

Allogeneic Blood Stem Cell Transplantation: Peripheralization and Yield of Donor-Derived Primitive Hematopoietic Progenitor Cells (CD34⁺ Thy-1^{dim}) and Lymphoid Subsets, and Possible Predictors of Engraftment and Graft-Versus-Host Disease

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Apheresis-derived hematopoietic progenitor cells have recently been used for allogeneic transplantation. Forty-one normal donors were studied to assess the effects of recombinant human granulocyte colony-stimulating factor (rhG-CSF) (12 μ g/kg/d) on the peripheralization of hematopoietic progenitor cells and lymphoid subsets. The white blood cell, polymorphonuclear cell (PMNC), and lymphocyte concentrations at the peak of rhG-CSF effect in the donor's peripheral blood (PB) exceeded baseline by 6.4-, 8.0-, and 2.2-fold, respectively. Corresponding concentrations of PB CD34⁺ cells and primitive subsets such as CD34⁺ Thy-1^{dim}, and CD34⁺ Thy-1^{dim} CD38⁻ cells increased by 16.3-fold, 24.2-fold, and 23.2-fold, respectively in eight normal donors. The percentage of CD34⁺ Thy-1^{dim} and CD34⁺ Thy-1^{dim} CD38⁻ cells among CD34⁺ cells increased as well, suggesting an additional peripheralization effect of rhG-CSF on primitive CD34⁺ subsets. The preapheresis PB CD34⁺ and CD34⁺ Thy-1^{dim} cell concentrations were predictive of their corresponding apheresis yield per liter of donor blood processed. PB lymphoid subsets were not significantly affected by rhG-CSF treatment. The mean apheresis-derived yield of CD34⁺, CD34⁺ Thy-1^{dim}, and CD34⁺ Thy-1^{dim} CD38⁻ cells per kilogram of recipient body weight and per liter of donor blood processed was 48.9 $\times 10^4$ (n = 41), 27.2 $\times 10^4$ (n = 10), and 1.9 $\times 10^4$ (n = 10), respectively. As compared with 43 single bone marrow (BM) harvests, the CD34⁺ cell yield of peripheral blood progenitor cell allografts of 41 normal donors exceeded that of BM allo-

grafts by 3.7-fold and that of lymphoid subsets by 16.1-fold (CD3⁺), 13.3-fold (CD4⁺), 27.4-fold (CD8⁺), 11.0-fold (CD19⁺), and 19.4-fold (CD56⁺CD3⁻). All PBPC allografts were cryopreserved before transplantation. The mean recovery of CD34⁺ cells after freezing, thawing, and washing out dimethylsulfoxide was 86.6% (n = 31) and the recovery of lymphoid subsets was 115.5% (CD3⁺), 121.4% (CD4⁺), 105.6% (CD8⁺), 118.1% (CD19⁺), and 102.4% (CD56⁺CD3⁻). All donors were related to patients: 39 sibling-to-sibling, 1 parent-to-child, and 1 child-to-parent transplant. Thirty-eight transplants were HLA fully identical, two transplants differed in one and two antigens. Engraftment occurred in 38 recipients; two patients died too early to be evaluated, and one patient did not engraft. The lowest CD34⁺ cell dose transplanted and resulting in complete and sustained engraftment was 2.5 $\times 10^6$ /kg of recipient body weight. There was no significant correlation between the total number of CD34⁺ cells transfused and the time to reach PMNC $>0.5 \times 10^9$ /L or platelets $>50 \times 10^9$ /L posttransplant, nor was there a correlation found between the total number of CD3⁺, CD4⁺, and CD8⁺ cells transfused and the development of acute graft-versus-host disease (GVHD). The actuarial probability of developing acute GVHD in 38 evaluable patients was 48%. In 13 patients followed longer than 100 days posttransplant, the actuarial probability of developing chronic GVHD was 66% (median follow-up, 264 days).

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SUCCESSFUL allogeneic transplantation of apheresis-derived peripheral blood progenitor cells (PBPCs) from normal, patient-related donors has been reported by several groups including our own.¹⁻³ There are practical advantages of using the circulating blood rather than the marrow as a stem cell source such as ease of access, less discomfort for the donor, and a higher yield of hematopoietic progenitor cells. The most striking attribute of PBPC transplantation has been the faster hematopoietic reconstitution after myeloablative treatment compared with bone marrow (BM) transplantation.¹ There is also possibly a more pronounced graft versus leukemia (GVL) effect given the larger lymphocyte dose in PBPC apheresis products. In addition, a higher stem cell clonogenicity of cytokine-stimulated apheresis products has been reported.⁴⁻⁶ In this report, we analyze (1)

the cytokine-induced expansion of the peripheral blood stem cell pool in normal donors, (2) the yield of CD34⁺ cells and lymphoid subsets as compared with single BM harvests from normal donors, (3) PB CD34⁺ cell concentration as a possible predictor of CD34⁺ cell yield in the apheresis product, (4) the recovery of lymphohematopoietic cells after freezing and thawing, as well as (5) possible predictors of clinical outcome.

