

Optimizing Dose and Scheduling of Filgrastim (Granulocyte Colony-Stimulating Factor) for Mobilization and Collection of Peripheral Blood Progenitor Cells in Normal Volunteers

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To define an optimal regimen for mobilizing and collecting peripheral blood progenitor cells (PBPC) for use in allogeneic transplantation, we evaluated the kinetics of mobilization by filgrastim (recombinant met-human granulocyte colony-stimulating factor [r-metHuG-CSF]) in normal volunteers. Filgrastim was injected subcutaneously for up to 10 days at a dose of 3 (n = 10), 5 (n = 5), or 10 $\mu\text{g}/\text{kg}/\text{d}$ (n = 15). A subset of volunteers from each dose cohort underwent a 7L leukapheresis on study day 6 (after 5 days of filgrastim). Granulocyte-macrophage colony-forming cell (GM-CFC) numbers in the blood were maximal after 5 days of filgrastim; a broader peak was evident for CD34+ cells between days 4 and 6. The 95% confidence intervals (CI) for mean number of PBPC per milliliter of blood in the three dose cohorts overlapped on each study day. However, on the peak day, CD34+ cells were significantly higher in the 10 $\mu\text{g}/\text{kg}/\text{d}$ cohort than in a pool of the 3 and 5 $\mu\text{g}/\text{kg}/\text{d}$ cohorts. Mobilization was not significantly influenced by volunteer age or sex. Leukapheresis products obtained at the 10 $\mu\text{g}/\text{kg}/\text{d}$

kg/d dose level contained a median GM-CFC number of $93 \times 10^4/\text{kg}$ (range, $50 \times 10^4/\text{kg}$ to $172 \times 10^4/\text{kg}$). Collections from volunteers receiving lower doses of filgrastim contained a median GM-CFC number of $36 \times 10^4/\text{kg}$ (range, $5 \times 10^4/\text{kg}$ to $204 \times 10^4/\text{kg}$). The measurement of CD34+ cells per milliliter of blood on the day of leukapheresis predicted the total yield of PBPC in the leukapheresis product ($r = .87$, $P < .0001$). Assuming a minimum GM-CFC requirement of $50 \times 10^4/\text{kg}$ (based on our experience with autologous PBPC transplantation), all seven leukapheresis products obtained at the 10 $\mu\text{g}/\text{kg}/\text{d}$ dose level were potentially sufficient for allogeneic transplantation purposes. We conclude that in normal donors, filgrastim 10 $\mu\text{g}/\text{kg}/\text{d}$ for 5 days with a single leukapheresis on the following day is a highly effective regimen for PBPC mobilization and collection. Further studies are required to determine whether PBPC collected with this regimen reliably produce rapid and sustained engraftment in allogeneic recipients.

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TRANSPLANTATION of autologous granulocyte colony-stimulating factor (G-CSF)-mobilized peripheral blood progenitor cells (PBPC) results in rapid and durable trilineage hematopoietic recovery after myeloablative chemotherapy.¹⁻⁴ This observation has led to interest in the use of such cells for allogeneic transplantation and the recent publication of a number of preliminary studies.⁵⁻⁷ However, the optimal dose of G-CSF for progenitor cell mobilization is not well defined in normal sibling donors.⁸ Furthermore, the kinetics of PBPC release induced by G-CSF have not been extensively evaluated in donors with normal bone marrow function.

It was with these issues in mind that the current phase I/II study was initiated. Three cohorts of normal volunteers received filgrastim for up to 10 days at increasing dose levels: 3, 5, or 10 $\mu\text{g}/\text{kg}/\text{d}$. The principal aims of this study were to define the kinetics of PBPC mobilization in normal volunteers to optimize PBPC collection and to confirm the tolerability of filgrastim given in this situation. The data indicate that the optimal timing for leukapheresis is after 5 days of filgrastim and that in the majority of normal donors receiving doses of 10 $\mu\text{g}/\text{kg}/\text{d}$ of filgrastim, potentially sufficient PBPC for allogeneic transplantation may be collected in a single leukapheresis.

MATERIALS AND METHODS

Recruitment and Eligibility

Normal volunteers were recruited through the Victorian branch of the Australian Bone Marrow Donor Registry. To be eligible, volunteers had to fulfil the following criteria: (1) age between 18 and 55 years, inclusive; (2) good general health with Eastern Cooperative Oncology Group (ECOG) performance status 0; (3) normal full blood examination, coagulation profile, and renal and liver function; and (4) written, informed consent.

Exclusion criteria included a past history of malignancy or psoriasis, current pregnancy or lactation, or regular medication other than

oral contraceptives. Sexually active women of child-bearing age were included only if they could use adequate contraception for the duration of the study. This study was approved by the Institutional Ethics Committee of the Royal Melbourne Hospital (Victoria, Australia) and conducted within the guidelines of the Australian National Health and Medical Research Council on Human Experimentation.

G-CSF Administration

Filgrastim (Amgen, Melbourne, Australia) was administered for 10 days, unless the volunteer experienced a toxicity greater than World Health Organization (WHO) grade 2 or the white blood cell (WBC) count exceeded specified limits (greater than $75 \times 10^9/\text{L}$ on any one of the first 6 days of the study and greater than $50 \times 10^9/\text{L}$ beyond day 6). Doses were based on ideal body weight. Volunteers were recruited in sequential cohorts, with 10 evaluated at the 3 $\mu\text{g}/\text{kg}/\text{d}$ dose level initially. Five of these received filgrastim as a continuous 24-hour subcutaneous (SC) infusion, and five received

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filgrastim as an SC bolus. As there were no clearcut differences in the kinetics and magnitude of PBPC response between routes, all subsequent volunteers at higher dose levels received filgrastim as an SC bolus. Five volunteers received 5 $\mu\text{g}/\text{kg}/\text{d}$, and 10 volunteers received 10 $\mu\text{g}/\text{kg}/\text{d}$ according to this protocol.

