Harvesting and Enrichment of Hematopoietic Progenitor Cells Mobilized Into the Peripheral Blood of Normal Donors by Granulocyte-Macrophage Colony-Stimulating Factor (GM-CSF) or G-CSF: Potential Role in Allogeneic Marrow Transplantation

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To explore the use of stem/progenitor cells from peripheral blood (PB) for allogeneic transplantation, we have studied the mobilization of progenitor cells in normal donors by growth factors. Normal subjects were administered either granulocyte-macrophage colony-stimulating factor (GM-CSF) at 10 μg/kg/d, or G-CSF at 10 μg/kg/d, or a combination of G- and GM-CSF at 5 μg/kg/d each, administered subcutaneously for 4 days, followed by leukapheresis on day 5. Mononuclear cells expressing CD34 (CD34+ cells) were selectively enriched by affinity labeling using Dynal paramagnetic microspheres (Baxter Isolab; Baxter Healthcare Corp, Santa Ana, CA). The baseline CD34+ cells in peripheral blood before mobilization was 0.07% ± 0.05% (1.6 ± 0.7/μL; n = 18). On the fifth day (24 hours after the fourth dose), the CD34+ cells were 0.99% ± 0.40% (61 ± 14/μL) for the 8 subjects treated with G-CSF, 0.25% ± 0.25% (3 ± 3/μL, both P < .01 v G-CSF) for the 5 subjects administered GM-CSF, and for the 5 subjects treated with G- and GM-CSF, 0.65% ± 0.28% (41 ± 18/μL, P < .5 v GM-CSF). Parallel to this increase in CD34+ cells, clonogenic assays showed a corresponding increase in CFU-GM and BFU-E. The total number of CD34+ cells collected from the G-CSF group during a 3-hour apheresis was 119 ± 65 × 10⁶ and was not significantly different from that collected from the group treated with G- and GM-CSF (101 ± 35 × 10⁶ cells), but both were greater than that from the group treated with GM-CSF (12.6 ± 6.1 × 10⁶; P < .01 for both comparisons). Analysis of the CD34+ subsets showed that a significantly higher percentage of cells with the CD34'+CD38' phenotype is found after mobilization with G- and GM-CSF. In the G-CSF group, immunomagnetic selection of CD34+ cells permitted the enrichment of the CD34+ cells in the apheresis product to 81% ± 11%, with a 48% ± 12% yield and to a purity of 77% ± 21% with a 51% ± 15% recovery in the G- and GM-CSF group. T cells were depleted from a mean of 4.5 ± 2.0 × 10⁶ to 4.3 ± 5.2 × 10⁶ after selection, representing 99.9% depletion. We conclude that it is feasible to collect sufficient numbers of PB progenitor cells from normal donors with one to two leukapheresis procedures for allogeneic transplantation. Subjects treated with the combination of G- and GM-CSF showed an equivalent mobilization of CD34+ cells and CFU-GM as G-CSF alone, and also demonstrated a significantly greater mobilization of cells with the CD34'+CD38' phenotype.

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Submitted June 10, 1994; accepted September 2, 1994.

Supported in part by University of California, San Diego (UCSD), Cancer Center Foundation; Baxter Healthcare Corporation, Immunotherapy Group, Santa Ana, CA; Cancer Research Coordinating Committee of University of California (UC), Grant No. 6-800575-441775-19900; Academic Senate of UC Award No. R5159M-Ho; Clinical Research Program, Grant No. M01 RR00827, of the National Center for Research Resources, National Institutes of Health.

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0006-4971/95/8501-0019$3.00/0


275
cells, which may increase the risk of graft-versus-host disease (GVHD), we also investigated the performance of a device designed to enrich CD34+ progenitor cells by immunomagnetic separation. Our results show that, using G-CSF or G-CSF combined with GM-CSF, sufficient numbers of progenitor cells can be mobilized and collected from the PB of normal individuals to perform or support allogeneic bone marrow transplantation (BMT) by one to maximally two leukapheresis procedures. The enrichment process also depletes the harvested product of sufficient numbers of T cells to diminish the likelihood of severe GVHD.

