Transplantation of Allogeneic Peripheral Blood Stem Cells Mobilized by Recombinant Human Granulocyte Colony-Stimulating Factor


Peripheral blood stem cells (PBSCs) are widely used in autologous transplantation because of ease of collection and rapid hematopoietic reconstitution. However, PBSCs have rarely been used for allogeneic transplantation because of concerns about donor toxicities from cytokine administration and the theoretical increased risk of graft-versus-host disease (GVHD) from the large number of T cells infused. Eight patients with advanced malignancies received allogeneic PBSC transplants from genotypically HLA-identical siblings. All donors received 5 days of recombinant human granulocyte colony-stimulating factor (rhG-CSF; 16 μg/kg/day) subcutaneously and were leukapheresed for 2 days. After treatment of the patient with total body irradiation and cyclophosphamide (n = 7) or etoposide, thiotepa, and cyclophosphamide (n = 1), PBSCs were infused immediately after collection and without modification. All patients received cyclophosphamide and either methotrexate (n = 6) or prednisone (n = 2) for GVHD prophylaxis. rhG-CSF was well tolerated with mild bone pain requiring acetaminophen occurring in two donors. All patients engrafted and in seven hematopoietic recovery was rapid, with 500 neutrophils/μL achieved by day 18 and 20,000 platelets/μL by day 12. Complete donor engraftment was documented by Y chromosome analysis in all four sex-mismatched donor-recipient pairs tested and by DNA analysis in two sex-matched pairs. One patient died on day 18 of veno-occlusive disease of the liver with engraftment but before chromosome analysis could be performed (results are pending in 1 patient). A second patient died of fungal infection 78 days after transplant. Grade 2 acute GVHD occurred in two patients and grade 3 GVHD occurred in one patient. One patient is 301 days from transplant in remission with chronic GVHD; the remaining five patients are alive and disease free 67 to 112 days after transplantation. Preliminary results indicate that allogeneic PBSCs mobilized by rhG-CSF can provide rapid hematologic recovery without an appreciably greater incidence of acute GVHD than would be expected with marrow. Further follow-up is required to determine the incidence of chronic GVHD and any potential beneficial effects on relapse after transplant.

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Table 1. Patient and Graft Characteristics

<table>
<thead>
<tr>
<th>UPN</th>
<th>Age (yr)/Sex</th>
<th>Disease</th>
<th>GVHD Prophylaxis</th>
<th>Day ANC &gt;500/μL</th>
<th>Day Platelets &gt;20,000/μL</th>
<th>Acute GVHD Grade</th>
<th>Days of Follow-Up</th>
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<tr>
<td>7962</td>
<td>38/M</td>
<td>CML-BC</td>
<td>CSA + Mtx</td>
<td>18</td>
<td>12</td>
<td>2</td>
<td>+301</td>
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<td>AML-rel</td>
<td>CSA + Mtx</td>
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<td>Wilms'</td>
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<td>CSA + Pred</td>
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<td>3</td>
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<tr>
<td>8849</td>
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<td>AML-rel</td>
<td>CSA + Mtx</td>
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<td>ALL-rel</td>
<td>CSA + Mtx</td>
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<td>10</td>
<td>0</td>
<td>18†</td>
</tr>
</tbody>
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Abbreviations: UPN, unique patient number; CML, chronic myelogenous leukemia—blast crisis; AML, acute myelogenous leukemia—relapse; Wilms', Wilms' tumor; HD, Hodgkin's disease; NHL, non-Hodgkin's lymphoma—relapse; ALL, acute lymphoblastic leukemia—relapse; CSA, cyclosporine; MTX, methotrexate; Pred, prednisone.

Table 2. Lymphocyte Subsets in Infused Cells

<table>
<thead>
<tr>
<th>UPN</th>
<th>CD3</th>
<th>CD4</th>
<th>CD8</th>
<th>CD56</th>
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<td>NA</td>
<td>NA</td>
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<td>557</td>
<td>433</td>
<td>119</td>
<td>234</td>
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</table>

Values are ×10⁶/kg recipient's body weight.

Abbreviation: NA, not available.

Table 2. Donor Mobilization

<table>
<thead>
<tr>
<th>UPN</th>
<th>Donor Age /yrl/sex</th>
<th>MNC/kg* (×10⁶)</th>
<th>CD34/kg* (×10⁶)</th>
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<td>47/M</td>
<td>12.8</td>
<td>21.6</td>
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<tr>
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<td>36/M</td>
<td>4.8</td>
<td>10.7</td>
</tr>
<tr>
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<td>12/M</td>
<td>9.6</td>
<td>10.5</td>
</tr>
<tr>
<td>8769</td>
<td>43/F</td>
<td>12.1</td>
<td>15.6</td>
</tr>
<tr>
<td>8849</td>
<td>29/M</td>
<td>6.0</td>
<td>10.6</td>
</tr>
<tr>
<td>8889</td>
<td>33/F</td>
<td>7.2</td>
<td>20.2</td>
</tr>
<tr>
<td>8756</td>
<td>30/M</td>
<td>16.7</td>
<td>21.0</td>
</tr>
<tr>
<td>8937</td>
<td>39/M</td>
<td>11.7</td>
<td>6.8</td>
</tr>
</tbody>
</table>

Abbreviation: MNC, mononuclear cells.