To closely define the peak time of PBPC mobilization induced by filgrastim, five further volunteers were evaluated at 10 $\mu\text{g}/\text{kg}/\text{d}$, including two who had previously received 3 $\mu\text{g}/\text{kg}/\text{d}$ (after washout periods of 10.5 and 11 months, respectively). This group received filgrastim for 7 days regardless of WBC count and did not undergo leukapheresis.

Specimens

Peripheral blood specimens were taken each day before each filgrastim injection. WBC counts, absolute neutrophil counts (ANCs), serum G-CSF assays, and toxicity assessments were performed daily from baseline (study day 1) to day 11 and on days 15 and 40. Blood samples for the clonogenic progenitor cell and CD34⁺ cell assays were taken at baseline and after 3, 5, 7, and 10 days of filgrastim injections (ie, study days 4, 6, 8, and 11). In the final five volunteers, additional samples were taken after four and six doses of filgrastim. Lymphocyte numbers and T-cell subsets were evaluated on study days 1 and 6. Biochemical screens were performed on days 1 and 4. WBC were measured using an automated cell counter (Sysmex NE8000; TOA, Kobe, Japan). ANC were estimated after a manual leukocyte differential.

Leukapheresis

In consenting subjects with adequate venous access, a single leukapheresis was performed on study day 6 (ie, after 5 days of filgrastim administration) using antecubital fossa veins. Seven liters of blood were processed using a Fenwal CS 3000 cell separator (Baxter, Deerfield, IL). Specimens were taken for progenitor cell assays and T-cell analysis. The cells were not infused into allogeneic recipients. For the purposes of this study, a target yield of greater than 50×10^4 GM-CFC per kilogram of recipient weight (based on a standard 70-kg recipient) was defined as sufficient to exceed the minimum required progenitor dose for allogeneic transplantation purposes.

Clonogenic Assays

Granulocyte-macrophage colony-forming cells (GM-CFC), erythroid colony-forming cells (burst-forming units-erythroid [BFU-E]), and mixed myeloid-erythroid colony-forming cells (Mix-CFC) were assayed in agar cultures as previously described.^{9,10}

Peripheral blood and leukapheresis light-density cells were assayed at 10^4 and 10^5 cells per 1 milliliter culture. GM-CFC cultures were stimulated by 500 U of recombinant human (rh) G-CSF, 100 ng rhGM-CSF, and 100 ng rh stem cell factor (SCF). BFU-E and Mix-CFC cultures were stimulated by 100 ng rhGM-CSF, 100 ng rhSCF, 100 ng rh interleukin-3 (IL-3), 100 ng rhIL-6, and 4 U rh erythropoietin (Epo). All recombinant cytokines were produced and provided by Amgen Inc (Thousand Oaks, CA). Triplicate cultures were scored using a dissection microscope at $35 \times$ magnification after 14 days of incubation in a fully humidified atmosphere of 5% CO₂ in air at 37°C. Mix-CFC were enumerated only if total colony numbers per culture were greater than 50 to ensure unambiguous assessment of colony composition. The numbers of progenitor cells per milliliter of blood and per milliliter of leukapheresis were calculated as previously described.¹¹

Flow Cytometric Analysis

CD34 cell estimations. Enumeration of CD34⁺ cells was performed on the mononuclear fractions of samples separated by Ficoll-

Hypaque (Pharmacia, Uppsala, Sweden) density gradient centrifugation. By loading the initial sample at a standard concentration (5×10^6 leukocytes per milliliter) and carefully documenting the yield of cells from the gradients, it was possible to calculate the numbers of CD34⁺ cells per milliliter in the starting population. Analysis was performed on a Coulter Profile II flow cytometer (Hialeah, FL). Pilot experiments using low orthogonal light scatter gating and single-color CD34 cell estimations were found to be unreliable in situations involving G-CSF or GM-CSF therapy, as the mobilization of large numbers of myeloid cells increased nonspecific background to levels that obscured the CD34 peak. To overcome this, a two-color method was used in which lineage-specific antibodies (CD3 [Leu4; Becton Dickinson, Mountain View, CA], CD2 [T11], CD14 [MY4], CD19 [B4], and CD20 [B1]; all from Coulter) labeled with Tricolor dye (Caltag, San Francisco, CA) were used to separate these cells from the lineage-negative CD34⁺ cells, which were stained with HPCA2-phycoerythrin. These could then be quantified accurately, and fluorescein-conjugated CD38 antibody (Caltag) used to determine CD38 subsets. The cut-off point for the fluorescein channel was determined from the negative control antibody profiles. All percentages were calculated relative to this cut-off point.

Leukocyte marker studies. Aliquots (0.5×10^6 to 1.0×10^6) of mononuclear cells were incubated with optimal concentrations of the following monoclonal antibodies (obtained from the American Type Culture Collection, Rockville, MD, unless otherwise indicated): anti-HLA-DR (2.06), CD2 (LyM1; a gift from Dr M. Sandrum, Austin Research Institute, Melbourne, Australia), CD3 (OKT3), CD4 (OKT4), CD5 (T1; Coulter), CD7 (3A1), CD8 (OKT8), CD15 (WEMG1), CD16 (Leu 11b; Becton Dickinson), CD19 (FMC63; a gift from Dr H. Zola, Flinders Medical Centre, Adelaide, Australia), CD25 (HB 8784), CD56 (NKH1; Coulter), CD57 (HNK1), and the negative control antibodies AG11 (IgG1) and AG12 (IgM). Fluorescein-labeled F(ab)₂ fragments of sheep anti-mouse immunoglobulin antibody (DDAF; Silenus, Melbourne, Australia) were used to indirectly stain the cells, which were then fixed in 1% formalin. Forward scatter and orthogonal light scatter parameters were used to define the lymphocyte region. Positivity for each antibody was determined relative to the relevant negative control antibody.