MATERIALS AND METHODS

Normal, healthy subjects were recruited using exclusion criteria similar to those used for blood donors.29 All subjects had a negative serologic test for hepatitis B and C, human immunodeficiency virus, human T-cell lymphotropic virus I/II (HTLV-I/II), and syphilis (RPR) and all had a normal complete blood count, including platelets and leukocyte differential (data not shown). Consent was obtained after careful explanation of the project. Subjects were given either recombinant human (rh) G-CSF (Amgen, Thousand Oaks, CA), or rhGM-CSF (Immunex, Seattle, WA), 10 μg/kg/d, or G-CSF plus GM-CSF, 5 μg/kg/d each, subcutaneously for 4 consecutive days. Injections were given between 8:00 AM and 10:00 AM. The project was reviewed and approved by the UCSD Human Subjects Committee.

A 10-L leukapheresis procedure was performed 24 hours after the fourth dose of cytokine using a Fenwal CS3000 (Deerfield, IL) or COBE Spectra instrument (COBE, Lakewood, CO). Acid-citrate-dextrose (ACD-A; Baxter Healthcare Corp, Santa Ana, CA) was kept at room temperature (22 to 24°C) and transported to the cell processing laboratory within an hour after collection.

PB counts, flow cytometry, and clonogenic assays were performed before the first injection of cytokine, daily for 4 days during growth factor administration, on the day of progenitor cell harvesting, and 24 hours after apheresis. Blood counts were performed in the routine laboratory using an automated cell counter (Coulter, Hialeah, FL) and leukocyte differential counts were performed manually on all blood samples with a leukocyte count greater than 10,000/μL. Mononuclear cells were prepared from EDTA-anticoagulated peripheral blood samples using Percoll (Sigma, St Louis, MO) density gradient separation.

Flow cytometry. Flow cytometry was performed on daily blood samples. Leukocytes in whole blood were stained at room temperature with appropriate antibodies for 20 minutes (fluorescein isothiocyanate- and phycoerythrin-labeled monoclonal antibodies [MoAbs] against CD3, CD5, CD14, CD19, CD33, CD34, CD45, or negative controls). Erythrocytes were lysed and leukocytes fixed with FACS Lyse (Becton Dickinson [BD] Immunocytometry Systems, San Jose, CA) according to instruction of the manufacturer. All MoAbs were obtained from BD unless otherwise noted. Two-color, four-paramater flow cytometric analysis was performed with a FACScan (BD) as previously described.30 Mononuclear cells were gated by forward- and side-scatter signals. Anti-CD45 was used to verify that all of the mononuclear cells were included in this gate. T cells are identified by the expression of CD3 and/or CD5, and B cells by CD19. At least 40,000 events were gated and data were analyzed using Lysis-II software (BD). The stained cell samples were analyzed within 72 hours after preparation.

Analysis of CD34+ subsets was performed on the leukapheresis product before and after enrichment of CD34+ cells, using antibodies to CD38, HLA-DR, CD15, and CD33. For CD34+ cell subsets analysis, the leukapheresis products, and CD34+ cell-enriched samples were lysed with NH4Cl buffer, washed, the MoAbs added at the appropriate dilutions, then incubated on ice for 30 minutes in the dark.30,32,33 Cells were washed twice, fixed with phosphate buffer containing 0.5% paraformaldehyde, and stored at 4°C protected from light. Three-color, five-parameter flow cytometric analysis was performed using a FACScan. At least 1 × 10^6 cells were analyzed and events were recorded in list mode first un gated to quantitate the relative frequencies of major cell types. The samples were reacquired using live gates with forward scatter, side scatter, and CD34 positivity as gating criteria. Data analysis was performed using Paint-a-Gate (BD), which permits transformation of orthogonal light-scattering signals and the identification of cell populations with multidimensional analysis.31

Clonogenic assays. Clonogenic assays for granulocyte-macrophage colony-forming unit (CFU-GM), erythroid burst-forming unit (BFU-E), and mixed CFU (CFU-GEMM) were performed as previously described30 using a methylcellulose assay system. Briefly, 1 × 10^6 mononuclear cells or 1 × 10^6 enriched CD34+ cells were aliquoted on duplicate 35-mm dishes containing Iscove's Modified Dulbecco's Medium (IMDM; GIBCO/BRL, Grand Island, NY) with 1% methylcellulose plates; erythropoietin 2.5 U/mL (Amgen, Thousand Oaks, CA); progenitor cell factor 150 ng/mL (Genzyme, Cambridge, MA); interleukin-3, 150 U/mL (Genetics Institute, Cambridge, MA); and GM-CSF, 50 ng/mL (Immunex, Seattle, WA). The dishes were placed in a humidified 5% CO2 incubator and clusters of greater than 50 cells were enumerated after 14 days.