DONORS, MATERIALS, AND METHODS

Donors

Forty-one normal PBPC donors were consecutively evaluated from October 1993 through December 1994 and compared with 43 normal BM donors (March 1992 through March 1994) as shown in Table 1. All donors were related to the recipients: 39 donors were siblings, 1 was a parent, and 1 was a child of the recipient. Thirty-eight transplants were HLA fully identical, 1 transplant differed in one antigen, and 1 differed in two antigens. Written informed consent was obtained from all donors and recipients as approved by the institutional review board of the M.D. Anderson Cancer Center. Donors were required to be in stable medical conditions, meeting eligibility criteria for volunteer blood component donation with the exception of age. None of the donors had serologic evidence of hepatitis or human immunodeficiency virus infection. None had a history of malignancy or exposure to any myelotoxic drugs.

In Vivo Expansion of the Circulating Blood Progenitor Cell Pool

All 41 normal PBPC donors underwent granulocyte colony-stimulating factor (G-CSF) treatment (Neupogen; Amgen, Thousand Oaks,

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Table 1. Characteristics of Blood Stem Cell and BM Donors

	Total No. of Donors	Mean Age	Mean Recipient Weight (kg)	Mean TBV (L)
Blood stem cell donors	41	40.1 (12-68)		
Female	18	40.7 (15-60)	66.6 (49-91)	4.2 (3.1-5.6)
Male	23	39.6 (12-68)	81.0 (55-114)	5.5 (4.3-7.1)
BM donors	43	37.1 (20-58)		
Female	22	36.8 (22-54)	75.4 (47-104)	
Male	21	37.4 (20-58)	69.6 (40-106)	

Ranges are given in parentheses.

Abbreviation: TBV, total blood volume.

CA) for peripheralization of progenitor cells. G-CSF was administered subcutaneously twice a day at a dose of 6 $\mu\text{g}/\text{kg}$ for 3 days and before the first apheresis procedure, which started on day 4. Aphereses were performed on a daily basis and G-CSF treatment was continued throughout completion of stem cell collection. Side effects of G-CSF treatment in normal donors were mild and included headache (76%), moderate bone discomfort (63%), bone ache (21%), ankle swelling (13%) and fluid retention/weight gain (28%). A localized skin rash has been observed in one donor. All donors successfully completed mobilizing treatment and PBPC collection.

Blood Stem Cell Sampling

Stem cell apheresis. White blood cells were collected by continuous-flow leukapheresis using a Cobe Spectra blood cell separator (Cobe BCT, Inc, Lakewood, CO). Venous access was achieved exclusively by venipuncture of both arms. No donor required placement of a central line for venous access to undergo apheresis. The whole blood:anticoagulant ratio was set as preprogrammed. Only anticoagulant citrate dextrose solution (ACD-A) was used as an anticoagulant except for one donor who had an artificial heart valve and also remained on previously prescribed warfarin sodium medication during the apheresis procedures. There were no major side effects observed from apheresis. CaCl_2 was given by continuous infusion through the return line at a rate of $[0.5 \times \text{ACD-A flow rate} \times \text{procedure time}] \text{ mg}/\text{min}$. Electrolytes were substituted intravenously through the return line in case of low magnesium or potassium levels at the beginning of apheresis. The $\text{CD}34^+$ cell target dose for collection was considered $4 \times 10^6/\text{kg}$ of recipient body weight. If necessary, daily apheresis procedures were repeated until this cell dose was collected.

BM harvest. BM was procured by multiple aspirations under general anesthesia from the posterior superior iliac crest bilaterally. The aspirated cell suspension was collected in 600-mL plastic transfer bags (Baxter Healthcare Corp, Deerfield, IL) using 60 mL ACD-

A and 10,000 IU heparin per bag. Syringes were rinsed with heparinized saline (20,000 IU heparin/200 mL saline).

Cell Processing

Cell freezing. Apheresis and BM-derived white blood cell (WBC) suspensions were diluted 1:1 with human serum albumin (HSA; 5%) containing dimethylsulfoxide (DMSO; Cryoserv, Research Industries Corp, Salt Lake City, UT). The final DMSO concentration was 10%. The apheresis-derived cells were frozen in Stericon freezing bags (Stericon, Inc, Chicago, IL) (100-mL volume/bag) and BM-derived cells in Cryocyte freezing bags (Baxter Healthcare Corp, Deerfield, IL) (60-mL volume/bag) using a controlled rate freezer (Cryomed, Marietta, OH).

Bags were stored in mechanical freezers (-130°C) or in the vapor phase of liquid nitrogen (-130°C).

Thawing and washing. Frozen bags were thawed in a 37°C water bath. If the total volume of thawed cell suspension exceeded 300 mL, cells were washed in a CS 3000 blood cell separator (Baxter Healthcare Corp) using saline and 5% HSA-5%, and concentrated to a transfusion volume of about 500 mL.

