

# Effect of G-CSF on Peripheral Blood Progenitor Cell Mobilization and Collection from Healthy Donors

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Granulocyte colony-stimulating factor (G-CSF) is a growth factor with the capacity to promote growth and maturation of myeloid cells and, particularly, proliferation and differentiation of neutrophil progenitors both *in vitro* and *in vivo*.<sup>1</sup> It has been used together with high dose chemotherapy to mobilize peripheral blood stem cells in autologous stem cell transplantation and is increasingly used for allogeneic stem cell transplantation. G-CSF alone can mobilize not only committed progenitors, such as colony-forming unit-granulocyte, macrophage (CFU-GM), burst forming unit-erythroid (BFU-E) and Mix-CFU (CFU-GEMM), but also long-term culture initiating cells (LTC-IC) into the peripheral blood of normal individuals.<sup>2</sup> Recent studies have shown that using peripheral blood progenitor cells (PBPC) instead of BM derived stem cells results in rapid hematological and immunological recovery which contributes to less platelet and red blood cell transfusion, reduction in febrile periods, earlier discharge

**SUMMARY** We studied granulocyte colony-stimulating factor (G-CSF) mediated peripheral blood progenitor cells (PBPC), which were mobilized and collected from healthy donors for allogeneic transplantation. A total of 26 donors, age ranged from 21-41 years were mobilized with G-CSF at a dose of 7.5 µg/kg/day subcutaneously for 5 days and the collection was started on day 5. The CD34<sup>+</sup> cell counts reached a maximum on day 5 and subsequently declined despite continually given G-CSF. White blood cells (WBC), absolute neutrophil counts (ANC), absolute lymphocytes (AL) and their subsets, absolute mononuclear cells (AMNC), colony-forming unit-granulocyte, macrophage (CFU-GM) and CD34<sup>+</sup> cells were increased about 6, 9, 2, 3, 34 and 40-fold, respectively, but red blood cells (RBC), hematocrit (Hct) and platelets (Plt) decreased on day 5 when compared to day 0. All parameters decreased after stem cell collection. For stem cell collection by Cobe Spectra, we used a blood volume of 19.27 ± 4.65 liters, flow rate of 60.53 ± 10.03 ml/minute, acid citrate dextrose solution (ACD)/blood ratio of 1:13.31, the final product volume was 314.14 ± 72.24 ml, collection time was 325.40 ± 73.36 minutes and one or two procedures were sufficient. The correlation between the number of CD34<sup>+</sup> cells/kg, CFU-GM/kg and MNC/kg found in the harvested product and CD34<sup>+</sup> cells can be used for determining the necessary amount of progenitor cells for transplantation.

from the hospital and lower transplantation cost.<sup>3</sup> In addition, the advantage of allogeneic peripheral blood stem cell transplantation over marrow transplantation is that the donor's risks associated with general anesthesia, certain amount of blood loss and discomfort caused by multiple bone punctures can be avoided. According to the report from the National Marrow Donor

Program (NMDP), adverse events experienced by 433 donors at 7-14 days after marrow harvest included pain at the donation site, fatigue, low back pain and difficulty in

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walking.<sup>4</sup> However, adverse effects of G-CSF administration in normal donors include bone pain, headache, body aches, fatigue, nausea, vomiting and several changes in serum chemistries.<sup>5</sup>

CD34<sup>+</sup> cells, which are the stem/progenitor cell compartment, from mobilized peripheral blood (MPB) resemble their steady-state peripheral blood (SSPB) counterparts and differ significantly from steady-state bone marrow (SSBM) CD34<sup>+</sup> cells.<sup>6</sup> CD34<sup>+</sup> cells in BM are characterized by restricted proportion of cells committed to myeloid differentiation (CD33<sup>+</sup>), intense metabolic activity as suggested by the expression of the transferrin receptor (CD71), high level of rhodamine 123 retention and active cycling status. On the other hand, the proportion of CD34<sup>+</sup> cells in PB expressing CD33 is higher in comparison to BM. Furthermore, very few PB CD34<sup>+</sup> cells express the CD71 antigen and their level of rhodamine retention is low. The proportion of PB CD34<sup>+</sup> cells expressing B cell antigens, such as CD10 and CD19, and T cell antigens such as CD7 is lower than in BM. Finally, the proportion of CD34<sup>+</sup> cells/Thy-1<sup>+</sup> cells (a subset rich in LTC-IC) in MPB leukapheresis products was shown to be greater than in bone marrow grafts and the number of CFU-GM was significantly higher in the leukapheresis product than normal bone marrow or cord blood.