Immunomagnetic selection of CD34+ cells. Cells expressing the CD34 antigen were enriched from leukapheresis products using the Baxter Isolex system.34 The principle of this method is as follows: CD34+ cells were enriched by affinity labeling using a murine MoAb (9C5; Baxter Immunotherapy Division, Santa Ana, CA) and subsequently with paramagnetic microspheres coated with goat-antimouse antibodies. The CD34+ cells were then captured by a magnet. The paramagnetic beads were released from the cells by treatment with chymopapain.

The platelet contents in the leukapheresis product were initially reduced by washing in RPMI (GIBCO; Grand Island, NY) containing 1% human serum albumin (HSA; Baxter/Hyland, Glendale, CA) (RPMI/HSA) twice at 200g for 10 minutes at ambient temperature. After the final wash, the cells were resuspended in RPMI/HSA containing 0.5% human Ig (Hig) (Baxter/Hyland) or other equivalent lg preparation. Nuclear cell concentration was measured using a Coulter Counter. The cells were sensitized with an anti-CD34 antibody (9C5; Baxter Immunotherapy) at 0.5 μg/1 × 10^6 cells for 30 minutes at 4°C under slow (~4 rpm) end-over-end rotation. Unbound antibody was removed by washing (three times at 400g, 10 minutes in cold), and the cells were resuspended in RPMI/HSA. Sensitized cells were resotted with Dynal paramagnetic microspheres (coated with sheep-antimouse IgG1 Fe) (Baxter/Fenwal, Deerfield, IL) at 2 cells/bead in a magnetic separation device for 30 minutes at ambient temperature at 1 to 5 × 10^6 cells/mL of RPMI/HSA/HIG. The device consists of a mixing chamber with integral inlet and outlet tubing for the introduction and removal of cells, a motor rocking/mixing for sensitized cells and beads, primary magnets for the capture of beads/rosettes, and secondary magnet for maximal entrapment of beads after selection and release. A microprocessor was incorporated into the device, which prompted the operator during each step of the selection procedure. After the conclusion of cell/bead mixing, the beads/rosettes were washed three times using 100 mL of RPMI/HSA to remove trapped nontarget cells, and resuspended in 40 mL of RPMI/HSA. Rosetted cells were released from the beads using chymopapain (prepared for Baxter Immunotherapy by Boots Pharmaceutical, Nottingham, UK)35,36 at 200 picokatal/mL for 15 minutes at room temperature under rocking/mixing, as above. The beads

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MOBILIZATION OF CD34 CELLS FROM NORMAL DONORS

were captured on the primary magnets. The released cells were passed over the secondary magnet to ensure maximal bead capture, washed, and resuspended in RPMI/HSA. Nucleated cell concentration was determined by Coulter Counter and the expression of CD34 and other leukocyte markers by flow cytometry.

Statistical analysis. Data are reported as the mean ± SD unless otherwise noted. Differences between mean values were evaluated for statistical significance by analysis of variance (ANOVA) and a two-tailed unpaired Student’s t-test, where appropriate, and were calculated on a personal computer using NCSS software (NCSS, Inc, Provo, UT).

RESULTS

Eighteen subjects were studied. Their ages ranged from 21 to 45 years (median age = 28), 8 were men and 10 were women. Eight received G-CSF, five received GM-CSF, and five received the combination of G-CSF and GM-CSF.

