Autologous Peripheral Hematopoietic Stem Cell Transplantation Restores Hematopoietic Function Following Marrow Ablative Therapy

By Anne Kessinger, James O. Armitage, James D. Landmark, Douglas M. Smith, and Dennis D. Weisenburger

From ten patients with advanced malignant disease involving the bone marrow, autologous hematopoietic stem cells were collected from the peripheral blood during eight four-hour pheresis procedures and cryopreserved. No manipulations to increase the number of stem cells circulating in the blood were used during the collections. Following marrow ablative chemotherapy or chemoradiotherapy, the autologous cells were thawed and infused intravenously (IV). WBCs reappeared in the circulation at a median of eight days (range seven to 11 days) after stem cell infusion. Two patients died early, whereas the other eight reached normal numbers of circulating granulocytes that have persisted for up to >20 months. These eight patients became independent of RBC transfusions (hemoglobin concentration >10 g/dL) at a median of 27 days (range 11 to 58 days) after transplantation. One patient received platelet transfusions for counts <50 x 10^9/L, one patient developed a clinical picture of idiopathic thrombocytopenic purpura, and six patients maintained a platelet count >20 x 10^9/L at a median of 23 days (range 14 to 26 days) following stem cell infusion. This technique allows patients ineligible for autologous bone marrow transplantation due to unacceptable anesthetic risks, prior pelvic irradiation, or bone marrow metastases to receive marrow ablative therapy.

© 1988 by Grune & Stratton, Inc.

From the Departments of Internal Medicine, and Pathology and Microbiology, University of Nebraska Medical Center; and American Red Cross Blood Services, Midwest Region Omaha.

Submitted June 29, 1987; accepted November 3, 1987.

Address reprint requests to Anne Kessinger, MD, University of Nebraska Medical Center, 42nd and Dewey Ave, Omaha, NE 68105.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

© 1988 by Grune & Stratton, Inc. 0006-4971/88/7103-0023$3.00/0
filled with peripheral blood at a rate of 40 to 80 mL/min. After the plasma was discharged and the buffy coat reached the shoulder of the bowl, the flow was decreased to 40 mL/min until the platelet band began to discharge. The flow rate was then reduced again to 20 mL/min. When half of the platelet band was discharged, cell collection began and continued until two minutes after the RBCs began to discharge. The discharged plasma and platelets were returned intravenously (IV) to the patient, and the procedure was repeated until four hours had passed. The product was allowed to settle between passes, and a portion of the RBCs was returned to the patient. Immediately following the four-hour collection, a cell count and differential count were done on an aliquot of the total product to determine the number of mononuclear cells collected.

Next, the product was centrifuged at 400 g for ten minutes in the processing laboratory. Enough plasma was removed to give a cell concentration of 1 x 10^9 cells/mL, and the product was cooled to 4°C. A precooled cryoprotectant mixture containing 40% dimethylsulfoxide (DMSO) and 6 µg/mL DNAse in Medium 199 (GIBCO, Grand Island, NY) was added at a ratio of one part cryoprotectant to three parts concentrated leukapheresis product, and the cells were frozen in a controlled rate liquid nitrogen freezer (Cryo-Med, Mt. Clemens, MI). The average total volume of cryopreserved cells for these patients was 1,278 mL.

Aliquots of all eight collections from each patient were thawed and pooled. CFU-GM assays were performed on the pooled specimens using an agar culture system with normal peripheral blood mononuclear cells (1 x 10^6 cells/mL) in an underlayer as a source of colony-stimulating factors (CSFs). One-milliliter aliquots of feeder cell suspension in 0.5% agar medium were incubated in 35-mm Petri dishes at 37°C in 7% CO2 humidified air for at least 24 hours. One-milliliter aliquots of target cells (1 x 10^5 cells/mL) in 0.3% agar medium were then pipetted onto the semisolid feeder layers. The cultures were incubated at 37°C in 7% CO2 humidified air for 14 days, stained with Wright’s stain, and counted (>50 cells = one colony). In our laboratory, the normal average of the pooled peripheral blood CFU-GM obtained by leukopheresis prior to cryopreservation is 26 ± 5.6 colonies/1 x 10^5 cells plated.