Measurement of Serum G-CSF Levels

Serum samples were stored in small aliquots at -20°C until use. G-CSF levels were measured by enzyme-linked immunosorbent assay (ELISA) as previously described,¹² with some modifications. Standards and samples were diluted in pooled normal human serum (Australian Red Cross, South Melbourne, Australia), which was selected to have a low background. The monoclonal anti-G-CSF antibody LMM201 was directly biotin-conjugated so that the rabbit-anti-mouse-Ig step was no longer required. The sensitivity of the ELISA in serum was 100 pg/mL.

Statistical Methods

For interval data (including WBC count, neutrophil counts, and serum concentrations of G-CSF), 95% confidence intervals (CIs) for the mean were calculated. Differences between groups were not considered significant if the 95% CIs overlapped ($P > .05$). PBPC data were distributed over wide ranges in all groups, and sample sizes precluded formal normality testing. To accurately represent the results, actual data points for GM-CFC and CD34⁺ cells are plotted in graphs (see Results). Comparisons of PBPC data between multiple groups were performed as for the other interval data after calculation of the 95% CIs for the means. Student's *t* test was used for comparisons of PBPC per milliliter blood levels on the day of leukapheresis

Table 1. Adverse Events in Normal Volunteers Receiving Filgrastim

Adverse Event	WHO Grade	Total (n = 30)*	3 $\mu\text{g}/\text{kg}/\text{d}$ (n = 10)	5 $\mu\text{g}/\text{kg}/\text{d}$ (n = 5)	10 $\mu\text{g}/\text{kg}/\text{d}$ (n = 15)
Bone pain	1	28	10	5	13
Lethargy/tiredness	1	19	7	5	7
Elevated alk phos	1	15	4	4	7
	2	6	0	0	6
Headache	1	10	2	3	5
Dizziness	1	6	0	1	5
Flu-like symptoms	1	5	3	1	1
Muscle pain	1	5	0	1	4
Perirectal abscess	2	1	0	1	0
Hyperventilation	2	1	0	1	0
Mouth ulcers	2	1	1	0	0
Painful right arm/ tingling hand	3	1	0	0	1

Events were included only if they were \geq WHO grade 2 or occurred in greater than five volunteers.

Abbreviation: alk phos, plasma alkaline phosphatase.

* 30 assessable episodes in 28 subjects.

between the 10 $\mu\text{g}/\text{kg}/\text{d}$ cohort and a pool of the 3 and 5 $\mu\text{g}/\text{kg}/\text{d}$ cohorts; *P* values are two-tailed.

Proportional data were analyzed by the χ^2 test. Associations between two interval variables were analyzed by Pearson product-moment correlation and linear regression.

RESULTS

Demographics

Thirty volunteers (13 female, 17 male) with a median age of 34 years (range, 24 to 51 years) entered the study. The results for two volunteers were not assessable and have been excluded: one volunteer withdrew for personal reasons on day 1, and the other volunteer received incorrect doses of filgrastim. Two volunteers were studied at both the 3 and 10 $\mu\text{g}/\text{kg}/\text{d}$ dose levels, giving a total of 30 evaluable episodes in 28 volunteers.

Safety

Filgrastim injections were generally well tolerated (Table 1). Most volunteers experienced mild bone pain and fatigue, particularly within the first 3 days of treatment. No volunteer required analgesia stronger than acetaminophen for pain relief. There was no relationship between filgrastim dose and the frequency or severity of symptoms. However, at the 10 $\mu\text{g}/\text{kg}$ dose, alkaline phosphatase levels were more frequently elevated; the levels normalized by day 15 of the study.

One volunteer experienced a transient hyperventilation episode that did not recur despite ongoing injections. One volunteer developed arm pain during treatment that worsened over the next month and was ultimately attributable to a C6-7 disc protrusion, and one volunteer developed a small perianal abscess on day 14 of the study, which resolved with antibiotics.

Effect of Filgrastim Dose on WBC Count, ANC, and Trough G-CSF Serum Levels

Filgrastim induced a significant rise in WBC count (Fig 1) and ANC (data not shown) in all volunteers. Clear evi-

dence of a direct relationship between dose and the level of the leukocytosis was not found. Although the mean WBC and neutrophil counts were generally higher for 10 $\mu\text{g}/\text{kg}/\text{d}$, for any given duration of filgrastim administration, the 95% CIs of the means for each cohort overlapped.

The mean trough serum concentrations of G-CSF demonstrated a trend to higher concentrations with higher doses of injected drug (Fig 2). However, no statistically significant differences were found between cohorts. A high degree of interindividual variation was evident, particularly at the highest dose level.

Kinetics of PBPC Mobilization

Figure 3 illustrates the increase and decrease in progenitor cell numbers during filgrastim injections for up to 10 days at the three different dose levels studied. Baseline levels of CD34+ cells and GM-CFC per milliliter blood among the three cohorts of volunteers were similar. Broad interindividual variation in PBPC levels over 5- to 15-fold ranges was observed on each day (including the baseline) and for each dose level of filgrastim. The general patterns for mobilization of total CD34+ cells and the clonogenic subsets GM-CFC, BFU-E, and Mix-CFC were similar, with a broad peak after 3 to 7 days of filgrastim, followed by a significant decrease despite ongoing G-CSF injections.