Baseline blood counts and leukocyte differentials of all subjects were within normal limits (data not shown). The administration of G-CSF and GM-CSF increased the leukocyte count and number of mononuclear cells in the PB (Fig 1, A and B). Both G-CSF and GM-CSF increased the leukocyte counts of the volunteers; however, G-CSF increased the total leukocyte count as well as the mononuclear cell count significantly more than an equal dose of GM-CSF. Injections of 5 μg/kg each of the combination of G-CSF and GM-CSF gave results intermediate between injections of 10 μg/kg of G-CSF or 10 μg/kg of GM-CSF. No significant change in platelet counts or hemoglobin was observed.

Side effects associated with the injections of cytokines were minor, consisting chiefly of myalgia and fatigue in all subjects. The side effects were mild and were partially or completely ameliorated in all subjects by acetaminophen. All subjects administered G-CSF and GM-CSF completed the full 4 days of therapy and the apheresis procedure. One of four subjects given the combination of G-CSF and GM-CSF (at reduced dosage) elected to discontinue the study because of myalgia and low-grade temperature.

Before cytokine administration the CD34+ cells represented 0.07% ± 0.05% of the peripheral blood mononuclear cells (1.6 ± 0.7 cells/μL; n = 18). This value was not different among the three treatment groups. After administration of the first dose of either G-CSF, GM-CSF, or the combination of G-CSF + GM-CSF there was a progressive increase in the number of CD34+ cells in the PB (Fig 2). These increases were significantly greater in subjects treated with G-CSF or G-CSF + GM-CSF than in those administered GM-CSF alone. There were significant differences in absolute CD34+ cells per microliter between subjects given G-CSF versus G-CSF + GM-CSF only on days 4 and +1 after cytokine administration. Numbers of circulating CD34+ cells started diminishing by 48 hours after the fourth dose of the cytokines.

Fig 1. Effect of cytokine administration on the leukocyte count (A) and mononuclear cell count (B). GM-CSF (Ⅲ, n = 5), G-CSF + GM-CSF (Ⅳ, n = 5), or G-CSF (Ⅴ, n = 8) were administered after blood was drawn on days 1, 2, 3, and 4.

Fig 2. Effect of cytokine administration on the number of CD34+ cells per microliter. GM-CSF (Ⅲ, n = 5), G-CSF + GM-CSF (Ⅳ, n = 5), or G-CSF (Ⅴ, n = 8) were administered after blood was drawn on days 1, 2, 3, and 4.
tered after blood was drawn on days 1, 2, 3, and 4. An instrument malfunction on one). Subjects in all three groups had similar yields of leukocytes (G-CSF = 1.9 ± 0.6 x 10^6, n = 7; GM-CSF = 1.4 ± 0.2 x 10^6, n = 4; G-CSF + GM-CSF = 2.0 ± 0.8 x 10^6, n = 4) and mononuclear cells (G-CSF = 1.7 ± 0.5 x 10^6; GM-CSF = 1.2 ± 0.1 x 10^6; G-CSF + GM-CSF = 1.8 ± 0.6 x 10^6). In contrast, the overall yield of CD34+ mononuclear cells in the leukapheresis product from subjects treated with G-CSF (119 ± 65 x 10^6 cells, n = 7) or with the combination of G-CSF + GM-CSF (101 ± 35 x 10^6 cells, n = 4) was significantly greater than that from subjects treated with GM-CSF (12.6 ± 6.1 x 10^6 CD34+ cells, n = 4, P < .05 for both comparisons) (Fig 4).

Subset analysis of CD34+ cells was performed in 10 of the leukapheresis products (4 mobilized with G-CSF, 3 with GM-CSF, and 3 with G-CSF + GM-CSF). The apheresis products from subjects treated with GM-CSF or G-CSF + GM-CSF contained a significantly higher percentage of CD34+ cells that coexpressed HLA-DR but did not coexpress CD38 (CD34+/HLA-DR+/CD38−) compared with those administered G-CSF (Table 1). When converted to absolute numbers, the combination of G-CSF + GM-CSF generated the highest amount of CD34+/HLA-DR+/CD38− cells in the leukapheresis products (1.41 ± 0.08 x 10^7) versus 0.36 ± 0.21 x 10^6 with G-CSF alone and 0.12 ± 0.05 x 10^6 with GM-CSF (P < .05 in all comparisons). There was no significant difference between subjects treated with G-CSF versus GM-CSF with respect to the percentage of CD34+/HLA-DR+/CD38− cells.