The high-dose therapy administered varied according to the type of neoplastic disease and prior treatment received. All antinecancer drugs were given IV. Four patients (patients 1, 2, 5, and 8 in Table 1) with breast cancer received cis-platinum, 125 mg/M² in a 24-hour infusion followed by two consecutive daily doses of cyclophosphamide, 60 mg/kg. On the following day, total body irradiation (TBI) was begun for five daily fractions of 2.2 to 2.5 Gy. Patients 3 and 4 received six doses of etoposide 150 mg/M² every 12 hours followed by cis-platinum and cyclophosphamide as given above. All three patients with Hodgkin’s disease received cyclophosphamide 6 g/M² in four divided doses over 4 days, six doses of etoposide, 150 mg/M² at 12-hour intervals over 3 days, and carmustine 300 mg/M² given on the day of the first doses of cyclophosphamide and etoposide. The patient with non-Hodgkin’s lymphoma received six doses of cytarabine 3 g/M² every 12 hours, followed 2 days later by cyclophosphamide 90 mg/kg. Forty-eight hours after the last dose of cyclophosphamide, 9 Gy of TBI fractionated into five doses was given over 2½ days. Following these various therapies, the patients’ peripheral stem cells were rapidly thawed in a 40°C water bath at the bedside and immediately infused IV in a one- to four-hour period. A bone marrow biopsy was done for patient 1 on day +7 (ie, seven days following stem cell infusion) to document marrow aplasia. Because this biopsy showed marrow recovery, subsequent biopsies to demonstrate aplasia were performed on day +4.

Patients received irradiated RBC transfusions when their hemoglobin concentrations were <10 g/dL and irradiated platelet transfusions when their platelet counts were <20 x 10^9/L except for patient 10, whose platelet count was maintained above 50 x 10^9/L because of pulmonary alveolar hemorrhage. All patients were nursed with a dedicated nursing staff in a special care unit in single rooms supplied with high-efficiency particulate-filtered (sterile) air at a rate of exchange that provided continuous positive pressure to the corridor. Appropriate antibiotics were administered for identified infections and for unexplained fevers >38.4°C. All patients received parenteral hyperalimentation from the time high-dose therapy was instituted until granulocyte recovery (>0.5 x 10^9/L granulocytes) occurred.

A complete response was defined as disappearance of all detectable malignant disease. Partial responses were defined as a decrease in tumor volume of ≥50%. Patients with bone metastases had a partial response if all measurable disease decreased in volume by 50% and persistent or improved bone abnormalities were present on radiographs.

RESULTS

A median of 8.4 x 10^9 mononuclear cells/kg patient weight was collected from 11 patients that contained a median of 8 x 10^9/kg CFU-GM after thawing. All patients who had a bone marrow biopsy done on day +4 had histopathologic evidence of marrow aplasia, and all ten patients developed WBC counts <0.1 x 10^9/L and became RBC and platelet transfusion dependent.

Following peripheral stem cell infusion, all patients demonstrated engraftment. Patients 1 and 8 died on day +12; bone marrow examination at autopsy showed that both patients had all hematopoietic cell lines. Circulating WBCs reappeared at a median time of day +8 (range day +7 to +11). Eight patients reached normal numbers of circulating granulocytes (ie, 2 x 10^9/L) that persisted for up to 20+ months. The median time to reach 0.5 x 10^9/L granulocytes was day +22 (range day +11 to +58). The patients maintained a hemoglobin concentration of 10 g/dL without transfusions at a median time of day +27 (range day +11 to +58). One patient (patient 2) developed a clinical picture of idiopathic thrombocytopenic purpura with adequate numbers of megakaryocytes in the bone marrow biopsy, one patient was transfused to maintain a platelet count of 50 x 10^9/L (patient 10), two patients had an early death, and six patients maintained a platelet count of >20 x 10^9/L at a median time of day +23 (range day +14 to +36). WBC counts and platelet counts not supported by transfusions are shown for each patient in Fig 1.

When normal peripheral counts were reached, they persisted until an event to explain cytopenia other than failure of the graft to endure occurred; the backup marrow was never used. One patient developed thrombocytopenia (28 x 10^9/L) and anemia (hemoglobin concentration 8.1 g/dL) on day +409 (patient 4). A bone marrow biopsy revealed extensive replacement with malignant cells, and she died of progressive disease 16 days later. Patients who received systemic therapy for relapsed disease also had the expected depression of circulating WBC and platelet numbers. Patient 7 developed thrombocytopenia with a viral illness. A bone marrow biopsy revealed normal numbers of megakaryocytes, and the platelet numbers rose spontaneously as he recovered from his illness.