At present, peripheral blood progenitor cells transplantation (PBPC) is increasingly used in place of bone marrow transplantation. Information on the effect of recombinant human granulocyte-colony stimulating factor (rhG-CSF) on peripheral blood progenitor cell mobilization and collection, how-

ever, varies among transplantation centers. So the effect of G-CSF on peripheral blood CD34<sup>+</sup> cell counts and hematological values, characteristic of peripheral blood progenitor cell collection and correlation of the CD34<sup>+</sup>, CFU-GM and mononuclear cells in the harvested product from our center were studied.

## MATERIALS AND METHODS

### Subjects and G-CSF administration

Twenty-six healthy donors (13 men and 13 women) for allogeneic peripheral blood stem cell transplantation were studied. The median age was 31 (21-41) years. Half of the donors received non-glycosylated G-CSF (Filgrastim) and the rest received glycosylated G-CSF (Lenograstim) by subcutaneous injection for 5 consecutive days at a dose of 7.5 µg/kg/day.

### PBPC collection

Leukapheresis were started on day 5 of G-CSF administration and performed with a Cobe Spectra (Cobe Laboratories, Inc., Lakewood, CO, USA). The Spectra system was set up and primed according to the manufacturer's instructions for leukapheresis. Body weight, height, gender and hematocrit (Hct) data were used to calculate the machine settings according to the manufacturer's program.

### Cell characterization

Peripheral blood samples were obtained from donors prior to G-CSF administration and immediately before and after the apheresis procedure (day 5). Complete blood counts were determined by automatic cell counter (Coulter Counter Model JT3).

CD34<sup>+</sup>, CD3<sup>+</sup>, CD4<sup>+</sup> and CD8<sup>+</sup> counts were performed by direct immunofluorescence staining using the flow cytometric technique on a Facsort flow cytometer (Becton Dickinson, USA). This technique was carried out by using 50 µl of cell suspension containing 1 x 10<sup>6</sup> WBC labeled with monoclonal antibody fluorescence dye as followed: 30 minutes at 2-8°C for CD34<sup>+</sup>, 15 minutes at room temperature for CD3<sup>+</sup>, CD4<sup>+</sup> and CD8<sup>+</sup> with 20 µl of CD34 (Anti-HPCA-2) PE/CD45-FITC, CD3-FITC/CD4-PE/CD45-PerCP and CD3-FITC/CD8-PE/CD45-PerCP, respectively. PE-conjugated mouse IgG1 was used as control for background non-specific staining. After labeling, red blood cells were lysed by adding 1 ml of FACS lysing solution for 10 minutes at room temperature in the dark. The suspension was washed with phosphate buffered saline (PBS) and fixed with 0.5 ml of 1% paraformaldehyde, mixed thoroughly and analyzed. For CD34<sup>+</sup> cell analysis, a gate for WBC excluding erythrocytes and debris was set according to a forward and side scattering cytogram, only mononuclear cells stained with monoclonal antibody to CD34-PE were gated. A minimum of 20,000 events were analyzed in each sample.

CFU-GM assay was performed in an agar culture medium as follows: blood samples were diluted with Hanks' balanced salt solution. Light density cells were separated by centrifugation over Isoprep. The mononuclear cells (MNC) obtained were washed and resuspended in 10% fetal bovine serum (FBS) in MEM α medium. One milliliter containing 2 x 10<sup>5</sup> MNC was cultured in MEM α medium supplemented with 0.3% Noble agar (Difco, MI, USA), 20%

heat-inactivated FBS and 10% condition medium of 5637 human bladder carcinoma cell line and maintained at 37°C in 5% CO<sub>2</sub> in air for 10 days. All assays were performed in duplicate, clusters (10-40 cells) and colonies (> 40 cells) were scored with an inverted microscope.

### Statistical analysis

Paired *t*-test was used for analysis of the differences of hematological values between, before and after G-CSF administration, as well as before and after leukapheresis. The correlation between CFU-GM/kg, MNC/kg and CD34<sup>+</sup>/kg in leukapheresis products was determined using linear regression.

