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 × 10^8 total nucleated cells/kg (range 20 to 25.5), 11.3 × 10^8 total mononuclear cells/kg (range 6.52 to 17.2), 113.1 × 10^6 total nucleated cells/kg (range 20 to 25.5). 1.3 × 10^8 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 × 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 the pheresis product. An average of 3.04 × 10^9 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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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 × 10⁷ 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 × 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 × 10⁹ and 20 × 10⁹ 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 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 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.

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

<table>
<thead>
<tr>
<th>UPN</th>
<th>Diagnosis</th>
<th>Preparative Regimen</th>
<th>Age</th>
<th>Sex</th>
<th>Status</th>
<th>Days After Transplant</th>
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</thead>
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<tr>
<td>7162</td>
<td>AML</td>
<td>Bu 16 mg/kg, Cy 120 mg/kg</td>
<td>60</td>
<td>M</td>
<td>Remission</td>
<td>214</td>
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<tr>
<td>7382A</td>
<td>Ewings sarcoma</td>
<td>Melphalan 100 mg/m², Bu14 mg/m²,</td>
<td>20</td>
<td>M</td>
<td>Remission</td>
<td>90</td>
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<tr>
<td></td>
<td></td>
<td>Thiopeta 500 mg/m²</td>
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<td></td>
<td></td>
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<tr>
<td>7382B</td>
<td>—</td>
<td>*Total body irradiation 12.0 Gy</td>
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<td></td>
<td></td>
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<tr>
<td>7448</td>
<td>Non-Hodgkin’s</td>
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<td></td>
<td>lymphoma</td>
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<tr>
<td>7440</td>
<td>Breast cancer</td>
<td>Bu 19 mg/kg, Cy 150 mg/kg</td>
<td>37</td>
<td>F</td>
<td>Stage IV</td>
<td>46</td>
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</table>

Abbreviations: AML, acute myeloid leukemia; Bu, busulfan; Cy, cyclophosphamide; TBI, total body irradiation; VP-16, etoposide.

* The lungs and liver were shielded to 10% of the administered dose; electron beam therapy was administered to the ribs.
Table 2. rhG-CSF—Mobilized PBMC Collection Characteristics in Normal Donors

<table>
<thead>
<tr>
<th>UPN</th>
<th>TNC (\times 10^6/\text{kg})</th>
<th>TMC (\times 10^6/\text{kg})</th>
<th>CFU-GM (\times 10^4/\text{kg})</th>
<th>CD34 (\times 10^4/\text{kg})</th>
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<td>25.5</td>
<td>17.2</td>
<td>63.2</td>
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<td>7448</td>
<td>20.2</td>
<td>8.2</td>
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<td>7.1</td>
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<tr>
<td>7382A</td>
<td>20.0</td>
<td>11.3</td>
<td>163.0</td>
<td>11.1</td>
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<tr>
<td>7382B</td>
<td>21.4</td>
<td>6.5</td>
<td>12.6</td>
<td>9.6</td>
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<tr>
<td>7440</td>
<td>20.5</td>
<td>17.2</td>
<td>46.7</td>
<td>8.2</td>
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</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>UPN</th>
<th>ANC &gt;0.1</th>
<th>ANC &gt;0.5</th>
<th>ANC &gt;1.0</th>
<th>Platelet &gt;200</th>
<th>Day to Discharge</th>
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<td>18</td>
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<td></td>
</tr>
<tr>
<td>7440</td>
<td>10</td>
<td>11</td>
<td>13</td>
<td>11</td>
<td>14</td>
</tr>
</tbody>
</table>

*Median 11, 13, 14, 10, 7-13, 10-15, 11-16, 7-70.

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

* Engraftment characteristics of 12 patients transplanted with rhG-CSF—mobilized autologous PBMCs.

† Transplant was performed in the outpatient department and the patient was never admitted to the hospital.

days before the collection of granulocytes. Feldman et al. have also reported a lack of toxicity in 8 normal donors given rhG-CSF 5 \(\mu\text{g}/\text{day}\) before granulocyte collections. Although no acute toxicities of rhG-CSF were observed and no long-term consequences are expected, unusual late complications cannot be ruled out without further follow-up.

We previously reported that a mean of 7.3 \(\pm 4.3 \times 10^6\) CD34\(^+\) cells/kg and 20.5 \(\pm 28.1 \times 10^4\) CFU-GM/kg collected after rhG-CSF administration resulted in prompt and durable neutrophil and platelet engraftment in autologous recipients following marrow ablative therapy. In that study, all patients experienced neutrophil engraftment before day 15; however, two patients did not become platelet transfusion-independent until day +49 and day +70. These two patients had the lowest number of CD34\(^+\) cells collected: 0.5 and 1.6 \(\times 10^6\) CD34\(^+\) cells/kg, respectively. The lowest number of syngeneic CD34\(^+\) cells infused was 1.6 \(\times 10^6/\text{kg}\) but that was not associated with delayed platelet recovery. It is unclear whether the delayed platelet transfusion recovery observed after autologous PBMC infusion is a result of an inadequate number of stem cells collected, or whether the stem cells may have been damaged by previous chemoradiation. Further analysis concerning the predictive value of the number of CD34\(^+\) cells infused is ongoing.

The question of whether PBMCs mobilized by rhG-CSF will result in permanent engraftment can only be resolved by direct evaluation of allogeneic transplantation. In the absence of genetic markers, it remains unknown as to whether the infusion of autologous or syngeneic marrow or PBMCs contributes to long-term engraftment because residual host stem cells surviving the marrow ablative regimen could be responsible for long-term recovery after myeloablative therapy. It is unlikely that further follow-up of the current four syngeneic recipients will be of value concerning this question because we have not observed late graft failure in recipients of autologous PBMCs receiving the same myeloablative regimens.

Studies of PBMC transplants in animals suggest that PBMCs can provide for long-term multilineage hematopoiesis. Molineaux et al. performed sex-mismatched allogeneic transplants in mice with G-CSF-mobilized PBMCs. After 10 months of follow-up, they were able to show that donor cells were present in the blood, spleen, thymus, and bone marrow of recipient mice. Fliedner et al. and Storb et al. have shown that the cells present in allogeneic PBMC collections are capable of hematopoietic reconstitution in lethally irradiated dogs. In the Fliedner studies, one dog survived over 791 days with confirmed donor cells. These studies show that the cells present in PBMC collections can provide for multilineage lymphohematopoiesis in mice and dogs for 1 to 2 years.

Only one study of allogeneic PBMC transplant has been performed in a human. This patient experienced rapid tri-lineage engraftment confirmed to be of donor origin at 27 days post-transplant, but subsequently died of infection.

We have determined that the number of lymphocytes expressing the CD3 antigen present in PBMC collected after the administration of rhG-CSF is between 1.99 and 5.61 \(\times 10^9\), or approximately 22% of the TNCs collected. This represents an approximate 1 log increase in the number of T lymphocytes in PBMC collections compared with marrow, where 1 to 2 \(\times 10^9\) total T lymphocytes are typically collected. The average number of T lymphocytes collected in the pheresis product of two normal donors was 3.04 \(\times 10^9\) as measured by CD3\(^+\) 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 \(\times 10^9/\text{kg}\) (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 \(\times 10^9/\text{kg}\) of TMCs compared with 3 \(\times 10^8\) for allogeneic transplants. Based on this empirical ratio, the collection of 15.0 \(\times 10^9/\text{kg}\) 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—mobilized PBMC products are found to be associated with increased graft-versus-host disease.
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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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