Flow Cytometry

Cell surface markers were determined by dual or three-color direct staining method using conjugated monoclonal antibodies CD34, CD3, CD4, CD8, $\text{TCR}\alpha\beta$, CD16, CD56, CD19 (Becton Dickinson Immunocytometry Systems, San Jose CA) and CDw90 (Thy-1), CD38 (PharMingen Inc, San Diego, CA). Antibodies were titrated against 1×10^5 to 1×10^6 cells and used at saturation levels; negative controls, autofluorescence, and isotype specific, were set up for each combination. Cells and monoclonals were incubated in ice for 30 minutes in the dark; after staining, erythrocytes were lysed in ammonium chloride hemolytic buffer for 10 minutes at room temperature. Cells were then centrifuged 800g for 5 minutes and washed once in phosphate-buffered saline (PBS) supplemented with 1% (vol/vol) goat serum and 0.1% (wt/vol) sodium azide (PBS-1% goat serum [GT]). Final cell pellet was resuspended in 500 to 1,000 μL PBS-1% GT and data (10,000 or 40,000 events) was acquired in list-mode using FACScan flow cytometer and LYSIS-II software (Becton Dickinson Immunocytometry Systems). Regions of fluorescence were identified for each fluorochrome and corresponding negative control and appropriate subset combinations were analyzed by logical gating with LYSIS-II software.

Statistical Evaluation

A standard two-tailed Student's *t*-test was performed on seven paired sets of donor data related to CD34 subsets in PB precytokine and postcytokine stimulation. The test was performed at an α level of .05 against the null hypothesis that there is no difference between the means of the groups analyzed. Actuarial method was used to

Table 2. Increase of WBC, PMNC, Lymphocyte, and Platelet Concentrations in the Donor's Peripheral Blood Over Pretreatment Level After 3 Days of G-CSF Treatment and Before the First Apheresis

	WBC $\times 10^9/\text{L}$	PMN $\times 10^9/\text{L}$	Lymphocytes $\times 10^9/\text{L}$	Platelets $\times 10^9/\text{L}$
Before G-CSF treatment	6.9 (3.7-13.6) (n = 41)	4.3 (1.6-8.8) (n = 28)	2.0 (1.0-3.6) (n = 28)	240.0 (142-348) (n = 41)
Before apheresis	44.4 (23.0-94.2) (n = 41)	34.2 (17.9-69.3) (n = 28)	4.3 (1.3-13.2) (n = 28)	232.5 (150-362) (n = 41)
Fold increase preapheresis over precytokine values	6.4 (3.7-14.6)	8.0 (4.5-23.8)	2.2 (0.6-6.6)	1.0 (0.5-1.3)
<i>P</i> value	.001	.001	.001	.20

Values given are means, with ranges in parentheses.

Table 3. Increase of CD34⁺ Cell and Subset Concentrations in the Donor's Peripheral Blood

	CD34 ⁺ Cells × 10 ⁶ /L	CD34 ⁺ Cells as % of TNC	CD34 ⁺ Thy-1 ^{dim} Cells × 10 ⁶ /L	CD34 ⁺ Thy-1 ^{dim} Cells as % of CD34 ⁺ Cells	CD34 ⁺ Thy-1 ^{dim} CD38 ⁻ Cells × 10 ⁶ /L	CD34 ⁺ Thy-1 ^{dim} CD38 ⁻ Cells as % of CD34 ⁺ Cells
Before G-CSF treatment	3.8 ± 0.8 (n = 14)	0.06	1.1 ± 0.3 (n = 14)	30.4	0.095 ± 0.07 (n = 14)	2.5
Before apheresis	61.9 ± 11.3 (n = 16)	0.93	26.6 ± 5.1 (n = 16)	43.1	2.2 ± 0.9 (n = 16)	3.5
Fold increase preapheresis over precytokine values	16.3		24.2		23.2	
P value	.009		.027		.087	

Increases were over pretreatment level after 3 days of G-CSF treatment and before apheresis. Change of the percentages of CD34⁺ cells were among TNC, and CD34⁺ subsets among CD34⁺ cells under G-CSF treatment. The *P* value is based on the evaluation of paired data from seven patients. Values given are mean ± SE.

compute the probability for development of acute or chronic graft-versus-host disease (GVHD) in this patient population.

RESULTS

Increase of PB Cell Concentration Over Pretreatment Level in 41 Normal Donors After 3 Days of G-CSF Treatment and Before Apheresis

As shown in Table 2, WBC, PMNC, and lymphocyte concentrations in the donors' PB increased under G-CSF treatment by 6.4-fold (*P* < .001), 8.0-fold (*P* < .001), and 2.2-fold (*P* < .001), respectively. Platelet concentration was not significantly affected rising by 1.0-fold (*P* = .20).