## RESULTS

### Effect of G-CSF on peripheral blood CD34<sup>+</sup> cell counts

The kinetics of CD34<sup>+</sup> cells in the donors' peripheral blood are shown in Fig. 1. Using a dose of 7.5 µg/kg/day, progenitor cell peaks

were always observed on day 5 of G-CSF administration with a 40-fold and 34-fold enrichment of CD34<sup>+</sup> cells and CFU-GM, respectively, compared with baseline values (Table 1).

### Effect of G-CSF on hematological values

G-CSF administration resulted in an sixfold increase in the peripheral WBC count, ninefold increase in the absolute number of neutrophils (ANC), twofold increase in the absolute number of lymphocytes (AL) and threefold increase in the absolute number of mononuclear cells (lymphocytes and monocytes) on day 5 compared with the baseline values, whereas RBC count, hematocrit and platelet count decreased (Table 1). After the first apheresis, the values of all parameters decreased.

On day 5 of G-CSF administration, the percentage of lymphocytes decreased but there was no change in the percentages of CD3<sup>+</sup>, CD4<sup>+</sup> and CD8<sup>+</sup> cells and the

CD4<sup>+</sup>/CD8<sup>+</sup> ratio (Table 2), although the absolute lymphocyte count and their subsets increased.

### Peripheral blood progenitor cell collection

Donors underwent leukapheresis for approximately 5.4 hours with blood inlet flow rates of 50 to 70 ml per minute. The average volume of blood processed was 19 liters, the ACD to blood ratio was 1:13.3. The mean collection time and final product volume were 325 minutes and 314 ml, respectively.

### Correlation of CD34<sup>+</sup>, MNC and CFU-GM in leukapheresis products

Fifty-six harvested products from 35 donors mobilized with G-CSF were studied. The correlation of numbers per kg patient's body weight of CD34<sup>+</sup>, MNC and CFU-GM were shown in Figs. 2-4.

Table 1 Effect of G-CSF on hematological values

Parameters	Day 0	Day 5 before collection	Day 5 after collection
WBC x 10 <sup>9</sup> /l	6.89 ± 1.37*	43.19 ± 9.31****	33.09 ± 9.38
RBC x 10 <sup>12</sup> /l	4.95 ± 0.58 **	4.73 ± 0.49***	4.34 ± 0.47
Hct %	41.87 ± 4.05**	39.95 ± 4.25***	36.58 ± 3.84
Plt x 10 <sup>9</sup> /l	260.33 ± 40.84**	237.13 ± 39.84***	83.53 ± 20.57
ANC x 10 <sup>9</sup> /l	3.89 ± 1.15*	34.81 ± 7.58****	28.94 ± 9.24
AL x 10 <sup>9</sup> /l	2.41 ± 0.42*	4.96 ± 1.77***	2.20 ± 0.91
AMNC x 10 <sup>9</sup> /l	2.77 ± 0.54*	7.83 ± 2.50***	3.81 ± 1.16
CFU-GM colonies/µl	0.16 ± 0.18*	5.38 ± 4.60	
CD34 <sup>+</sup> cells/µl	1.29 ± 1.76*	51.36 ± 36.12	

Mean ± ISD: Difference from day 5 before collection with *p* < 0.001\*, *p* < 0.05\*\*  
Difference from day 5 after collection with *p* < 0.001\*\*\*, *p* < 0.05\*\*\*\*