Corresponding to the total number of CD34+ cells collected, the number of CFU-GM per leukapheresis was also higher in the G-CSF-mobilized products (Table 2). Interestingly, in subjects treated with G-CSF, the plating efficiencies for CFU-GM (based on the content of CD34+ cells) were significantly lower than the plating efficiencies of clonogenic cells collected from subjects treated with GM-CSF (Table 3). A similar, but statistically insignificant, trend was noted for CFU-GM (P = .08 v GM-CSF). The plating efficiency of BFU-E in subjects treated with G-CSF + GM-CSF was significantly lower than that in subjects treated with either G-CSF or GM-CSF alone.

Table 1. CD34+ Cell Subsets in Leukapheresis Harvests From Normal Donors Treated With G-CSF and GM-CSF

<table>
<thead>
<tr>
<th>Subsets</th>
<th>G-CSF (n = 4)</th>
<th>GM-CSF (n = 4)</th>
<th>G-CSF + GM-CSF (n = 4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD34+/CD38−</td>
<td>1.19 ± 0.33</td>
<td>0.24 ± 0.22*</td>
<td>0.34 ± 0.18</td>
</tr>
<tr>
<td>CD34+/CD38+</td>
<td>0.81 ± 0.22</td>
<td>4.42 ± 3.40</td>
<td>4.73 ± 2.72*</td>
</tr>
<tr>
<td>CD34+/HLADR−</td>
<td>20.7 ± 6.9</td>
<td>20.3 ± 2.9</td>
<td>24.0 ± 5.3</td>
</tr>
<tr>
<td>CD34+/HLADR+/CD38−</td>
<td>0.37 ± 0.19</td>
<td>1.10 ± 0.22*</td>
<td>1.86 ± 0.34*</td>
</tr>
</tbody>
</table>

* P < .05 v G-CSF.

Table 2. Colony-Forming Potential of Cells Collected by Leukapheresis

<table>
<thead>
<tr>
<th>Total Colonies Collected (G-CSF, n = 7)</th>
<th>(GM-CSF, n = 4)</th>
<th>(G-CSF + GM-CSF, n = 4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CFU-GM</td>
<td>11.8 ± 9.4</td>
<td>3.2 ± 2.3*</td>
</tr>
<tr>
<td>BFU-E</td>
<td>30.3 ± 15.3</td>
<td>16.2 ± 17.1</td>
</tr>
<tr>
<td>CFU-GM</td>
<td>1.5 ± 0.7</td>
<td>0.9 ± 1.1</td>
</tr>
<tr>
<td>(CD34+ x 10^6)</td>
<td>119 ± 65</td>
<td>12.6 ± 6.1*</td>
</tr>
</tbody>
</table>

* P < .05 v G-CSF and G-CSF + GM-CSF.

1 P < .05 v G-CSF + GM-CSF.
Before the enrichment of CD34+ cells, the percentages of CD34+ cells in the G-CSF, GM-CSF, and G-CSF + GM-CSF mobilized products were 1.2% ± 0.4%, 0.2% ± 0.1%, and 0.6% ± 0.3%, respectively. The purity after enrichment was 81% ± 11% for G-CSF (n = 6), 22% ± 7% for GM-CSF (n = 3), and 77% ± 21% for the combination (n = 3), whereas the recovery was 48% ± 12%, 79% ± 32% and 51% ± 15%, respectively. Therefore, the enrichment of CD34+ cells was 73- ± 31-fold for the group treated with G-CSF, 138- ± 36-fold for GM-CSF, and 156- ± 86-fold for GM-CSF + GM-CSF. The percentages of T and B cells as characterized by CD34, CD3, and CD19 before and after enrichment process are shown in Table 4. The total numbers of T and B cells in each leukapheresis product were reduced to approximately 0.1% of the original. Correspondingly, the clonogenic cells were also enriched after the selection process (Table 5).

**DISCUSSION**

Recent reports have produced encouraging data to support the use of allogeneic hematopoietic transplantation with the supplementary or exclusive use of blood-derived PCs. Several potential advantages are associated with the use of blood-derived PCs versus marrow-derived cells. First, this technique is likely to enhance donor acceptance and eliminates the cost and side effects of general anesthesia. Second, the use of PCs mobilized into the PB might be associated with accelerated hematopoietic reconstitution, as extensively shown in the autologous setting. However, blood-derived PC preparations contain a massive number of T cells that might aggravate the development of GHVD.