Circulating CD34⁺ cells as well as less differentiated CD34⁺ subsets were both peripheralized under G-CSF treatment (Table 3). The circulating CD34⁺ cell pool expanded by 16.3-fold (*P* = .009). The more primitive CD34⁺Thy-1^{dim} and CD34⁺Thy-1^{dim}CD38⁻ subsets^{7,8} increased by 24.2-fold (*P* = .027) and 23.2-fold (*P* = .087, not significant), respectively. This additional expansion of primitive CD34⁺ cell subsets over CD34⁺ cell expansion was significant for CD34⁺Thy-1^{dim} (*P* = .018), but not for the CD34⁺Thy-1^{dim}CD38⁻ subset, possibly because of the greater diversification of data. The percentage of CD34⁺ cells among total nucleated cells (TNCs) in the donor's PB increased from a mean of 0.06% precytokine to 0.93% preapheresis (after 3 days of G-CSF treatment). The percentages of primitive CD34⁺ subsets such as CD34⁺Thy-1^{dim} and CD34⁺Thy-1^{dim}CD38⁻ among CD34⁺ cells increased from precytokine to the preapheresis stage by 13% and 1%, respectively (Table 3).

As shown in Table 4, the PB concentrations of lym-

phoid subsets including a putative suppressor cell subset (CD3⁺CD4⁻CD8⁻TCR⁺)⁹ and natural killer (NK) cells exceeded the cytokine pretreatment level by only between 1.1-fold and 1.5-fold (*P* > .05).

Correlation Between Precytokine and Preapheresis PB CD34⁺ Cell and Subset Concentrations and Apheresis CD34⁺ Cell and Subset Yield

The CD34⁺ cell concentrations before cytokine treatment at steady state and before apheresis at the peak of G-CSF mobilization effect were correlated with the CD34⁺ cell yield in the apheresis products per liter of donor's blood processed. No correlation was observed between precytokine PB CD34⁺ cell concentration and CD34⁺ cell yield per liter of blood processed. Positive correlations were found between preapheresis PB CD34⁺ and PB CD34⁺Thy-1^{dim} cell concentrations, and CD34⁺ and CD34⁺Thy-1^{dim} cell yields per liter of blood processed, respectively (*r*² = .73 and *r*² = .61) (Figs 1 and 2).

Yield of Apheresis-Derived CD34⁺ Cells and Subsets

The total CD34⁺ cell and CD34⁺ subset yields per allograft and, for reasons of comparison, per liter of donor blood processed, are depicted in Table 5. It is noteworthy that the percentage of CD34⁺ cells among TNCs was not significantly different from that of PB CD34⁺ cells at the peak of G-CSF mobilization effect before first apheresis, nor were the percentages of primitive CD34⁺ cell subsets among CD34⁺ cells.

Table 4. Increase of Lymphoid Subset Concentrations in the Donor's Peripheral Blood Over Pretreatment Level After 3 Days of G-CSF Treatment and Before Apheresis

	CD3 ⁺ Cells × 10 ⁶ /L	CD4 ⁺ Cells × 10 ⁶ /L	CD8 ⁺ Cells × 10 ⁶ /L	CD3 ⁺ CD4 ⁻ CD8 ⁻ TCR ⁺ Cells × 10 ⁶ /L	CD19 ⁺ Cells × 10 ⁶ /L	CD3 ⁺ CD16 ⁺ CD56 ⁺ Cells × 10 ⁶ /L
Before G-CSF treatment	1,351.6 ± 166.5 (n = 5)	908.8 ± 126.1 (n = 5)	325.9 ± 72.2 (n = 5)	12.8 ± 2.6 (n = 5)	252.0 ± 52.4 (n = 5)	168.6 ± 62.7 (n = 5)
Before apheresis	1,732.6 ± 181.2 (n = 8)	1,148.6 ± 139.3 (n = 8)	479.5 ± 79.0 (n = 8)	14.3 ± 4.0 (n = 8)	351.2 ± 67.1 (n = 8)	183.9 ± 25.4 (n = 8)
Fold increase preapheresis over precytokine values	1.3	1.3	1.5	1.1	1.4	1.1
P value	.35	.26	.21	.62	.32	.26

Values given are means ± SE.