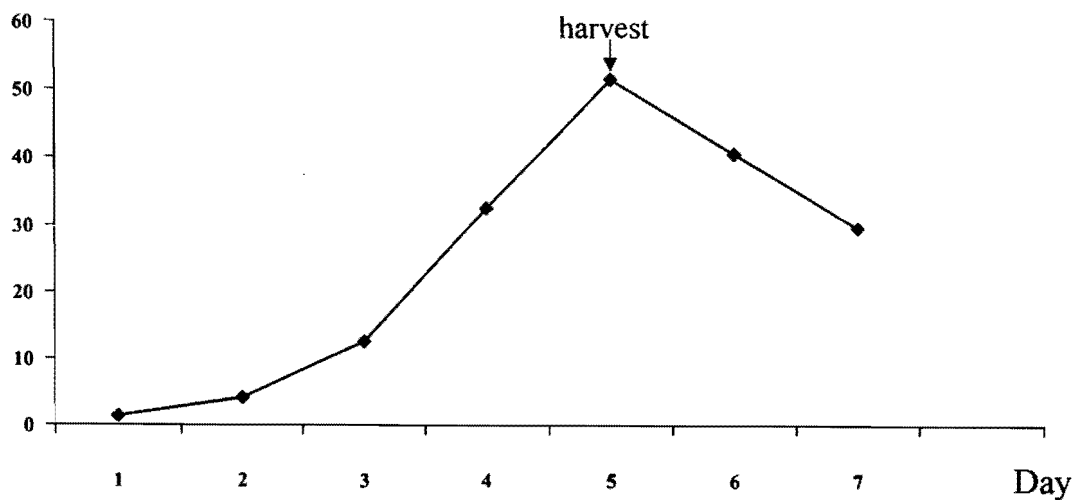
**Table 2** Effect of G-CSF on lymphocyte subsets

Parameter(s)	%		Absolute count $\times 10^9/l$	
	Day 0	Day 5	Day 0	Day 5
Lymphocyte	35.22 $\pm$ 7.27*	12.00 $\pm$ 3.09	2.41 $\pm$ 0.42*	5.14 $\pm$ 1.75
CD3 <sup>+</sup>	62.28 $\pm$ 6.90	63.22 $\pm$ 6.30	1.50 $\pm$ 0.31*	3.25 $\pm$ 1.12
CD4 <sup>+</sup>	35.44 $\pm$ 5.77	35.72 $\pm$ 6.86	0.86 $\pm$ 0.22*	1.83 $\pm$ 0.72
CD8 <sup>+</sup>	26.39 $\pm$ 6.20	27.22 $\pm$ 6.46	0.63 $\pm$ 0.18*	1.40 $\pm$ 0.57
CD4 <sup>+</sup> /CD8 <sup>+</sup>	1.42 $\pm$ 0.44	1.41 $\pm$ 0.53		

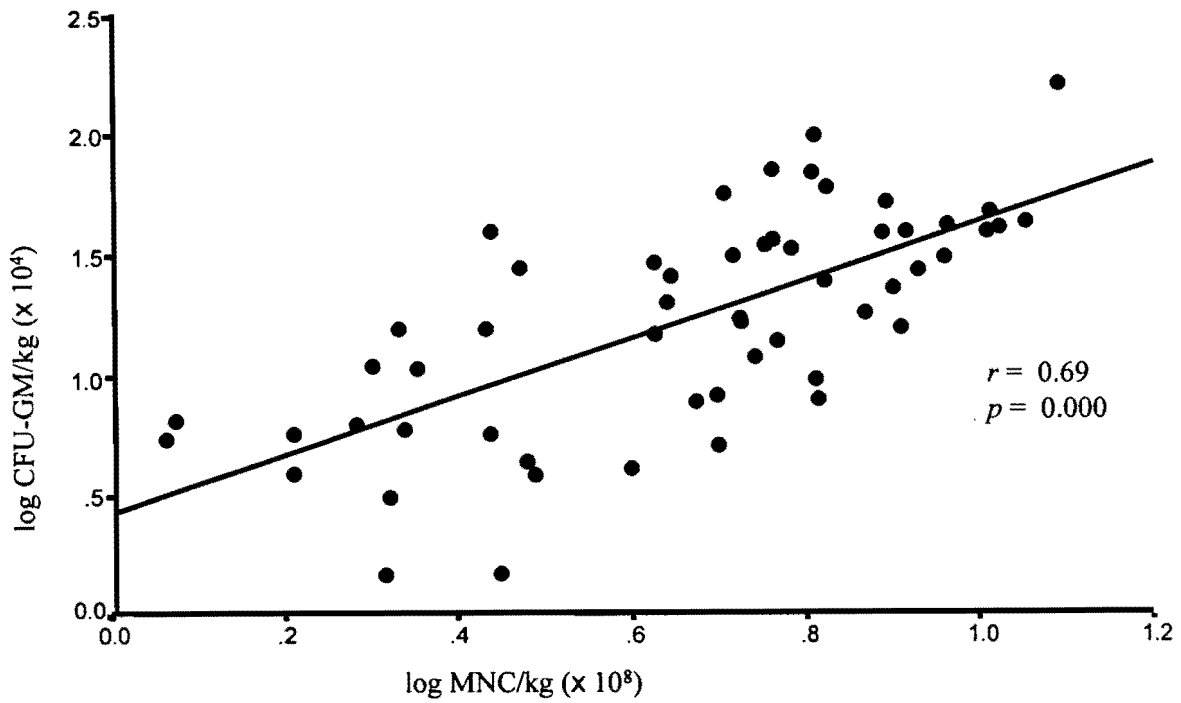
Mean  $\pm$  1 SD: Difference between day 0 and day 5 with  $p < 0.001^*$