At 3 $\mu\text{g}/\text{kg}/\text{d}$, total CD34+ cell numbers appeared to peak after 5 days and GM-CFC, after 7 days of G-CSF. At 10 $\mu\text{g}/\text{kg}/\text{d}$, peak levels were observed for both GM-CFC and CD34+ cells after 5 days of G-CSF. To more closely define a peak time of PBPC mobilization, the final five volunteers received 10 $\mu\text{g}/\text{kg}/\text{d}$ of filgrastim, and progenitor cell levels were measured on days 4 to 8, inclusive (Fig 4). A definite peak was observed for GM-CFC after 5 days of filgrastim. However, the pattern for CD34+ cells in the same volunteers was not as consistent and suggested a broad peak after 4, 5, and, perhaps, 6 days of filgrastim.

Effect of Filgrastim Dose on PBPC Mobilization

A trend to increased PBPC numbers measured as GM-CFC or CD34+ cells with increased dose of filgrastim was evident after 3 and 5 days (Fig 3A and B). However, on each study day, the 95% CIs for the means for each dose cohort overlapped, indicating the differences were not statistically significant. A comparison of peripheral blood CD34+ cell numbers per milliliter on the day of leukapheresis (after 5 days of G-CSF) between the 10- $\mu\text{g}/\text{kg}/\text{d}$ cohort (mean, 54,222; 95% CI, 35,930 to 72,514) and a pool of the lower dose cohorts (mean, 20,927; 95% CI, 7,323 to 34,521) did suggest that the highest dose mobilized more PBPC (*P* = .01). The same comparison for peripheral blood GM-CFC per milliliter (10 $\mu\text{g}/\text{kg}/\text{d}$: mean, 15,175; 95% CI, 7,915 to 22,435; pool of 3- and 5- $\mu\text{g}/\text{kg}/\text{d}$ cohorts: mean, 7,970; 95% CI, 2,914 to 11,434) did not demonstrate a statistically significant difference (*P* = .06) but was consistent with a dose effect. Overall, filgrastim induced a median increase of 157-fold in circulating GM-CFC (range, 52- to 3,940-fold) and 22-fold in CD34+ cells (range, 8-fold to 105-fold) after 5 days of injections of 10 $\mu\text{g}/\text{kg}/\text{d}$.

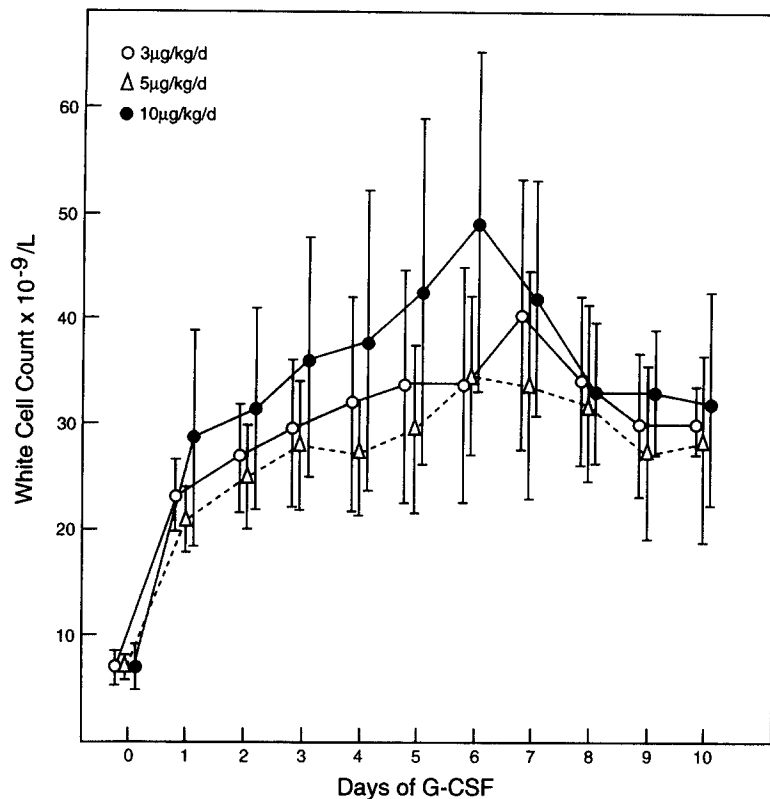


Fig 1. The mean WBC count in normal volunteers during filgrastim administration at different dose levels: 3 µg/kg/d, n = 10; 5 µg/kg/d, n = 5; 10 µg/kg/d, n = 10. Error bars indicate standard deviations (SD).

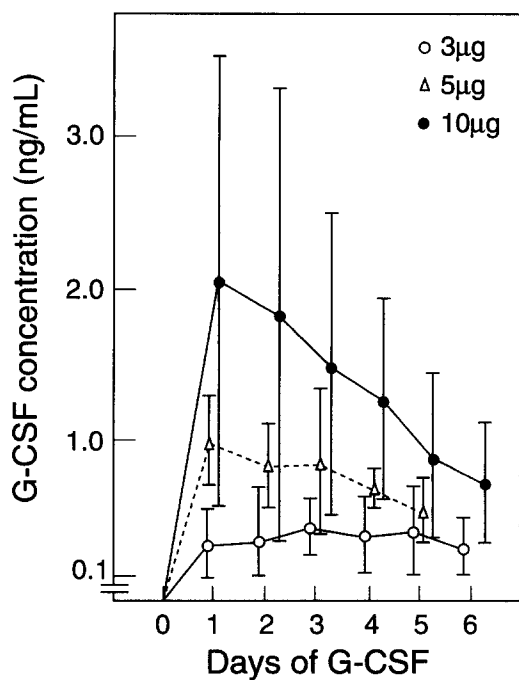


Fig 2. The mean trough serum G-CSF concentrations in normal volunteers during filgrastim administration at different dose levels. Error bars indicate SD.