**Table 4. Effect of Enrichment on Lymphocyte Subsets in Leukapheresis Harvests From Normal Donors Treated With G-CSF (n = 10)**

<table>
<thead>
<tr>
<th>Marker</th>
<th>% of Cells Total x 10^6</th>
<th>% of Cells Total x 10^6</th>
<th>% Reduction</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD3</td>
<td>56 ± 9</td>
<td>4.5 ± 2.0</td>
<td>99.9 ± 0.1</td>
</tr>
<tr>
<td>CD6</td>
<td>59 ± 12</td>
<td>4.9 ± 2.3</td>
<td>99.9 ± 0.1</td>
</tr>
<tr>
<td>CD19</td>
<td>21 ± 13</td>
<td>1.8 ± 1.4</td>
<td>99.9 ± 0.1</td>
</tr>
</tbody>
</table>

Includes subjects for whom complete data was available, including 5 subjects administered G-CSF, 2 subjects administered GM-CSF, and 3 subjects administered G-CSF + GM-CSF. There were no appreciable differences among groups, hence the data are combined in the table.

The present report has demonstrated the feasibility of using PBPC mobilized by G-CSF or G-CSF + GM-CSF for this purpose. We also found that the CD34+ PC harvested by apheresis can be significantly enriched and more than 99% of the T cells were eliminated from the enriched product by positive selection of the CD34+ cells.

We have shown that, with the current schedule and analysis times, CD34+ cells and CFU-GM mobilization are greater with G-CSF than GM-CSF as single agents. The possibility cannot be excluded that if the peak mobilization of CD34+ cells or CFU-GM numbers is not yet reached during the time of analysis. Such variables are currently being explored. However, a significantly higher percentage of CD34+/HLA-DR-/CD38- was found among the CD34+ cells mobilized by GM-CSF compared with those by G-CSF alone, suggesting a higher proportion of early progenitors in GM-CSF–mobilized cells. Thus, subjects treated with the combination of G-CSF + GM-CSF showed an equivalent mobilization of CD34+ cells and CFU-GM numbers as with G-CSF alone, and also demonstrated a significantly greater mobilization of cells with CD34+/CD38- or CD34+/HLA-DR+/CD38- phenotype. Moreover, the plating efficiency (percent of CD34+ cells giving rise to colonies) of CFU-GM as well as BFU-E was higher in cells stimulated by GM-CSF than those by G-CSF. Whether this finding is associated with more rapid engraftment is not known. Nevertheless, this observation shows that CD34+ cells are heterogeneous, especially when mobilized by different growth factors. The total number of CD34+ cells by itself might not correlate with clonogenic assays or with clinical reconstitution. More sophisticated assays analyzing the CD34+ subsets are necessary and are currently underway in our laboratory.

Based on experience reported in the literature, we have adopted the present G-CSF mobilization schedule. However, as the CD34+ cells and clonogenic progenitors continued to increase 24 hours after the fourth and final dose of G-CSF or GM-CSF in this study, 1 or more additional days of cytokine administration might eventually give rise to a higher concentration of PC. Weaver et al. reported that 16 μg/kg of G-CSF was well tolerated and might result in a better yield of CD34+ cells. It is possible that the addition of GM-CSF might stimulate more pluripotent progenitors as discussed above. A combination of these approaches, ie, change in schedule, increase in cytokine dosage, and combinations of cytokines, might improve on the current regimen.