**Table 3** Characteristics of peripheral blood stem cell collection (Mean  $\pm$  1 SD)

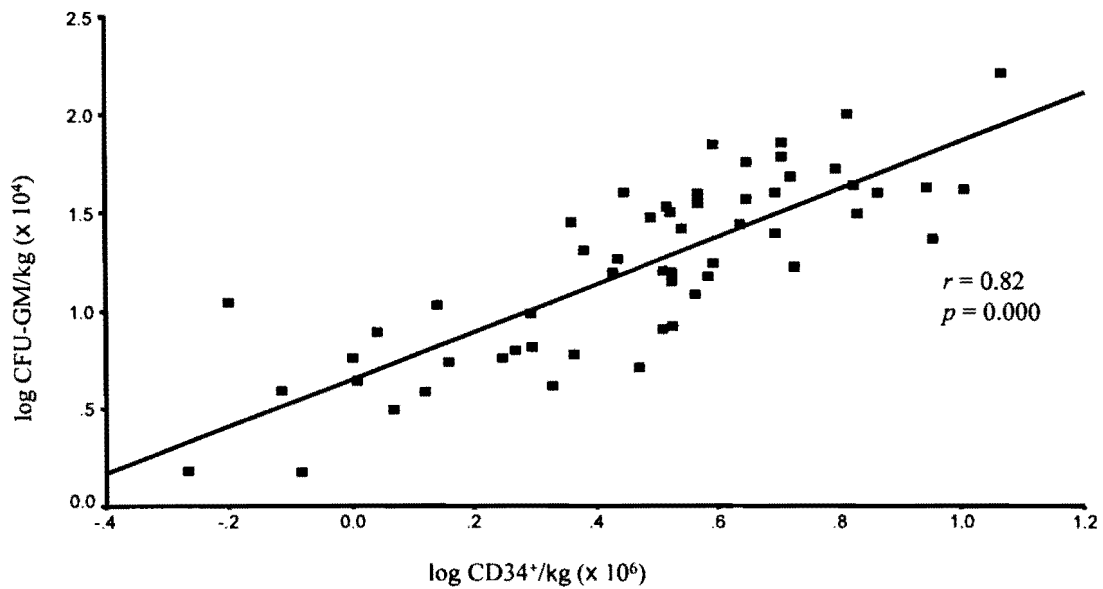
Volume (liters)	Flow rate (ml/minute)	ACD/Blood ratio	Final volume (ml)	Time (minutes)
19.27 $\pm$ 4.65	60.53 $\pm$ 10.03	1:13.31 $\pm$ 0.47	314.14 $\pm$ 72.24	325.40 $\pm$ 73.36

CD34<sup>+</sup> cells/ $\mu$ l

**Fig. 1** Effect of G-CSF on peripheral blood CD34<sup>+</sup> cell counts. G-CSF was administered on days 1-7 (7.5  $\mu$ g/kg/day) and PBPC were collected on day 5.



**Fig. 2** Correlation between CFU-GM  $\times 10^4$ /kg and MNC  $\times 10^8$ /kg in 56 leukapheresis products.



**Fig. 3** Correlation between CFU-GM  $\times 10^4$ /kg and CD34<sup>+</sup>  $\times 10^6$ /kg in 56 leukapheresis products.