Two volunteers who had demonstrated relatively poor PBPC mobilization with 3 µg/kg/d were retested with 10 µg/kg/d filgrastim after an interval of more than 6 months (Fig 5). In both subjects, significantly higher elevations in trough serum levels of G-CSF and WBC count were seen at the higher dose. One subject also demonstrated a substantial increase in PBPC mobilization, while the other subject showed no additional increase in GM-CFC per milliliter blood (or CD34+ cells; data not shown) at the higher dose.

Effect of Other Variables on PBPC Mobilization

No significant correlations were found between progenitor cell numbers in the blood after 5 days of filgrastim 10 µg/kg/day and either volunteer age ($r = .42$, $n = 14$; $P = .13$) or trough serum level of G-CSF on that day ($r = .13$, $n = 13$; $P = .67$). No significant differences were observed when these results were grouped by sex ($P = .71$).

Leukapheresis Yields

Within each cohort, several volunteers underwent leukapheresis after five doses of filgrastim to determine whether potentially sufficient cells for allogeneic transplantation could be obtained from a single collection. No complications of the procedure were observed. Results for the 15 leukaphereses are shown in Table 2. All seven volunteers receiving 10 µg/kg/d achieved the target yields of progenitor cells (GM-CFC greater than 50×10^4 /kg) with a single leukapheresis compared with three of four at 5 µg/kg/d and one of four at 3 µg/kg/d ($\chi^2 = 7.33$, $P = .03$).

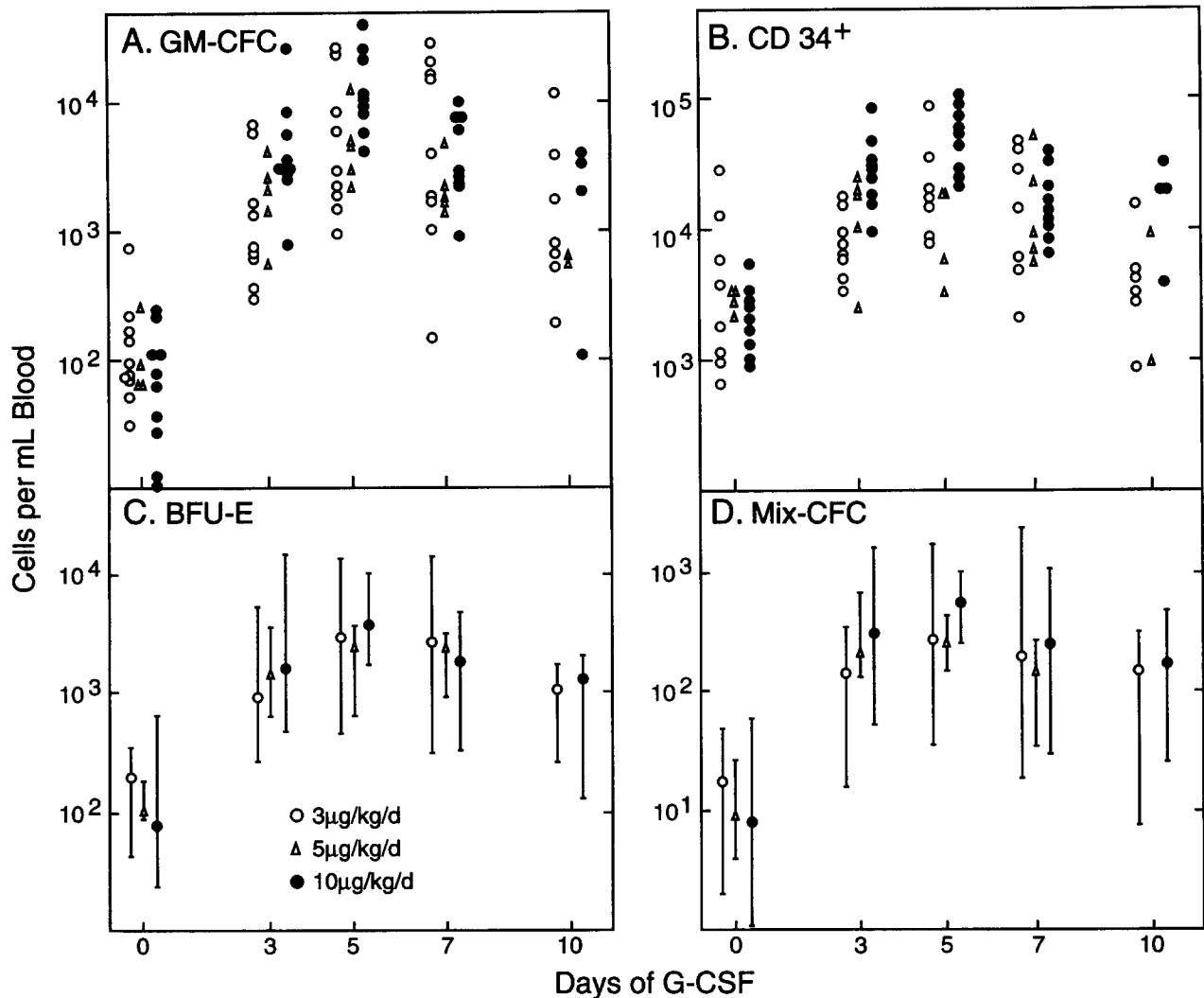


Fig 3. The progenitor cell levels in the peripheral blood in normal volunteers during filgrastim administration at different dose levels. (A and B) Individual results are graphed to emphasize the variability of results within dose cohorts. (C and D) Median results are graphed, and error bars represent the range of progenitor cell levels.