Complete responses occurred in the patient with non-Hodgkin’s lymphoma and in a patient with Hodgkin’s disease. Four breast cancer patients and two Hodgkin’s disease patients had partial responses. Two patients were not evalua-
<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Sex</th>
<th>Age (yr)</th>
<th>Malignancy</th>
<th>Days to First WBC (days)</th>
<th>Days to &gt;2 x 10^9/L GRN and Time Determined</th>
<th>Days to &gt;20 x 10^9/L HG and Time Determined</th>
<th>Days to &gt;150 x 10^9/L PLTS and Time Determined</th>
<th>Tumor Response (days)</th>
<th>Survival (days)</th>
<th>Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>F</td>
<td>41</td>
<td>Breast cancer</td>
<td>8</td>
<td>0.05 (day 12)</td>
<td>NE (day 102)</td>
<td>NE (day 102)</td>
<td>NE</td>
<td>NE</td>
<td>12</td>
</tr>
<tr>
<td>2</td>
<td>F</td>
<td>54</td>
<td>Breast cancer</td>
<td>8</td>
<td>19.0 (day 102)</td>
<td>34 (day 102)</td>
<td>10.3 (day 102)</td>
<td>NA</td>
<td>Never</td>
<td>20</td>
</tr>
<tr>
<td>3</td>
<td>F</td>
<td>35</td>
<td>Breast cancer</td>
<td>11</td>
<td>0.4 (day 152)</td>
<td>47 (day 152)</td>
<td>9.3 (day 152)</td>
<td>25</td>
<td>40</td>
<td>23</td>
</tr>
<tr>
<td>4</td>
<td>F</td>
<td>43</td>
<td>Breast cancer</td>
<td>7</td>
<td>3.6 (day 409)</td>
<td>23 (day 409)</td>
<td>14.5 (day 409)</td>
<td>15</td>
<td>59</td>
<td>18</td>
</tr>
<tr>
<td>5</td>
<td>F</td>
<td>36</td>
<td>Breast cancer</td>
<td>9</td>
<td>3.0 (day 707)</td>
<td>58 (day 707)</td>
<td>9.4 (day 707)</td>
<td>36</td>
<td>58</td>
<td>198</td>
</tr>
<tr>
<td>6</td>
<td>F</td>
<td>18</td>
<td>Hodgkin's disease</td>
<td>8</td>
<td>10.8 (day 539)</td>
<td>26 (day 478)</td>
<td>11.1 (day 478)</td>
<td>25</td>
<td>59</td>
<td>78</td>
</tr>
<tr>
<td>7</td>
<td>M</td>
<td>53</td>
<td>Non-Hodgkin's lymphoma</td>
<td>9</td>
<td>11.6 (day 347)</td>
<td>27 (day 347)</td>
<td>12.8 (day 347)</td>
<td>21</td>
<td>347</td>
<td>170</td>
</tr>
<tr>
<td>8</td>
<td>F</td>
<td>47</td>
<td>Breast cancer</td>
<td>9</td>
<td>0.06 (day 12)</td>
<td>NE (day 12)</td>
<td>NE (day 12)</td>
<td>NE</td>
<td>NE</td>
<td>0</td>
</tr>
<tr>
<td>9</td>
<td>M</td>
<td>40</td>
<td>Hodgkin's disease</td>
<td>7</td>
<td>9.6 (day 201)</td>
<td>19 (day 201)</td>
<td>8.5 (day 201)</td>
<td>14</td>
<td>29</td>
<td>268</td>
</tr>
<tr>
<td>10</td>
<td>F</td>
<td>26</td>
<td>Hodgkin's disease</td>
<td>8</td>
<td>10.8 (day 271)</td>
<td>11 (day 271)</td>
<td>13.2 (day 271)</td>
<td>NE</td>
<td>43</td>
<td>87</td>
</tr>
</tbody>
</table>

Days, days following transplantation; NE, not evaluable; NA, not available; PLTS, platelets; GRN, granulocytes; HG, hemoglobin.
ble for response due to early death. Details of each patient's course are given in Table 1.

**DISCUSSION**

The number of mononuclear cells and CFU-GM have been used as indicators, but specific cell numbers that accurately reflect the number of pluripotent stem cells in a peripheral blood collection may or may not exist. For our ten patients, no statistically significant correlation between the number of mononuclear cells or the number of CFU-GM administered and the length of time to hematopoietic recovery of any cell line was found (Spearman rank correlation coefficient), but the sample size was not large enough to detect correlations of 0.6. Recently, autologous PSCT in a small number of patients with diseases other than CGL has been reported. Most of these transplants were done with cells collected when peripheral blood counts were beginning to recover following nonmarrow lethal chemotherapy; the number of CFU-GM circulating in the peripheral blood is increased at that time when compared with the normal population. Five of those PSCT were successful, and two of the five were done with a number of mononuclear cells equivalent to the number our patients received. The number of cells infused for two transplants were not specified, and the fifth successful PSCT was done with a relatively small number of mononuclear cells (1.9 x 10⁶/kg). However, two PSCTs using cells collected at the time of marrow recovery resulted in incomplete hematopoietic reconstitution; for these two transplants, the numbers of CFU-GM collected (some may have been lost in the freezing–thawing process) were similar to the numbers given to our patients, but the numbers of mononuclear cells infused (1.3 and 3.0 x 10⁶/kg) were considerably less than our patients received. This information suggests that the number of mononuclear cells given may be either a more sensitive predictor of engraftment or a more accurate reflection of the number of pluripotent stem cells present than the number of CFU-GM infused.