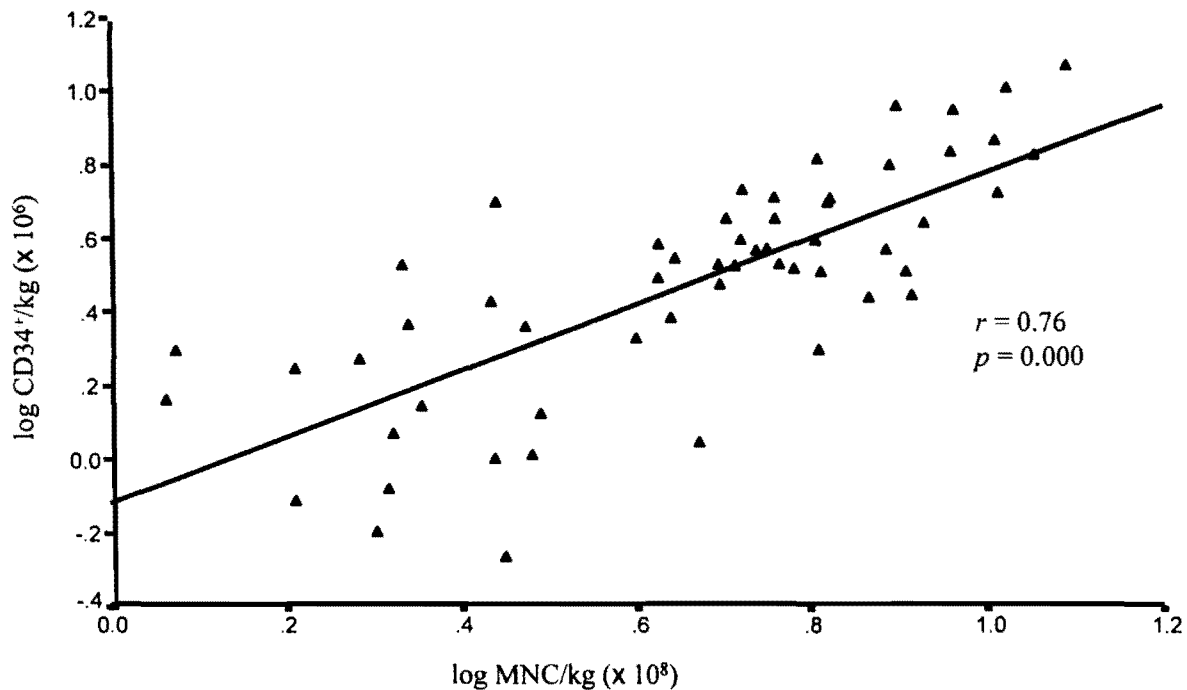


Fig. 4 Correlation between  $CD34^+ \times 10^6/kg$  and  $MNC \times 10^8/kg$  in 56 leukapheresis products.

## DISCUSSION

The advantage of PBPC donation in comparison to bone marrow aspiration is the avoidance of risks and discomfort associated with the surgical procedure. The disadvantages are side effects from the exposure to a hematopoietic growth factor and the need for one or more leukapheresis session(s). The side effects include bone pain, headache, general fatigue, nausea and changes of their own laboratory data such as elevation in lactate dehydrogenase and alkaline phosphatase.<sup>5,7-9</sup> According to Murata *et al.*,<sup>9</sup> the incidence of bone pain increased when G-CSF was given at a dose of 8.8  $\mu g/kg/day$  or more, headaches were frequent in donors younger than 35 years and the incidence of nausea or vomiting was high in female donors. On the basis

of available data, it appears that for doses between 5 and 10  $\mu g/kg/day$ , a relationship exists between the G-CSF dose and the degree of progenitor cell mobilization.<sup>5,10-12</sup> In this study, we used G-CSF at 7.5  $\mu g/kg/day$ . The number of  $CD34^+$  cells reached a maximum on day 5 with a 40-fold increase and began to decrease although G-CSF administration was continued. This is consistent with the previous studies of Dreger *et al.*<sup>12</sup> and Stroncek *et al.*<sup>5</sup> which showed that progenitor cell peaks were always observed on day 5 with the dose of 10  $\mu g/kg/day$  of G-CSF administration and the quantities of  $CD34^+$  cells collected from the peripheral blood of healthy people could be increased by increasing G-CSF dosage but not duration of G-CSF treatment. Mutsumaga *et al.*<sup>13</sup> reported that the increase of  $CD34^+$  cells occurred 6-

7 days after G-CSF administration at a dose of 2.5  $\mu g/kg/day$  for 6 days and at 5.0  $\mu g/kg/day$  for the following 4 days. Majolino *et al.*<sup>14</sup> reported that after administration of rhG-CSF 16  $\mu g/kg/day$  for 4 days the  $CD34^+$  cells peaks to  $147.0 \times 10^6/l$  on day 4 with a median increase of 65.3 times. These studies suggest that the peak of  $CD34^+$  cells depends on the dose of G-CSF administration. Moreover, the timing of the peak in the number of  $CD34^+$  cells and the quantity of  $CD34^+$  cells collected, varied among individuals.