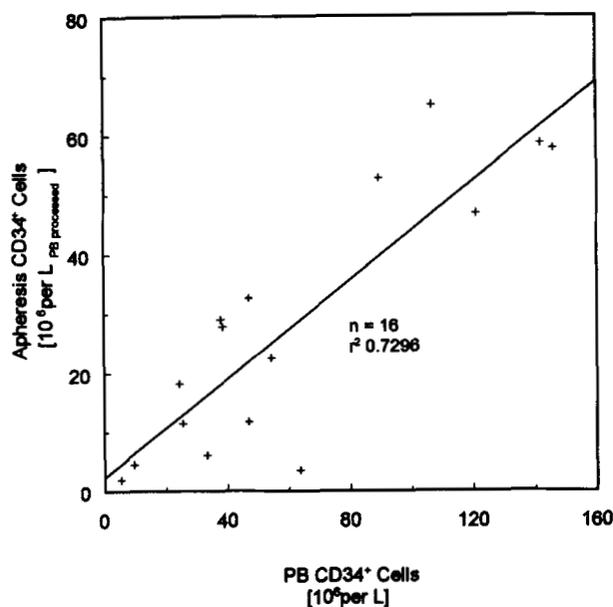


Fig 1. Correlation between peripheral blood CD34⁺ cell concentration before apheresis and apheresis-derived CD34⁺ cell yield.

Cell Yield Collected by Apheresis From 41 Normal Donors Compared With 43 Normal BM Donors

Flow cytometric analyses were performed on PBPC allografts from 41 normal donors and compared with BM allografts from 43 normal donors (Table 6). The fold-increases of cell yield in PBPC over BM allografts were between 3.7 (CD34⁺ cells) and 27.4 (CD8⁺ cells). The average total number of CD56⁺CD3⁻ (NK) cells in the blood stem cell

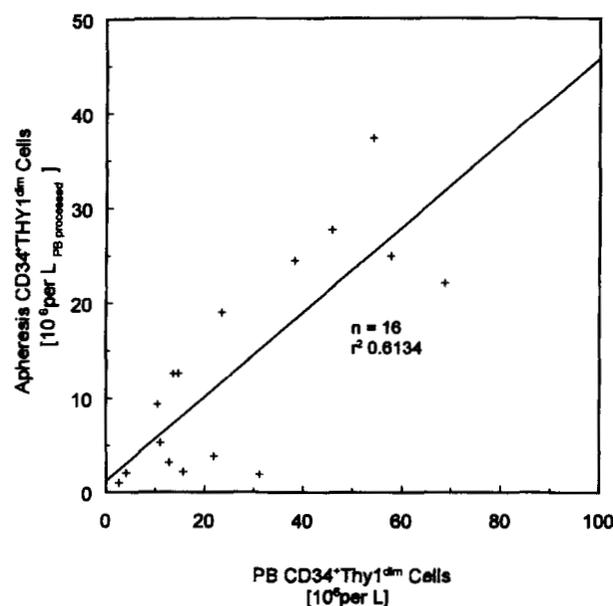


Fig 2. Correlation between peripheral blood CD34⁺ Thy-1^{dim} cell concentration before apheresis and apheresis-derived CD34⁺ Thy-1^{dim} cell yield.

Table 5. Mean Yield of CD34⁺ Cells and Subsets in the Apheresis Products Collected Under G-CSF Treatment From Each of 10 Blood Stem Cell Donors

	G-CSF-Mobilized Apheresis Products
CD34 ⁺ cells × 10 ⁴ /kg/L	62.9 ± 17.9
Total CD34 ⁺ cells × 10 ⁶ /kg	7.4 ± 2.2
CD34 ⁺ cells as % of TNC	0.75
CD34 ⁺ Thy-1 ^{dim} cells × 10 ⁴ /kg/L	27.2 ± 7.7
Total CD34 ⁺ Thy-1 ^{dim} cells × 10 ⁶ /kg	3.3 ± 1.0
CD34 ⁺ Thy-1 ^{dim} cells as % of CD34 ⁺ cells	45
CD34 ⁺ Thy-1 ^{dim} CD38 ⁻ cells × 10 ⁴ /kg/L	1.9 ± 0.9
Total CD34 ⁺ Thy-1 ^{dim} CD38 ⁻ cells × 10 ⁶ /kg	0.2 ± 0.09
CD34 ⁺ Thy-1 ^{dim} CD38 ⁻ cells as % of CD34 ⁺ cells	2.6

Values given are means ± SE.

Abbreviations: kg, kilograms of recipient body weight; L, liters of donor blood processed.

allografts exceeded that of BM allografts by a factor of 19.4. The mean number of CD34⁺ cells in the apheresis products from 41 normal donors was 11.7 × 10⁶/kg of recipient body weight (range, 4.2 to 40.1 × 10⁶) or 11.4 × 10⁶/kg donor body weight. The respective increase over BM allografts was 3.7-fold and 3.6-fold. The mean number of aphereses performed per PBPC allograft was 2.0 (range, 1 to 4) as compared with a single BM harvest.