Leukapheresis products also contained large numbers of T cells (median, 42×10^8 ; range, 10×10^8 to 132×10^8), the immunophenotypes of which were not different from resting peripheral blood lymphocytes (data not shown).

Correlations of PBPC Numbers and Leukapheresis Yields

The two assays (GM-CFC and CD34+ cells) demonstrated a good correlation for estimates of progenitor cell numbers in the blood ($r = .86$, $n = 110$; $P < .0001$) and in the leukapheresis products ($r = .8$, $n = 14$; $P = .0006$). The number of GM-CFC in the leukapheresis product was correlated with the number of progenitor cells in the blood, measured either as GM-CFC ($r = .83$, $n = 15$; $P = .0001$) or as CD34+ cells ($r = .87$, $n = 14$; $P < .0001$; Fig 6). The 95% CIs for the predicted number of GM-CFC in a leukapheresis given a newly measured CD34+ cell level in the peripheral blood are also plotted in Fig 6.

DISCUSSION

Previous studies^{5-7,13,14} of PBPC mobilization and collection in normal donors or volunteers have used varying approaches. The G-CSF dose has varied between 5 and 16 $\mu\text{g}/\text{kg}/\text{d}$; leukaphereses have commenced after 3 or 4 days of G-CSF; and one to three collections have been performed. Unfortunately, the substantial differences in techniques for measuring CD34+ cells and GM-CFC between institutions make it difficult to use comparative data across studies to draw firm conclusions about which regimens are superior. The results of the current study speak directly to the question of what constitutes the minimum effective dose of filgrastim for potentially adequate PBPC mobilization in the majority of normal donors.

Our data indicate that the kinetics and magnitude of mobilization were influenced by both the dose and duration of filgrastim. Mobilization occurred in a wave of progenitor

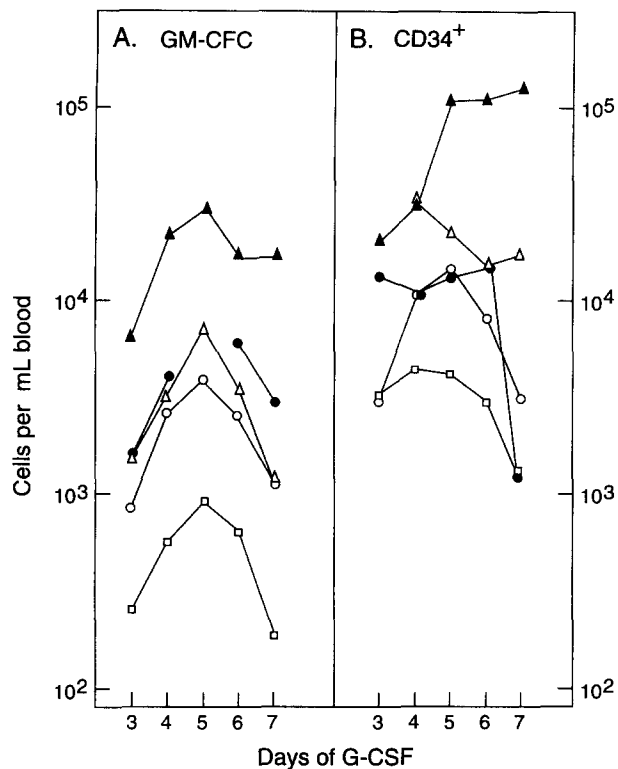


Fig 4. The progenitor cell numbers per milliliter peripheral blood in five volunteers injected with filgrastim 10 $\mu\text{g}/\text{kg}/\text{d}$ for 7 days. Volunteers did not undergo leukapheresis. Individual results for each volunteer are presented.

cell release from the bone marrow, and peak levels were not sustained despite ongoing filgrastim injections. Definition of effects of dose and duration on circulating progenitor cell levels was confounded by the broad interindividual variation in the capacity of normal subjects to mobilize progenitor cells. We have previously shown that this variability is not an artefact of imprecision of the assays used.¹¹ Within the limitations imposed by this biologic heterogeneity and the group sizes, a trend for higher PBPC levels in volunteers receiving the highest dose, 10 $\mu\text{g}/\text{kg}/\text{d}$, was evident. Comparison of results from this group and a pool of the two lower doses suggested a significant difference on the day of leukapheresis. More convincingly, all seven volunteers who received 10 $\mu\text{g}/\text{kg}/\text{d}$ and who underwent a single leukapheresis mobilized greater than our defined minimum target number of cells for allogeneic transplantation purposes, whereas collections from volunteers receiving lower doses of filgrastim were less reliable. These observations are broadly consistent with the results of a smaller study of the effect of filgrastim dose on PBPC mobilization.¹⁵

We also addressed the kinetics of PBPC release. Our data indicate that at 10 $\mu\text{g}/\text{kg}/\text{d}$, the peak level of GM-CFC in the blood is reached after 5 days of filgrastim. Although as sharp a peak was not observed for CD34⁺ cells, a broader peak encompassing days 4 to 6 was defined. Again, these results are consistent with two smaller studies that evaluated a total of 12 donors receiving 10 $\mu\text{g}/\text{kg}/\text{d}$ of G-CSF.^{7,14,15}

Filgrastim was well-tolerated in normal subjects in doses up to 10 $\mu\text{g}/\text{kg}/\text{d}$ for 10 days. The leukapheresis process was also well tolerated, although it is important to note that volunteers with adequate peripheral venous access were selected. By contrast, in one allogeneic transplant study, five of eight unselected donors required a central catheter for venous access.⁶ Because complications such as pneumothorax, subclavian vein thrombosis, and infection are not infrequent with central venous catheters and are unacceptable in volunteer donors, there is a real need to reduce requirements for central catheters by minimizing the number of leukaphereses required.