After 5 days of G-CSF administration, we found that WBC, ANC, AL, AMNC, CFU-GM,  $CD34^+$  and absolute lymphocyte subsets increased but RBC, Hct and platelet counts decreased. This was compatible with the studies of An-

derlini *et al.*,<sup>15</sup> Murata *et al.*,<sup>9</sup> Matsunaga *et al.*,<sup>13</sup> and Gabrilove *et al.*<sup>16</sup> In contrast, Stroncek *et al.*<sup>17</sup> and Dreger *et al.*<sup>12</sup> found that WBC and ANC increased one day after G-CSF injection until day 6 but the platelet counts, RBC counts and Hct remained unchanged from the pretreatment values. However, Stroncek *et al.*<sup>17</sup> also showed that platelet production was decreased in healthy donors given G-CSF for only 5 days before collection of PBPC. The decrease in platelet counts was not sufficient to preclude the apheresis procedure.<sup>15</sup> On day 5 after PBPC collection, all hematological values in our study decreased; this was compatible with the study of Stroncek *et al.*<sup>17</sup> They also found that platelet counts, RBC and Hct returned to pretreatment values on days 16, 27 and 27, respectively, but ANC was still lower. However, all neutropenia was asymptomatic and had resolved on follow up evaluation.<sup>9</sup>

The PBPC collected contained  $2-4 \times 10^9/l$  T-lymphocytes ( $CD3^+$ ) which was higher than the number of T-lymphocytes in harvested bone marrow ( $1 \times 10^9/l$ ) used for transplantation in an adult.<sup>6</sup> The increased number of T-lymphocytes could result in peripheral blood stem cell transplantation (PBSCT) than in bone marrow transplantation (BMT), particularly in aggravating the development of graft versus host disease.

In this study, we used a new continuous flow cell separator (Cobe Spectra) with large volume leukapheresis on day 5 of G-CSF mobilization. On average, 19 liters of blood were processed with a mean flow rate of 60 ml/minute. One or two leukapheresis sessions were adequate to collect the number of  $CD34^+$  cells required for allo-

genic stem cell transplantation ( $4 \times 10^6$   $CD34^+$  cells/kg). This resulted in a slight reduction in donor hematocrit and approximately 50 percent depletion in circulating platelets, the result of which was similar to the study by Hillyer *et al.*<sup>18</sup> who also found that the platelet count returned to baseline level within 7 days after apheresis. CFU-GM were increased 2-fold, which confirmed the reports that large volume leukapheresis increases progenitor cell concentration and yield in animals<sup>19</sup> and human.<sup>20,21</sup>

We observed a positive correlation between the number of MNC,  $CD34^+$  and CFU-GM per kilogram body weight in the leukapheresis products. Similar results were found in leukapheresis products after treatment with chemotherapy and G-CSF in adult patients<sup>22</sup> and pediatric patients.<sup>23</sup> Although CFU-GM assays have been used as a standard method, it takes 2 weeks to obtain the results. Enumeration of  $CD34^+$  cells which can be performed within 2 hours should thus be better than CFU-GM assay and should be used as a guide for estimating the quantity of progenitor cells in PBPC collection, in agreement with previous reports.<sup>23-25</sup>

With the G-CSF dose at 7.5  $\mu\text{g}/\text{kg}/\text{day}$  and one or two large volume leukapheresis sessions, we can collect adequate numbers of  $CD34^+$  cells for allogeneic peripheral blood progenitor cell transplantation. It is not necessary to use a higher dose of G-CSF (10-16  $\mu\text{g}/\text{kg}/\text{day}$ ) as indicated in other reports.<sup>3,23,26</sup>

Although the collection of blood stem cells for allografting has practical advantage over the traditional marrow harvest, long term effects of G-CSF in normal donors

are presently still unknown and long term monitoring of donors will be required to answer this question definitively.

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