RAPID COMMUNICATION

Syngeneic Transplantation With Peripheral Blood Mononuclear Cells Collected After the Administration of Recombinant Human Granulocyte Colony-Stimulating Factor


Five syngeneic transplants were performed in four patients following myeloablative therapy using unmodified peripheral blood mononuclear cells (PBMCs) collected after the administration of recombinant human granulocyte colony stimulating factor (rhG-CSF) to normal donors. The only toxicity experienced by the four normal donors was bone pain. Four patients received two collections of PBMCs, and a second transplant was performed in one patient with one collection. The patients received a median of 20.53 \times 10^8 total nucleated cells/kg (range 20 to 25.5), 11.3 \times 10^8 total mononuclear cells/kg (range 6.52 to 17.2), 113.1 \times 10^6 CFU-GM (range 46.7 to 211.8) and 9.6 \times 10^6 CD34+ cells/kg (range 1.6 to 12.6). Post-transplant growth factors were not administered. The median time to an absolute neutrophil count greater than 0.5 \times 10^9/L was 14 days (range 10 to 18). The median time to platelet transfusion independence was 11 days (range 10 to 13). Two patients had the number of CD3+ T lymphocytes determined in thepheresis product. An average of 3.04 \times 10^6 CD3+ cells were collected per pheresis. This represents an approximate 1 log increase over the number of T lymphocytes in a typical bone marrow transplant. RhG-CSF can be used to mobilize peripheral blood progenitor cells from normal donors with minimal toxicity. Studies of allogeneic transplants using PBMCs collected after rhG-CSF administration to determine permanent grafting ability and the incidence and severity of graft-versus-host disease are warranted.

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AUTOLOGOUS PERIPHERAL blood mononuclear cells (PBMCs) collected after the administration of recombinant human granulocyte colony stimulating factor (rhG-CSF) results in prompt and probably durable engraftment after myeloablative therapy.1 Allogeneic transplants using PBMCs collected after rhG-CSF administration may provide a substitute for aspirated bone marrow cells. Available data from autologous transplants suggests that allogeneic PBMCs could provide for more rapid neutrophil and platelet engraftment, reducing platelet requirements after transplant and ultimately reducing the cost and length of the hospital stay. Additionally, the morbidity associated with PBMC collection may be less than with traditional bone marrow harvests.2,4 As a preliminary step to allografting with PBMCs, the results of five syngeneic transplants in four patients using PBMC’s collected from syngeneic normal donors after the administration of rhG-CSF were analyzed and are reported here.

PATIENTS AND METHODS

Four patients with syngeneic donors were referred to the Fred Hutchinson Cancer Research Center (FHCRC) for consideration of marrow transplantation. As an alternative to marrow harvest, normal donors were offered the alternative of receiving rhG-CSF followed by the collection of PBMCs by leukapheresis. Informed consent was obtained using protocols and forms approved by the FHCRC institutional review board for patients and donors.

Four normal donors underwent the administration of rhG-CSF (Amgen, Thousand Oaks, CA) by subcutaneous injection (16 \mu g/kg) daily for 4 days. Leukapheresis using a percutaneous Mahurkar catheter was initiated 18 to 24 hours following the third dose of rhG-CSF and performed on 2 consecutive days. RhG-CSF was administered after leukapheresis on day 4. The donor for unique patient number (UPN) 7382 underwent a second PBMC collection using a vein-to-vein technique 60 days after collections for the first transplant. One leukapheresis was performed on day 5 of rhG-CSF administration. This second transplant was performed as consolidation treatment. UPN 7382 had normal peripheral blood counts at that time. Leukapheresis was performed with the use of automated continuous-flow blood cell separators (Spectra; Cobe Laboratories, Engelwood, Co. CS-3000; Fenwal Inc, Round Lake, IL) as previously described.1 CD34+ cells in the leukapheresis product were measured as previously described.1 Lymphocyte quantities were measured in all samples in the following manner. A 1 ml sample of the leukapheresis product was hemolyzed with ammonium chloride hemolytic buffer and washed twice with phosphate buffered saline containing 2% human AB serum, and aliquoted into tubes at 1 \times 10^6 cells per tube. Each tube was incubated with either CD3, CD4, CD8, CD19, or CD20 monoclonal antibody conjugated to phycoerythrin or fluorescein for 20 minutes at 22°C, then washed twice. Cells were analyzed on a FACSCAN (Becton-Dickinson, Palo Alto, CA) and the data analyzed using a cell analysis program (Reproman; Truefacts Software Inc, Seattle, WA). From the analysis of forward and 90 light scatter, a gate was established to include all white blood cells, excluding platelets and red blood cells. A second gate was established to include only lymphocytes and mononuclear cells, excluding granulocytes. The cells in the second gate were analyzed for fluorescence of cells labeled with the particular MoAb. The percentage of cells labeled was then multiplied by the total white blood cell count.

