To the Editor:

We have previously shown in normal donors that administration of either recombinant human granulocyte-macrophage colony-stimulating factor (GM-CSF) (Immunex, Seattle, WA) at 10 μg/kg/d, or granulocyte colony-stimulating factor (G-CSF) (Amgen, Thousand Oaks, CA) at 10 μg/kg/d, or a combination of G-CSF and GM-CSF at 5 μg/kg/d each, administered subcutaneously for 4 days and followed by leukapheresis on day 5 will enable mobilization and collection of sufficient hematopoietic progenitor cells to perform allogeneic transplantation. We also showed that these cells can then be selectively enriched by immunoselection of CD34⁺ progenitors by affinity labeling using dynal paramagnetic microspheres (Baxter Isolex™ 300 System; Baxter Healthcare Corp, Immunotherapy Division, Irvine, CA) and that the positive selection technique would remove up to 3 logs of T cells, which might be sufficient to reduce the incidence and severity of acute graft-versus-host disease (GVHD). We have successfully used this methodology to obtain...
selected CD34+ cells for allogeneic transplant from HLA fully matched sibling donors for allogeneic transplant in two patients with chemoresistant advanced-stage (with bone marrow [BM] involvement) intermediate-grade non-Hodgkin's lymphoma.

CASE REPORTS

Patients were conditioned with high-dose cyclophosphamide (120 mg/kg administered over 2 days) and fractionated total body irradiation (total dose, 14 Gy, hyperfractionated over 4 days in 2 Gy fractions). Cyclosporine (Sandoz, East Hanover, NJ) was given in standard dose commencing at 2 mg/kg intravenously every 12 hours before transplant from day −4 and adjusted to maintain therapeutic blood levels as prophylaxis against GVHD. G-CSF 5 μg/kg/d was administered subcutaneously from day 1 posttransplant to accelerate granulocyte recovery. Donor A initially failed to mobilize adequately with G-CSF (10 μg/kg/d for 5 days before and during apework) alone; consequently, G-CSF and GM-CSF were administered sequentially for mobilization of both donors. GM-CSF (10 μg/kg/d subcutaneously) was given for 3 days followed by G-CSF (10 μg/kg/d subcutaneously) for the next 2 days, after which time apheresis was started (day 5). G-CSF was continued until the day before the final apheresis. Peripheral blood progenitor cell (PBPC) collection characteristics and CD34+ selection results are shown in Table 1. More than 4 × 10^6 CD34+ cells/kg recipient body weight remained after immunomagnetic selection of CD34+ cells, which were cryopreserved for transplant. Data for CD3+ and natural killer (NK) cells were available for collections from donor B and showed a 99.99% (4-log) depletion for both cell types after CD34+ selection. The total of CD3+ cells infused with the enriched CD34+ cells to patient B was 0.3 × 10^9/kg recipient body weight. Precise CD3+ and NK cell data were not available for patient A's graft, but approximately 3 × 10^9 CD3+ cells/kg recipient body weight were infused.

Engraftment of both patients was as rapid as is usually observed with unmanipulated PBPCs in allogeneic or syngeneic transplant, with no more toxicity than the former. Engraftment has been complete and sustained in both patients (273 and 126 days posttransplant, respectively, as of this writing). (Table 2 with patient data were not available for patient A's graft, but approximately 3 × 10^9 CD3+ cells/kg recipient body weight were infused.

Engraftment of both patients was as rapid as is usually observed when using unmanipulated PBPCs in allogeneic or syngeneic transplant, with no more toxicity than the former. Engraftment has been complete and sustained in both patients (273 and 126 days posttransplant, respectively, as of this writing). (Table 2 with patient and donor characteristics). Both patients were discharged from the hospital within 14 days of transplant. DNA fingerprinting using PCR analysis of short tandem repeats showed complete donor chimera in both patients by days 14 and 9, respectively. Both patients developed acute GVHD. Patient A had grade 2 acute GVHD with skin and upper gastrointestinal tract involvement proven by biopsy, and patient B had grade 1 skin acute GVHD. Both patients responded to steroid therapy. Both patients have achieved complete remission of their lymphomas as determined by full restaging, including BM examination. Patient A had BCL-2 gene rearrangement detectable in his marrow by polymerase chain reaction before transplant, however this marker was no longer detectable 67 days posttransplant.

COMMENTARY

Recent reports support the conclusion that unmanipulated PBPCs are superior to cytotoxic PBPCs in syngeneic transplantation. This is one of the first reports of successful allogeneic donors mobilized with cytokines to provide sustained engraftment after transplantation. 2,5 This is one of the first reports of successful allogeneic donors mobilized with cytokines to provide sustained engraftment after transplantation. 2,5 This is one of the first reports of successful allogeneic donors mobilized with cytokines to provide sustained engraftment after transplantation. 2,5 This is one of the first reports of successful allogeneic donors mobilized with cytokines to provide sustained engraftment after transplantation. 2,5 This is one of the first reports of successful allogeneic donors mobilized with cytokines to provide sustained engraftment after transplantation. 2,5 This is one of the first reports of successful allogeneic donors mobilized with cytokines to provide sustained engraftment after transplantation. 2,5 This is one of the first reports of successful allogeneic donors mobilized with cytokines to provide 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Table 1. PBPC Collection Characteristics

<table>
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<tr>
<th>Phenex No.</th>
<th>Total Nucleated Cells</th>
<th>Pre (10^6)</th>
<th>Post (10^6)</th>
<th>%</th>
<th>CD34+</th>
<th>Pre (10^6)</th>
<th>Post (10^6)</th>
<th>Yield</th>
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*G-CSF was administered alone for mobilization.
† This pheresis product was not infused into the recipient.
associated with cytokine mobilization, which was consistent with our previous experience with normal donors. Venous access was a problem with donor B, and necessitated temporary placement of a central venous apheresis catheter.

Although they received T-cell-depleted grafts and were immune-suppressed with cyclosporine, both patients had mild (grade 1) or moderate (grade 2) acute GVHD. However, it has been shown that as few as 1 x 10^7 donor T cells/kg recipient body weight in the marrow graft in patients receiving a short course of cyclosporine posttransplant is sufficient to cause grade 1 or 2 acute GVHD. It is uncertain at this time whether CD34+ positive selection is comparable with other forms of T-cell depletion (TCD) in preventing GVHD. Although it has been postulated from animal studies that accessory cells may be required to facilitate engraftment and to suppress GVHD, both patients had mild (grade 1) or moderate (grade 2) acute GVHD. However, it has been shown that with an adequate conditioning regimen, allogeneic transplantation with enriched CD34+ PBSCs from fully matched sibling cytokine-mobilized donors is both feasible and safe. Depletion of NK cells may be disadvantageous because NK cells may contribute to graft-versus-leukemia effects, however the overall importance of this effect remains unclear. Finally, CD34+ PBPC selection may not only reduce the incidence and severity of GVHD, but may be useful for matched unrelated donor transplants and in the future for other types of graft engineering including gene transduction and adoptive immunotherapy with selective donor T-cell expansion and infusion. Further study is required.

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Robert E.T. Corrgham
Anthony D. Ho
Departments of Medicine and Patholgy
University of California at San Diego
School of Medicine
San Diego, CA
and Baxter Healthcare Corporation
Immunotherapy Division
Irvine, CA

REFERENCES


Bell's palsy as a sign of Burkitt's lymphoma in children [letter]

J Katz, A Polliack, I Harushouski, R Ben Oliel and Y Marmary

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