Our study has demonstrated that filgrastim at 10 $\mu\text{g}/\text{kg}/\text{d}$ for 5 days with a leukapheresis on the following day is an efficient regimen for mobilization and collection of large numbers of PBPC. Whether sufficient cells for reliably rapid and sustained engraftment in allogeneic recipients can be collected with this regimen remains to be proven. The study was closed after recruitment of the 10 $\mu\text{g}/\text{kg}/\text{d}$ cohort, and consequently no conclusion can be made regarding the relative efficacy of higher doses of filgrastim used in studies from Houston, TX (12 $\mu\text{g}/\text{kg}/\text{d}$)⁶ and Seattle, WA (16 $\mu\text{g}/\text{kg}/\text{d}$)⁵ to mobilize progenitor cells.

Previous studies have generally prescribed two or more leukaphereses to ensure adequate collections.⁵⁻⁷ By timing the leukapheresis to occur on the peak day of PBPC levels, one collection may prove to be sufficient. Further studies are required to test this hypothesis. The exact proportion of donors requiring only one leukapheresis will depend ultimately on (1) the minimum target set and (2) the duration of the leukapheresis.

The minimum number of PBPC for rapid and durable engraftment in the allogeneic setting has not been established. We defined a GM-CFC target of $50 \times 10^4/\text{kg}$ as safe and reasonable. Although an arbitrary estimate, this level is conservatively based on approximately twice the minimum dose of GM-CFC required to ensure rapid (platelet count greater than $20 \times 10^9/\text{L}$ in less than 15 days) and sustained engraftment in our autologous transplantation experience.⁴ While successful engraftment in the allogeneic setting has been reported with as few colony-forming units-granulocyte/macrophage (CFU-GM) as $37.8 \times 10^4/\text{kg}$ ¹⁶ and as few CD34⁺ cells as $2.2 \times 10^6/\text{kg}$,⁷ variations between laboratories in the flow cytometric methods for determining numbers of CD34⁺ cells and in the assay conditions for culturing GM-CFC make it difficult to delineate universal guidelines in this respect.

In the current study, satisfactory progenitor cell collections were achieved despite relatively small volumes (7 L) of processed blood. Other studies^{5-7,13,14} have used larger volumes in the order of 10 to 15 L. Unpublished data (May 1995) from our autologous transplantation program indicate that the rate of yield of progenitor cells remains constant over prolonged leukaphereses; therefore, it is likely that larger volume leukaphereses in our subjects would have enabled collection of even larger numbers of progenitor cells.

An additional benefit of being able to reliably schedule a single sufficient collection is the avoidance of the need for cryopreservation. Apart from saving cost, the use of fresh