The time following PSCT until reappearance of circulating WBCs in these ten patients (median eight days) compares favorably with the time until reappearance of circulating WBCs for 83 patients (median 11 days) who have had ABMT at our institution (P < .00001 by the two-sided Mann-Whitney U test). This shorter period of aplasia may be important since infections during aplasia are common. Although granulocytes reappeared earlier in these patients, comparisons of the time to reach 0.5 x 10⁹/L granulocytes following PSCT (median 22 days) and ABMT (median 23 days) (P = .44), the time to maintain 10 g/dL hemoglobin after PSCT (median 27 days) and ABMT (median 24 days) (P = .21), and the time to recover 20 x 10⁹/L platelets following PSCT, (median 23 days) and ABMT (median 24 days) (P = .44) at our institution failed to suggest an additional advantage for PSCT.

Because each patient received autologous cells and no marker to discriminate between autologous peripheral stem cell and autologous marrow stem cell progeny existed, recovery of marrow function rather than engraftment by peripheral stem cells could not be disproved. However, no explanation seems likely to account for peripheral evidence of hematopoiesis eight days following nine or more Gy of TBI (patients 1, 2, 5, 7, and 8) other than peripheral stem cell engraftment. Probably no current high-dose therapy is consistently permanently marrow ablative. Following high-dose therapy and allogeneic marrow transplantation, one patient began to produce autologous cells at 4 months and the proportion of donor cells continued to decrease through subsequent months from 90% to 50%, whereas another patient continued to produce cells exclusively of donor origin.
PERIPHERAL STEM CELL TRANSPLANTATION

>1 year later. Autologous marrow transplantation is used to accelerate marrow recovery after high-dose therapy, thereby making such therapy feasible as originally described by Appelbaum et al. Peripheral stem cell transplantation resulted in more rapid return of circulating cells than we have seen with ABMT; early hematopoiesis may have resulted from engraftment of committed rather than pluripotent stem cells and later hematopoiesis from autologous marrow recovery.

The peripheral cells were not examined for tumor cell contamination. Circulating tumor cells may have been collected, cryopreserved, and reinfused. However, no patient had an initial tumor relapse in a site different from known areas of metastases at the time high-dose therapy was given, suggesting that residual disease rather than reinfused tumor stem cells was responsible for the regrowth of malignancy. One could speculate that PSCT offers less chance of returning tumor stem cells capable of reestablishing malignancy to the patient than does ABMT. Recently, tumor stem cells were found in long-term cultures of histologically normal bone marrow collected from four of nine patients with breast cancer. The reported high plating efficiency of these tumor stem cells suggests that they could be especially virulent. Malignant cells in human circulation, however, are not necessarily capable of establishing metastatic disease. Animal studies have demonstrated that most tumor cells entering the circulation are immediately trapped by the lungs, and only a small fraction of them continue in the circulation. If the small number of tumor cells remaining in the circulation are there because of an inability to extravasate, they could not serve as metastatic progenitors.

PSCT permits patients with unacceptable anesthetic risks, prior pelvic irradiation, or metastatic disease in the bone marrow to receive marrow ablative therapy. PSCT might prove better than ABMT for all patients receiving marrow ablative therapy since the trauma associated with harvesting marrow is eliminated, the period of aplasia following transplantation is shortened, and the risk of reestablishing malignancy may be lessened; therefore, a randomized prospective study comparing PSCT to ABMT for patients with similar malignancies receiving identical high-dose therapy is underway.

REFERENCES

1. McCredie KB, Hersh EM, Freireich EJ: Cells capable of colony formation in the peripheral blood of man. Science 71:293, 1971
5. Hershko C, Gale RP, Ho WG, Cline MJ: Cure of aplastic anemia in paroxysmal nocturnal hemoglobinuria by marrow transfusion from identical twin; failure of peripheral-leukocyte transfusion to correct marrow aplasia. Lancet 1:945, 1979
15. To LB, Haylock DN, Kimmer RJ, Juttner CA: High levels of circulating haemopoietic stem cells in very early remission from acute non-lymphoblastic leukaemia and their collection and cryopreservation. Br J Haematol 58:399, 1984
Autologous peripheral hematopoietic stem cell transplantation restores hematopoietic function following marrow ablative therapy

A Kessinger, JO Armitage, JD Landmark, DM Smith and DD Weisenburger