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to give the concentration of antibody + cells in the peripheral blood with a concentration factor for gated cells. To calculate the absolute number of antibody + cells in the leukapheresis product, the percentage of antibody + cells was multiplied by the percentage of gated cells times the total number of white blood cells in the product to give a concentration of antibody + cells in the product. The concentration of tagged cells was then multiplied by the volume of product collected to give the total number in the product. Colony-forming units-granulocyte-macrophage (CFU-GM) were measured by plating 1 x 10^5 peripheral blood mononuclear cells in a 1.25% methylcellulose solution containing 10% lymphocyte-conditioned media, 10% human plasma, 20% fetal calf serum, 9% Iscoves medium, and 5 x 10^-4 mol/L M2-mercaptoethanol. Colonies of greater than 50 cells were counted on an inverted microscope after 14 days.

The characteristics of the four patients receiving syngeneic PBMC transplants are shown in Table 1. Patients received 1 of 3 different preparative regimens followed by the infusion of noncryopreserved PBMCs as the sole source of stem cells on each of 2 successive days immediately following the collections. Day 0 was the day of first PBMC infusion. Post-transplant growth factors were not administered.

Supportive care included private rooms, red blood cell and platelet transfusion, and antibiotics as previously described, except for the second transplant in one patient (UPN 7382B), which was performed entirely in the outpatient department. Daily complete blood cell counts were performed and time to engraftment was assessed by determining the day after transplant on which patients achieved 0.1, 0.5, 1.0 neutrophils x 10^9 and 20 x 10^9 platelets/L, independent of transfusion support.

RESULTS

Effect of PBMC collection on normal donors. All four normal donors received a short Mahurkar catheter placed percutaneously without complications. PBMCs were collected for the second transplant for patient UPN 7382 using a standard vein-to-vein technique. There were no catheter-related infections or thromboses in the four normal donors. All four normal donors reported minor bone pain during the 5-day administration of rhG-CSF, and this was relieved by acetaminophen in all cases. There were no additional side effects observed during or following the administration of rhG-CSF.

PBMC collection characteristics. The number of total nucleated cells (TNC), total mononuclear cells (TMC), CFU-GM, and CD34+ cells collected with 2 consecutive collections are shown in Table 2. The values for patient UPN 7382B are from a single collection. The number of T lymphocytes were determined in the PBMC collections for patient UPN 7382B and 7440. The PBMC collection contained an average of 3.04 x 10^10 (4.5 x 10^8/kg) CD3+, 1.80 x 10^10 (2.6 x 10^8/kg) CD4+, and 0.99 x 10^10 (1.4 x 10^8/kg) CD8+ lymphocytes per pheresis.

Table 3 shows the engraftment characteristics of the five syngeneic transplants. When compared with a historical control group of 12 patients receiving autologous rhG-CSF-mobilized PBMCs, there was no difference in time to ANC greater than 0.1, greater than 0.5, greater than 1.0, or to platelets greater than 20 x 10^9/L. One patient (UPN 7440) experienced a decrease in the platelet count to 25 x 10^9/L on day +32, after initially achieving platelet transfusion independence on day +11 and a peak platelet count of 170 x 10^9/L on day +19. This patient had no evidence of infection, and was not taking any medications that could explain the abrupt drop in her platelet count. She received a platelet transfusion on day +32 and day +35. Her platelet count has increased steadily and was 68 x 10^9/L on day +43.