Liters of Donor Blood Processing Needed to Collect an Alloengraftment Dose PB CD34⁺ Cells

Of 41 normal blood stem cell donors, the mean donor total blood volume was estimated at 4.9 L (range, 3.1 to 7.1).¹⁰ The mean total blood volume processed per donor was 27.1 L (range, 11.5 to 54.6). The number of CD34⁺ cells collected per liter of donor blood processed and per kg of recipient body weight was 48.9 × 10⁴ (range, 10.6 to 121.8 × 10⁴) or 47.1 × 10⁴ per kg of donor body weight (range, 10.4 to 158.5 × 10⁴), respectively. Based on a freshly collected alloengraftment target dose of 4 × 10⁶ CD34⁺ cells/kg of recipient body weight and based on 48.9 × 10⁴ CD34⁺ cells collected per kg of recipient body weight, a total of 8.2 L donor blood needs to be processed, on the average, to reach that goal. With a usual donor's blood volume processed per run being in the range of 10 to 17.5 L, one single apheresis appears to be sufficient, on the average, to collect even more than the CD34⁺ alloengraftment dose of 4 × 10⁶ CD34⁺ cells/kg of recipient body weight.

Recovery of Frozen-Thawed-Washed CD34⁺ Cells and Lymphoid Subsets

The mean recovery of CD34⁺ cells after freezing, thawing, and washing was 86.6% (range, 24.7 to 257.8) (n = 31). In eight patients the recovery of lymphoid subsets was determined to be 115.5% (range, 75.7 to 249.4) for CD3⁺ cells, 121.4% (range, 80.7 to 209.3) for CD4⁺ cells, 105.6% (range, 67.1 to 215.0) for CD8⁺ cells, 118.1% (range, 32.0 to 247.5) for CD19⁺ cells, and 102.4% (range, 43.5 to 212.0) for CD3⁻CD56⁺ cells. There was no selective loss of lymphoid subsets caused by the cryopreservation procedure.

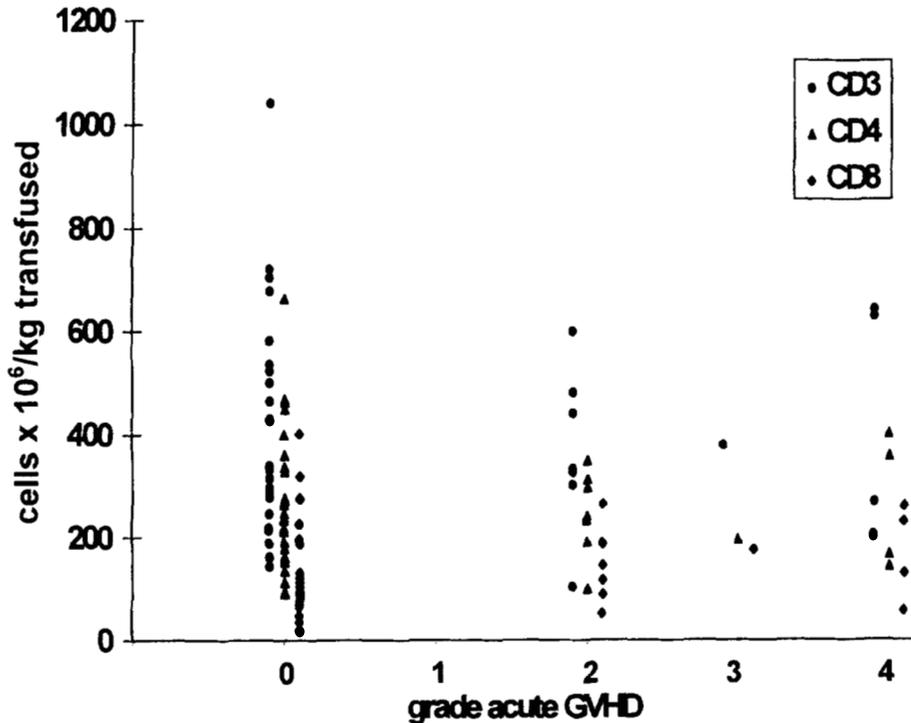


Fig 3. Correlation between total number of CD3⁺, CD4⁺, or CD8⁺ cells transfused and development of acute GVHD.

Engraftment and Transplant Toxicity

The lowest CD34⁺ cell dose successfully transplanted was 2.5×10^6 /kg of recipient body weight. One patient with the diagnosis of myelodysplastic syndrome who was transplanted with her son's one antigen/mismatched PBPCs did not engraft despite a sufficient amount of CD34⁺ cells transfused (8.6×10^6 /kg of recipient body weight). A subsequent BM transplant (4.3×10^6 CD34⁺ cells/kg of recipient body weight) 39 days later using the same donor also failed to engraft. The patient died 45 days after the initial PBPC transplantation. Two patients died too early to be evaluated for engraftment: one patient died of respiratory syncytial virus infection at day 4 posttransplant, and one patient died of transplant regimen-related central nervous system toxicity at day 6. Thirty-eight patients showed complete and sustained engraftment.

Correlations Between CD34⁺ Cells Recovered After Freezing and PMNC or Platelet Recovery

The number of CD34⁺ cells transfused after cryopreservation was correlated with the time to reach a PMNC level greater than 1.0×10^9 /L or platelet level greater than 50×10^9 /L. No such correlation was observed.

