.—Polymorphonuclear leukocyte from patient's blood 22 hours after chronic myelogenous leukemia transfusion. Ingested Staphylococi and a nuclear appendage can be seen.

Table 2.

<table>
<thead>
<tr>
<th>Time After Transfusion 2</th>
<th>-1 hr.</th>
<th>0.5 hr.</th>
<th>2.5 hr.</th>
<th>5 hr.</th>
<th>7 hr.</th>
</tr>
</thead>
<tbody>
<tr>
<td>(P_{\text{O}_2})</td>
<td>56</td>
<td>58</td>
<td>60</td>
<td>68</td>
<td>70</td>
</tr>
<tr>
<td>(O_2\text{SAT})</td>
<td>87</td>
<td>88</td>
<td>89</td>
<td>92</td>
<td>93</td>
</tr>
</tbody>
</table>

that the recipient had, as expected in a male, no "drumsticks" present in his leukocytes, whereas following the second transfusion and up to the time of death the number of "drumsticks" present in his leukocytes did not differ significantly from the number of the donor blood. In addition, a chromosome preparation prepared twenty-two hours after the second transfusion when the peripheral white blood cell count was rising rapidly showed six out of six metaphase plates positive for the Philadelphia 1 chromosome. It was also noted (Fig. 2) that circulating polymorphonuclear leukocytes observed in the patient's blood twenty-two hours following the second transfusion were packed with ingested Staphylococi which stained characteristically dark purple with Giemsa stain as well as with gram stain. Leukocyte alkaline phosphatase stains of granulocytes obtained at this time had values of 8, 11, and 4 on a scale with a maximum of 400/100 cells. Figure 3 reveals a polymorphonuclear leukocyte observed at that time which contains a few ingested Staphylococi and a typical "drumstick" nuclear appendage.

Although the dose of white blood cells administered to the patient was massive (194 Gm. in thirteen hours) there was no demonstrable effect of transfusion on pulmonary function (Table 2). A small rise in blood \(P_{\text{O}_2}\) and oxygen saturation followed this massive transfusion.
DISCUSSION

White blood cell transfusions have previously been shown to be effective in bone marrow failure associated with neoplastic states. The possibility that the underlying neoplasm or secondary immunosuppressive therapy in these patients was responsible for the success of the transfusion has always been present.9 The data presented here suggest that the transfusion of biologically active white blood cells may be performed in patients without these conditions. The possibility cannot be denied, however, that this young man with overwhelming sepsis and possible antecedent viral disease may have had a modified immune response.

Following each leukocyte transfusion, the white blood cell count from this patient developed a transient peak followed by a plateau. These phenomena suggest that the transfused leukocytes were rapidly sequestered in tissue sites as originally described by Weisberger8 and later documented by Bierman.9 They then apparently slowly returned to circulation. The fact that the second transfusion, which was only 40 per cent greater in cell count than the first, produced a threefold rise in the peak and an eightfold rise in the plateau suggests that part of the depleted "tissue space" of this patient had been filled by the first transfusion and hence the second was more effective because permanent sequestration was limited. This phenomena has also been observed by Morse et al.4 Such data emphasize the importance of continued transfusion even when an initial transfusion fails to elevate the peripheral white blood cell count and the importance of the total dose of the white blood cells.

It is possible to assume from the data accumulated in the last sixteen hours of life that many of the sequestered white blood cells which were originally trapped in the small vessels of the body had started to recirculate. Since the Philadelphia chromosome studies performed preterminally showed that all metaphases seen were Philadelphia-chromosome-positive, it is very doubtful that the late rise in white cell count was due to the production of autologous granulocytes. It is also possible, however, that maturation or proliferation of the transfused myelocytes and metamyelocytes could account for some of the observed rises in the white blood count even though the majority of the transfused cells were mature polymorphonuclear leukocytes. The patient's brief clinical course (thirty-six hours after the first transfusion) would seem to limit the extent of such a homograft, however.

With regard to the concept of tissue sequestration, several authors10,11,12 have noted that transfused white blood cells are particularly sequestered in the lungs. It was therefore possibly hazardous to transfuse a large mass of chronic myelogenous leukemia white cells into a patient with such minimal respiratory function. However, it was felt that the site where white blood cells were most needed was indeed the lungs and that close observation of blood PO2 during, and immediately after, the transfusion would provide a warning of any deleterious effects. Clearly the infused white blood cells did not measurably depress the low pulmonary reserves in this patient, although the autopsy data showed that immature myelocytes did concentrate in the lungs.

The large numbers of Staphylococci in the circulating polymorphonuclear
leukocytes may well have been due to the free access of these organisms into the blood stream secondary to the alveolar and vascular necrosis noted in the lungs. Alternately, the cells found may have previously been resident in the lungs adjacent to massive concentrations of Staphylococci only to have emerged into the circulation shortly prior to sampling.

Unfortunately, this patient did not survive. The autopsy data would suggest that the pulmonary necrosis was so marked that when therapy was started the recovery was impossible. Had it been possible to start white blood cell transfusions before this necrosis was advanced he might have survived. However, early white blood cell transfusion requires an available source of chronic myelogenous leukemia cells and, moreover, unless this source is consistently available, any advantages from therapy of this type will depend upon circumstance. In this case, the logistics for transfusion were fortuitous in that a chronic myelogenous leukemia donor presented herself shortly after the patient was shown to be aplastic and septicemic. While the maintenance of a group of untreated patients with chronic myelogenous leukemia as sources of white blood cell transfusions may not be justified, the possibility of a physician finding a suitable donor would be greatly increased by having an up-to-date registry of newly diagnosed cases of chronic myelogenous leukemia in various geographic centers. Another approach to this problem may be suggested by the evidence that chronic myelogenous leukemia cells may be preserved in a frozen state with either dimethyl sulfoxide or glycerol as antifreeze. There is further evidence that such cells upon thawing may be biochemically and biologically active. If such preservation technics could be adequately refined, a chronic myelogenous leukemia "cell bank" could be envisioned. Newly diagnosed chronic myelogenous leukemia patients could easily donate eight or ten plasmaphoresis units before treatment with little risk. The use of continuous plasmaphoresis centrifuges of the types currently under development would further reduce any risk and simplify this procedure. In fact, with the advent of these centrifuges, the possibility of using normal white blood cells processed in large centralized blood collection centers could be considered.

**Summary**

Morphologic and clinical evidence for the in vivo activity of white blood cell transfusions in a previously well young man with marrow depression and staphylococcal septicemia is presented. No underlying malignancy or chronic immunosuppressive therapy was present.

Characteristics and problems of white blood cell transfusions are discussed with special emphasis on the site and clinical importance of tissue sequestration of the transfused cells.

**SUMMARIO IN INTERLINGUA**

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*We have recently successfully treated a patient with an aplastic marrow secondary to methotrexate therapy for choriocarcinoma with chronic myelogenous leukemia leukocytes frozen with dimethyl sulfoxide.*
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e septicemia staphylococcal. Nulle subjacente malignitate esseva presente. Chronic therapia immunosuppressive esseva etiam absente.

Es discutite le caracteristicas e le problemas de transfusiones leucocytic, con accentuation special del importantia del sito seligite e del signification clinic de sequestration tissular del transfusionate cellulas.

REFERENCES

Morphologic Evidence for the In Vivo Activity of Transfused Chronic
Myelogenous Leukemia Cells in A Case of Massive Staphylococcal
Septicemia

STEPHEN B. SHOHET