* Recipient body weight.

RESULTS

Table 1 summarizes patient characteristics, GVHD prophylaxis, engraftment, acute GVHD, and follow-up. All patients had advanced malignancies and had failed conventional therapy. None had received a previous marrow or PBSC transplant.

Donors tolerated the rhG-CSF well, with bone pain relieved with acetaminophen as the only side effect in two donors. No donor had to discontinue rhG-CSF because of symptoms. PBSBC collections were performed on days 5 and 6 after initiation of rhG-CSF administration. With 10 to 12 L of whole blood processed per collection. Collection data are presented in Tables 2 and 3. A median of 10.6 (range, 4.8 to 16.7) × 10⁶ mononuclear cells/kg recipient's weight were collected yielding a median of 13.1 (range, 6.9 to 21.6) × 10⁶ cells/kg recipient's weight. The PBSC contained a median of 385 × 10⁶ CD34+ cells/kg recipient's weight. The PBSC was infused intravenously to recipients without incident immediately after each collection. Engraftment was prompt in seven of eight patients who recovered to an absolute neutrophil count (ANC) greater than 500/μL by days 9 to 18. A platelet count greater than 20,000/μL unmaintained by transfusion was achieved by days 10 and 12. There were no secondary graft failures. Patient UPN 8747, who had a previous nephrectomy, developed renal failure on day 7 and required dialysis. She had high plasma methotrexate levels on day 9 and did not receive further methotrexate. On day 21, she had an ANC of 200/μL and began rhG-CSF therapy. The ANC increased...
rapidly to 500/μL on day 22 and to 1,000/μL on day 23 and she became temporarily platelet independent on day 42. Subsequently, she developed pulmonary hemorrhage requiring continued platelet support after day 50 and eventually died of disseminated aspergillus on day 73. Marrow examinations showed normal cellularity, with 98.5% donor cells by Y chromosome analysis.

Donor engraftment was documented by in situ Y probe cytogenetics in four patients (blood for 1 patient and marrow for 3 patients) on days 30 to 80 posttransplant. One patient died on day 18 of veno-occlusive disease of the liver with normal peripheral counts but before cytogenetics were performed. VNTR analysis of marrow from patient UPN 7962 and blood from patient UPN 8781 performed 80 days after transplant indicated greater than 99% donor-specific bands. Results from the one sex-matched pair are pending at this time.

Acute GVHD grade 2 to 3 developed in three patients. Patient UPN 8769 developed grade 3 skin GVHD on day 26 without gastrointestinal or liver involvement. He was unable to tolerate cyclosporine because of renal insufficiency and GVHD occurred while on steroids alone. His skin GVHD cleared quickly with antithymocyte globulin (ATG). The other two patients with grade 2 GVHD responded to initial therapy with steroids or ATG. One patient developed biopsy proven grade 1 gastrointestinal GVHD 30 days after transplant and responded quickly to oral beclomethasone.

Two patients, UPN 7962 and UPN 8781, are beyond 100 days from transplant. Patient UPN 7962 remains in remission and the cyclosporine dosage was tapered off 245 days after transplant. He developed chronic GVHD of the skin and liver on day 260 and was treated with cyclosporine and prednisone with a good response.

DISCUSSION

In this study, we have shown that rhG-CSF–mobilized PBSCs are able to provide rapid engraftment in allogeneic recipients and do not lead to an intolerable increment in severe acute GVHD. Patients engrafted quickly, except for 1 patient with renal insufficiency who probably had marrow toxic levels of methotrexate that may have affected the tempo of engraftment. The prompt engraftment observed in seven patients was probably caused by the relatively high numbers of CD34+ cells infused. We elected to use 16 μg/kg of rhG-CSF for mobilization of PBSCs because of our previous experience in the autologous and syngeneic setting showing that larger numbers of CD34+ cells can be collected using higher doses of rhG-CSF.1,11 rhG-CSF was well tolerated by the donors and allowed sufficient numbers of cells to be collected in only two leukapheresis. The minimum number of allogeneic CD34+ cells necessary to achieve consistent sustained engraftment using HLA-matched siblings is unknown. However, it has been estimated that 2.5 to 5.0 × 10^6 CD34+ cells/kg are necessary for consistently rapid engraftment in the autologous setting.11,12 By extrapolation from marrow transplant studies one would anticipate that consistent allografts would require three times the minimum autograft dose, i.e., 5 to 15 × 10^6 CD34+ cells/kg. However, these estimates may not be relevant when considering PBSC allografts because the large number of lymphocytes infused may enhance engraftment by the elimination of host lymphocytes. Only further studies comparing different cell doses will resolve this issue.