Correlations Between CD3⁺, CD4⁺, and CD8⁺ Cells and Development of Acute GVHD

Using the same anti-GVHD prophylaxis acute GVHD seemed to develop independently from the number of apheresis-derived, frozen/thawed T cells transfused (Fig 3). Despite a mean 16-fold higher total amount of CD3⁺ cells contained in the PBPC allograft as compared with a BM

allograft (Table 6), the actuarial probability of developing acute GVHD in 38 evaluable patients was only 48% (median follow-up, 70 days (range, 35 to 182)). In 13 patients with a follow-up longer than 100 days posttransplant, the actuarial probability of developing chronic GVHD was 66% at a median follow-up of 264 days (range, 151 to 476).

DISCUSSION

The G-CSF-mobilizing regimen for PBPC donors was based upon the kinetics of WBC and PB CD34⁺ cell concentration setting the upper limit of WBC concentration at 50×10^9 /L. With starting aphereses on the fourth day of G-CSF treatment in the present study, a mean WBC count of 44×10^9 /L was reached. The concentration of CD34⁺ cells in the donor's PB increased under G-CSF treatment at the time of apheresis by 16.3-fold over baseline, coinciding with the peak level of PB CD34⁺ cell concentration.⁴ A 9- and 25-fold increase of PB CD34⁺ cell concentration by day 4 and 5, respectively, has been reported by Weaver et al¹¹ documented in patients using a higher-dose G-CSF (16 μ g/kg/d). This study provides previously unpublished observations that (1) G-CSF peripheralizes primitive CD34⁺ subsets such as CD34⁺ Thy-1^{dim} and CD34⁺ Thy-1^{dim} CD38⁻ in normal donors, and (2) the percentage of those primitive CD34⁺ subsets among CD34⁺ cells at the peak of G-CSF effect significantly exceeded the PB precytokine and steady-state percentage, suggesting an additional mobilization effect of G-CSF on those primitive stem cell subsets. Those early blood-derived CD34⁺ subsets putatively encompass self-renewing stem cells that initiate and sustain lymphohematopoietic reconstitution.^{5,12} The mobilizing effect of G-CSF on early blood stem cell subsets is further documented by a

Table 6. TNCs, CD34⁺ Cells, and Lymphoid Subsets Collected From 41 Normal PBPC Donors Compared With 43 Normal BM Donors

Stem Cell Allograft	n	TNC	CD34 ⁺	CD3 ⁺	CD4 ⁺	CD8 ⁺	CD19 ⁺	CD56 ⁺ CD3 ⁻
PB cells × 10 ⁶ /kg	41	1,336.6 (563-3113)	11.7 (4.2-40.1)	393.2 (104.6-1039.9)	257.4 (91.9-663.4)	153.6 (21.5-402.3)	79.0 (25.5-189.0)	54.4 (13.7-193.1)
BM cells × 10 ⁶ /kg	43	292.5 (28.1-674.4)	3.2 (0.5-10.9)	24.4 (15.8-33.7)	19.3 (10.5-30.3)	5.6 (1.7-11.5)	7.2 (1.9-14.1)	2.8 (1.1-5.1)
Fold increase blood over marrow cells in stem cell allografts		4.6	3.7	16.1	13.3	27.4	11.0	19.4

Values given are means, with ranges in parentheses.
Abbreviation: kg, kilograms of recipient body weight.

murine *in vivo* allogeneic transplantation study reported by Molineux et al¹³ showing that G-CSF peripheralized progenitor cells (1) are capable of long-term lymphohematopoietic reconstitution, and (2) exhibit a significantly higher clonogenicity as compared with nonstimulated PBPCs. In humans, we and others¹⁻³ recently reported complete and sustained hematopoietic engraftment after PBPC transplantation using G-CSF for stem cell peripheralization, providing evidence that early pluripotent stem cells with sustained self-renewal capacity are contained in the G-CSF-mobilized PBPC allografts. Lymphocyte subpopulations were only mildly affected by G-CSF treatment with T-lymphocytes increasing 1.1 to 1.5 times baseline by day 4 of G-CSF treatment. In Weaver's study,¹¹ CD3⁺, CD4⁺, CD8⁺, CD19⁺, and CD20⁺ PB lymphocytes increased by day 3 of 16 μg/kg/d G-CSF treatment with T lymphocytes increasing 1.5 to 2.0 times over baseline. It is noteworthy in their study on 11 patients and 2 normal donors that all lymphocyte phenotypes returned to below pretreatment levels on days 4 and 5 of G-CSF administration.

The optimal dose of G-CSF for PBPC mobilization in normal donors is unknown. The G-CSF priming dose of 12 μg/kg/d in the present study was higher than what is reported by Matsunaga et al (2.5 to 5.0 μg/kg/d),¹⁴ Fritsch et al (5 μg/kg/d),¹⁵ and Tjonnfjord et al (10 μg/kg/d).⁴ Weaver et al,¹⁶ on the other hand, used 16 μg/kg/d for stem cell priming. Side effects of the higher-dose G-CSF (12 μg/kg/d) were well tolerated in this study, and no apheresis procedure needed to be cancelled because of severe G-CSF-related adverse effects. The procedure was well tolerated, even in older donors, the oldest being age 68 in our study.