DISCUSSION

Transplants with PBMCs have several advantages compared with bone marrow. PBMCs appear to result in faster engraftment of neutrophils and platelets than does marrow, or marrow plus post-transplant GM-CSF in the autologous setting. Additional advantages of PBMCs include a decrease in platelet and red blood cell transfusions, a decrease in the duration of hospital stay, and a reduction in the cost of transplantation. Before proceeding with allogeneic PBMC transplants, the ability to harvest PBMCs from normal donors without complications, and a measurement of the number of T lymphocytes collected should be determined because the number of donor T lymphocytes transplanted may have an impact on both sustained engraftment and graft-versus-host disease. Much of this information can be obtained from autologous PBMC transplants, especially in patients with limited chemotherapy exposure. However, evaluation of syngeneic transplants is a logical step before proceeding with allogeneic transplants using PBMCs.

The toxicity of rhG-CSF 16 μg/kg/day for 5 days was evaluated on five occasions in four syngeneic normal donors. The only toxicity observed in these donors was minor bone pain relieved by acetaminophen in all cases. No catheter-related infections or thromboses were encountered. In a previous study, we reported a lack of side effects in eight normal donors given rhG-CSF (3.5-6 μg/kg/day) for 9 to 14
cells. Therefore, it may be advantageous to limit PBMC collections to one procedure because there is a doubling of the lymphocyte quantity with each collection.

Based on an analysis of platelet recovery after autologous PBMC transplants, we have set the minimum dose of CD34+ cells for autografting at 5.0 \( \times 10^6 \) kg (unpublished observations). The dose of CD34+ PBMCs necessary for allografting is unknown but is likely to be greater than for autografts. For autologous marrow transplants we attempt to obtain a minimum of 1 \( \times 10^9 \) kg of TMCs compared with 3 \( \times 10^8 \) for allogeneic transplants. Based on this empirical ratio, the collection of 15.0 \( \times 10^9 \) kg of CD34+ cells would be a reasonable goal. With appropriate timing this should be feasible with one collection by leukapheresis. The effect on graft-versus-host disease of increased numbers of T lymphocytes present in PBMC collections is unknown. Strategies such as T-cell depletion or CD34+ selection may be necessary if the increased numbers of T lymphocytes present in the rhG-CSF-mobilised PBMC products are found to be associated with increased graft-versus-host disease.

### Table 2. rhG-CSF–Mobilized PBMC Collection Characteristics in Normal Donors

<table>
<thead>
<tr>
<th>UPN</th>
<th>TNC ( \times 10^6 ) kg</th>
<th>TMC ( \times 10^6 ) kg</th>
<th>CFU-GM ( \times 10^6 ) kg</th>
<th>CD34 ( \times 10^6 ) kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>7162</td>
<td>25.5</td>
<td>17.2</td>
<td>63.2</td>
<td>1.6</td>
</tr>
<tr>
<td>7448</td>
<td>20.2</td>
<td>8.2</td>
<td>211.9</td>
<td>7.1</td>
</tr>
<tr>
<td>7382A</td>
<td>20.0</td>
<td>11.3</td>
<td>163.0</td>
<td>11.1</td>
</tr>
<tr>
<td>7382B</td>
<td>21.4</td>
<td>6.5</td>
<td>—</td>
<td>12.6</td>
</tr>
<tr>
<td>7440</td>
<td>20.5</td>
<td>17.2</td>
<td>46.7</td>
<td>9.6</td>
</tr>
</tbody>
</table>

All patients received two collections of PBMCs, except UPN 7382B who received one.

Abbreviations: TNC, Total nucleated cells; TMC, Total mononuclear cells.

### Table 3. Engraftment Characteristics of Patients Receiving rhG-CSF–Mobilized PBMCs

<table>
<thead>
<tr>
<th></th>
<th>ANC ( \times 10^9 ) L</th>
<th>Day to Discharge</th>
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</thead>
<tbody>
<tr>
<td>7162</td>
<td>13</td>
<td>25</td>
</tr>
<tr>
<td>7448</td>
<td>12</td>
<td>14</td>
</tr>
<tr>
<td>7382A</td>
<td>9</td>
<td>10</td>
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<tr>
<td>7382B</td>
<td>12</td>
<td>19</td>
</tr>
<tr>
<td>7440</td>
<td>10</td>
<td>11</td>
</tr>
</tbody>
</table>

*Median 11, 13, 14, 10.

Range 7-13, 10-15, 11-16, 7-70.

Abbreviation: ANC, absolute neutrophil count \( \times 10^9 \) L.

† Transplant was performed in the outpatient department and the patient was never admitted to the hospital.
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Syngeneic transplantation with peripheral blood mononuclear cells collected after the administration of recombinant human granulocyte colony-stimulating factor

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