In one previous study, a patient received a series of 10 Buffy coat infusions that were T-cell depleted after collection from a donor who did not receive a growth factor.13 Although neutrophils recovered by day 11 and a marrow examination showed trilineage engraftment on day 27, death from infection occurred on day 32 and platelet engraftment and GVHD could not be evaluated. Dreger et al14 infused rhG-CSF–mobilized allogeneic PBSCs for graft failure without reconditioning in a patient who had molecular evidence of donor engraftment.14 That 47-year-old patient developed normal hematopoeisis and grade 2 GVHD.

PBSCs are rapidly replacing marrow as the preferred source of autologous hematopoietic stem cells after myeloablative therapy for malignant disease because of the advantages of more rapid hematologic recovery and ease of collection. However, concerns about exposing normal donors to colony-stimulating factors and the theoretical potential for severe GVHD due to the large numbers of T cells infused have generated caution among groups considering such an approach for allogeneic transplantation. The concerns regarding GVHD have been raised because of previous experience demonstrating that unmodified Buffy coat infusions added to marrow resulted in an increased incidence of chronic but not acute GVHD in patients with aplastic anemia receiving cyclosporine plus methotrexate.15 Patients with high-risk leukemia receiving Buffy coat in addition to marrow and methotrexate only for GVHD prophylaxis had an increased incidence of acute GVHD.16

In the current study, although patients received approximately 1.7 log greater numbers of CD3, CD4, and CD8 cells compared with marrow,17 acute GVHD did not develop in three of seven evaluable patients. Three other patients developed mild to moderate (grade 1 to 2) GVHD and responded quickly to first-line therapy. Only one patient developed grade 3 acute GVHD and responded to ATG. The reasons for the apparent lack of severe GVHD are presently unknown. The combination of cyclosporine and methotrexate could be effective in suppressing acute GVHD despite the large number of lymphocytes infused. A low incidence of acute GVHD (20%) was seen in patients with aplastic anemia receiving marrow and unmodified Buffy coat cells followed by cyclosporine and methotrexate.12 However, these patients developed a high incidence of chronic GVHD (72%) after tapering of the cyclosporine dose. In another study, patients with advanced leukemia receiving marrow and Buffy coats and immunosuppression with methotrexate alone had a high incidence of acute GVHD.16 Additionally, rhG-CSF may alter the function of lymphocytes collected in PBSCs, making them unable to respond to host antigens.

We have measured daily peripheral blood T-cell subsets in patients receiving high-dose rhG-CSF and found no major difference in the absolute numbers of CD3, CD4, and CD8 cells found in the peripheral blood as compared with baseline.7 Thus, the number and composition of T cells infused will depend on the number of collections performed. In one
recent series, five patients received marrow plus rhG-CSF–
mobilized PBSCs using posttransplant cyclosporine and
prednisone. Engraftment was rapid in four of five patients
and grade 2 acute GVHD developed in three of five patients,
with three developing extensive chronic GVHD after day
100. One patient in the present study developed chronic
GVHD when immunosuppression was discontinued and the
other five are too early to evaluate.

Only two previous patients have been reported who re-
ceived rhG-CSF–mobilized PBSCs as the initial source of
allogeneic stem cells for transplant.5,4 Those patients re-
ceived 2 and 5 PBSC infusions after PBSCs were mobilized
with 10 and 3 μg/kg rhG-CSF, respectively. The donors
tolerated the drug and procedures well. Engraftment tempo
was similar to or slower than that observed with marrow.
Acute GVHD was not observed in one patient and was mod-
e rate (grade 2) in the other. Chronic GVHD did not develop
in one patient with a follow-up of more than 1 year, whereas
the other died of relapse on day 102. Other studies using
allogeneic PBSCs are in progress.19

These preliminary data indicate that the likelihood of de-
veloping severe acute GVHD after transplantation of unmod-
ified, allogeneic PBSCs from HLA-identical donors mobil-
ized with rhG-CSF is probably not significantly greater than
with marrow. However, a larger series of patients will have
to be observed to determine the probability of developing
chronic GVHD and to measure whether the benefits of early
engraftment outweigh any adverse effects. It will be of major
interest to determine the effect, if any, of infusing large
numbers of lymphocytes on chronic GVHD and posttrans-
plant relapses. In patients at high risk of relapse, the develop-
ment of chronic GVHD could have a beneficial effect.

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REFERENCES

Transplantation of allogeneic peripheral blood stem cells mobilized by recombinant human granulocyte colony-stimulating factor [see comments]

WJ Bensinger, CH Weaver, FR Appelbaum, S Rowley, T Demirer, J Sanders, R Storb and CD Buckner