We tried to characterize a predictor for CD34⁺ cell yield in the G-CSF mobilized apheresis product. The CD34⁺ and CD34⁺ Thy-1^{dim} PB cell concentrations before apheresis at the maximum of G-CSF stimulation predicted for apheresis-derived stem cell yield. Thus, costly apheresis procedures can be avoided by evaluating PB CD34⁺ cell concentrations before apheresis. Precytokine, steady-state CD34⁺ cell levels, on the other hand, were not predictive, most probably because of sensitivity limitations of the flow cytometry technique.

Lymphohematopoietic cell subsets in blood stem cell allografts and BM allografts procured from normal donors were found to be significantly different. The average yield of CD34⁺ cells in the PBPC allograft collected from two G-CSF-mobilized aphereses was 3.7 times higher than that in an average single BM harvest. The lymphoid subsets CD3⁺, CD4⁺, CD8⁺, CD19⁺, and CD3⁻CD56⁺ in the PBPC allograft numerically exceeded the BM allograft to an even higher

extent, namely between 11- and 27-fold. In particular, the total amount of NK cells was found to be 19-fold higher in the PBPC allograft. This observation was confirmed in four normal donors who donated both a BM allograft and a PBPC allograft for the same recipient. Transplanting significantly higher amounts of lymphoid cells contained in the PBPC allograft did not increase the incidence of acute GVHD,¹⁻³ whereas the impact on chronic GVHD, GVL effect, and long-term disease-free survival is under current investigation. Preliminary data on 38 evaluable patients undergoing allo-PBPC transplantation in our series show that the actuarial rate of acute GVHD (48%) and chronic GVHD (66%) is not higher than what is expected after BMT using unmanipulated allografts.¹⁷

With a mean of about 50 × 10⁴ CD34⁺ cells/kg of recipient body weight collected per liter of donor blood processed, and a target CD34⁺ cell alloengraftment dose of 4 × 10⁶/kg of recipient body weight, a single apheresis procedure under G-CSF treatment seems to be sufficient to collect a safe engraftment dose in a majority of cases. Nonetheless, 10 of 41 blood stem cell donors required two to four apheresis procedures. The lower CD34⁺ apheresis yield could not be associated with the donor's age, sex, or other characteristics. For example, the 68-year-old blood stem cell donor yielded 52 × 10⁴ CD34⁺ cells/kg of recipient body weight and per liter of donor blood processed in the first apheresis product, a cell dose sufficient for engraftment.

Because of a possible need to perform more than one apheresis on a given donor, we decided to cryopreserve all apheresis products before transplant, which, in addition, allowed us to schedule the transplant procedure independently from the collection date. After thawing and washing out DMSO, the median CD34⁺ cell recovery was near 85%. Cryopreservation of BM allografts has been reported to reduce the risk of acute GVHD, possibly through selective depletion of or induction of anergy in GVHD-inducing cells.^{18,19} This may have played a role in the outcome observed in our patients. However, the percentage recovery of CD3⁺, CD4⁺, CD8⁺, CD19⁺, and CD3⁻CD56⁺ subsets in our study was found to be in a similar range without evidence of selective loss of any subset. Nevertheless, in the present study, no experiments were performed to particularly address the question of whether frozen/thawed PB lymphoid subsets exhibit the same functional properties *in vitro*. The impact of cryopreservation on alloreactivity of allogeneic PBPCs needs to be further investigated.

The lowest CD34⁺ cell dose in our series transplanted and resulting in full alloengraftment was 2.5 × 10⁶/kg of recipient body weight similar to 2.2 × 10⁶/kg reported by Schmitz

et al.² This patient needed 16 and 17 days post allotransplant to reach 0.5 and 1.0×10^9 PMNC/L, respectively, and 39 days to reach 20×10^9 /L platelets. At day 61, the patient's platelet count was still below 50×10^9 /L. GVHD prophylaxis for this patient consisted of cyclosporin-A and methotrexate as compared with our series using the less myelosuppressive regimen methylprednisolone and cyclosporin-A.¹

Further evaluation is necessary to determine whether the substantially higher T-cell dose in allogeneic PBPC transplants will result in a higher rate of chronic GVHD. Although not significantly different than with BM transplantation, the rate of acute GVHD remains substantial. Measures to modify the composition of PBPC allografts to potentially reduce acute and chronic GVHD have been initiated.

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Allogeneic blood stem cell transplantation: peripheralization and yield of donor-derived primitive hematopoietic progenitor cells (CD34+ Thy-1dim) and lymphoid subsets, and possible predictors of engraftment and graft-versus-host disease

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