**Table 5. Enrichment of Clonogenic Cells by CD34+ Cell Selection (n = 10)**

<table>
<thead>
<tr>
<th>CFU-GM</th>
<th>BFU-E</th>
<th>CFU-GEMM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial CFU/10^6 cells</td>
<td>46 ± 33</td>
<td>120 ± 88</td>
</tr>
<tr>
<td>Enriched</td>
<td>2,792 ± 3,249</td>
<td>5,558 ± 2,818</td>
</tr>
<tr>
<td>Fold enrichment</td>
<td>68 ± 56</td>
<td>78 ± 60</td>
</tr>
<tr>
<td>Recovery</td>
<td>34 ± 36</td>
<td>32 ± 17*</td>
</tr>
</tbody>
</table>

Includes subjects for whom complete data were available, including 5 subjects administered G-CSF, 2 subjects administered GM-CSF, and 3 subjects administered G-CSF + GM-CSF. There were no appreciable differences among groups, hence the data are combined in the table. *P < .05 v G-CSF-GM.
such that only one leukapheresis procedure is required.\textsuperscript{57,46} Experiments are concurrently underway to address these issues.

The minimal dose of blood-derived hematopoietic PC required for successful engraftment is a critical issue that has yet to be defined. Because the concentration of PCs in PB from normal individuals in steady state is 1/100th to 1/1000th of that of bone marrow, “mobilization” procedures using hematopoietic growth factors, alone or in combination with chemotherapy, have been developed for patients with malignancies.\textsuperscript{7,18,40,41} For obvious reasons, only the use of growth factors is acceptable for normal donors. It has been suggested that doses of at least $4 \times 10^6$ mononuclear cells/kg, $>2 \times 10^7$ CFU-GM/kg, and between 2 and $5 \times 10^8$ CD34\textsuperscript{+} cells/kg are required for autologous PC transplantation.\textsuperscript{18,50}

For allogeneic transplantation the minimum number of blood-derived PC have not been established. Guidelines for donor cell doses when performing allogeneic BMT indicate that a minimum of $1 \times 10^6$ mononuclear cells/kg, $5 \times 10^7$ CFU-GM/kg, or $2 \times 10^7$ CD34\textsuperscript{+} cells/kg is necessary.\textsuperscript{51} With this presumption, our present data suggest that a sufficient number of CD34\textsuperscript{+} cells for engraftment would have been collected in two of the seven subjects from a single leukapheresis, and in all seven from two leukapheresis procedures. As recovery of CD34\textsuperscript{+} from the leukapheresis after separation ranged from 25% to nearly 100%, a median of three with a range from two to four leukapheresis procedures might be required using the present mobilization regimen. Weaver et al\textsuperscript{52} have recommended even higher doses of CD34\textsuperscript{+} cells to ensure engraftment. These findings highlight the need to further investigate more effective doses and schedules of mobilizing agents and optimal timing of the apheresis procedures.

Other than the presently described procedure, several methods have been developed to separate and enrich the CD34\textsuperscript{+} population on a clinical scale. Most of them are based on incubation with MoAbs against CD34 and subsequent binding of the labeled cells using the biotin-avidin system,\textsuperscript{53} or cell selection flasks coated with anti-CD34 antibody.\textsuperscript{53} Counterflow centrifugal elutriation has also been used to concentrate CD34\textsuperscript{+} cells and CFU-GM progenitors on a large scale.\textsuperscript{54} According to our own preliminary evidence, the use of paramagnetic microspheres has thus far produced one of the purest products (99.2%; unpublished results, June 1994). However, we are not yet aware of any data on comparisons of these various methods.

The incidence and severity of GVHD in the setting of allogeneic transplantation is dependent on the number of host T cells administered to the recipient.\textsuperscript{55} The positive selection technique used in this study removed up to 3 logs (99.9%) of the T cells originally present in the apheresis product and resulted in approximately $5 \times 10^6$ residual T cells in the enriched product. This would result in an average dose of $0.7 \times 10^7$ T cells/kg to a 70-kg allogeneic transplant recipient and is well below the 1 to $5 \times 10^7$ T cells/kg range reported to reduce GVHD.\textsuperscript{55,56} The role of T-cell depletion in allogeneic transplant is still controversial. The use of a technique which separates CD34\textsuperscript{+} cells from the remaining cells harvested from the PB should facilitate the investigation of the role of specific T-cell subsets in mediating engraftment while minimizing GVHD.

ACKNOWLEDGMENT

The authors thank Mary Cobb, Barbara Vickers, and Susan Shina-gawa for their excellent administrative assistance; Dr David Ward and Odette Ada, RN, for performing the leukapheresis procedures; and the staff of the UCSD Clinical Research Center for the daily care of the experimental subjects.

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