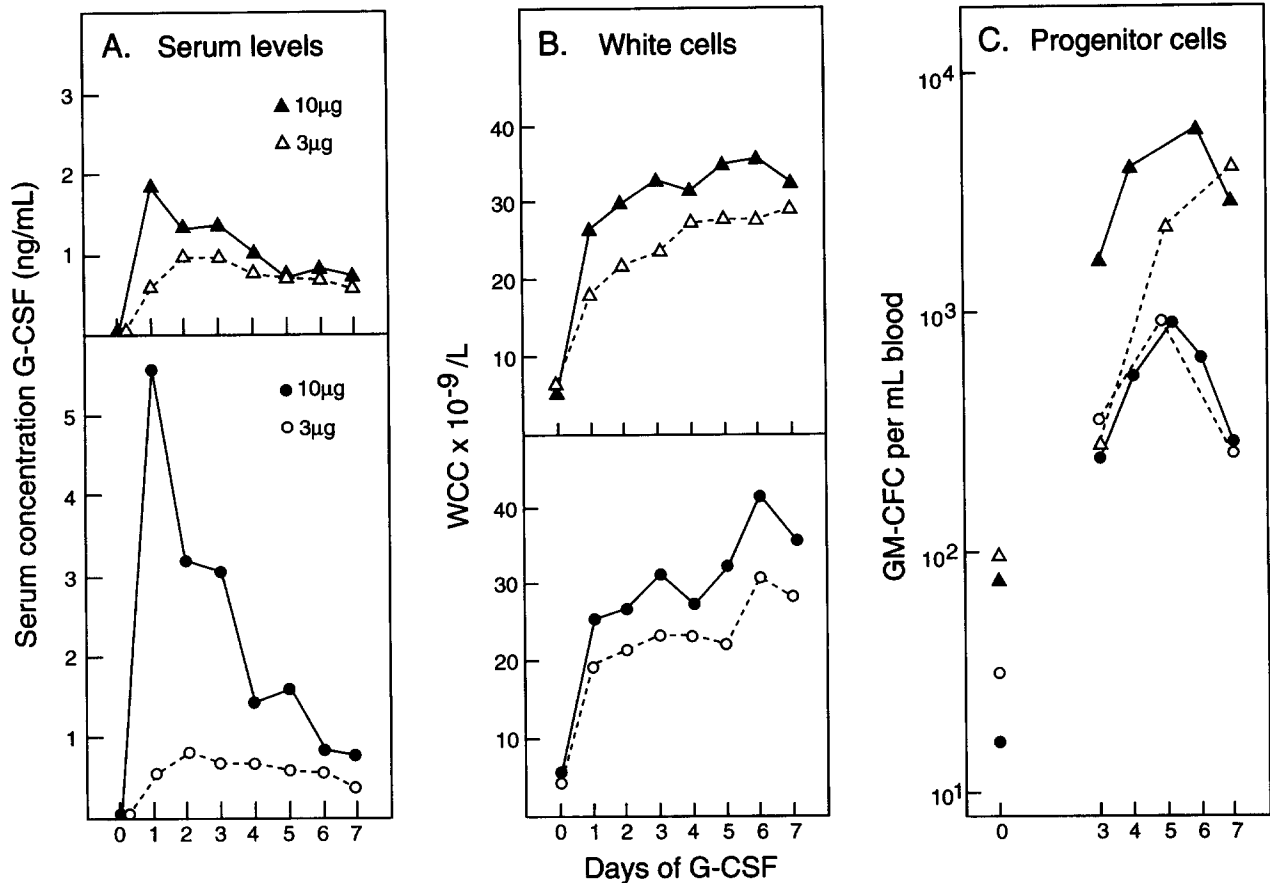


Fig 5. The (A) trough serum G-CSF concentrations, (B) WBC counts, and (C) numbers of GM-CFC per milliliter blood in two volunteers who were evaluated initially at 3 μg/kg/d filgrastim for 10 days and who were subsequently reevaluated at 10 μg/kg/d for 7 days.

Table 2. Progenitor and T-Cell Yields at Leukapheresis

Dose Level and Volunteer No.	Leukapheresis Product/kg*			
	GM-CFC × 10 ⁴	CD34 ⁺ × 10 ⁶	CD34 ⁺ 38 ⁺ × 10 ⁶	CD2 T Cells × 10 ⁶
3 μg/kg/d				
3	13	0.5	—	14
5	36	0.6	—	37
8	204	6.9	—	57
12	5	0.7	—	17
5 μg/kg/d				
11	93	—	—	57
12	20	1.2	—	149
14	65	0.4	—	—
15	74	2.1	—	57
10 μg/kg/d				
16	50	2.0	—	106
20	64	2.0	—	66
21	93	7.1	0.4	71
22	80	5.1	0.4	189
23	97	4.6	0.2	60
24	172	10.6	0.95	183
25	134	2.7	0.3	151

* Cell yields expressed per kilogram body weight assuming a 70-kg recipient.

product may be advantageous to the recipient. In the two recent studies describing allogeneic PBPC transplantation and using cyclosporin A and methotrexate as graft-versus-host disease (GVHD) prophylaxis, platelet and neutrophil engraftment were notably more rapid in the study using fresh PBPC⁵ than in the study using thawed cryopreserved product.⁶

Our data clearly indicate that some volunteers are intrinsically poor responders to filgrastim and that dose escalation may not increase their responsiveness. Consequently, it remains essential to rapidly assess the adequacy of either PBPC mobilization or the yield in the leukapheresis products. Currently, no useful baseline predictors of response to G-CSF have been proven. We were unable to confirm the observation of Dreger et al¹⁵ that age was inversely related to PBPC mobilization. We were able, however, to demonstrate a close correlation between the number of circulating CD34⁺ cells and the number of GM-CFC in the leukapheresis product (Fig 6). Although the 95% CIs are quite wide, it is likely that assessment of peripheral blood CD34⁺ numbers will enable a rapid and reasonably accurate assessment of the quality of the leukapheresis to be performed later that day. This may assist in planning for poor responders who may require a second apheresis. As progenitor cells remain elevated after 6 days of filgrastim, the second collection may

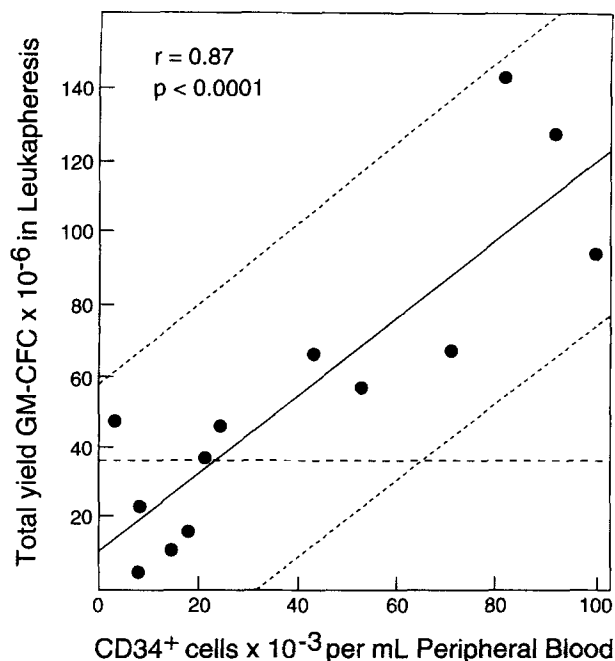


Fig 6. Correlation and regression of peripheral blood CD34+ cells on the day of leukapheresis and the total yield of progenitor cells. The solid line indicates the line of best fit, and the parallel broken lines represent the 95% CIs of estimates of leukapheresis yield for a newly measured level of CD34+ cells in the blood. The minimum target leukapheresis yield corresponding to 50×10^4 GM-CFC per kilogram in a 70-kg recipient is marked by the horizontal broken line.

be conveniently scheduled on the following day after an additional injection of filgrastim.

Implicit in this study and our previous studies,^{1,4} as well as those of others,^{2,3,5-7} is the assumption that the peak of GM-CFC release into the blood (after 5 days of G-CSF) and total CD34+ cell release (after 5 ± 1 days) also represents the peak release of primitive stem cells responsible for durable engraftment. It remains unknown whether GM-CFC represent the cells responsible for rapid in vivo engraftment and, similarly, whether GM-CFC or CD34+ cells are good surrogate markers for the long-term repopulating stem cells that are crucial for the success of allotransplantation.⁸ However, in humans receiving chemotherapy plus G-CSF, both long-term culture-initiating cells (LTC-IC) and CFC appear to be released at the same time.¹⁷ In repopulation experiments in mice, true stem cells and CFC mobilized by G-CSF alone (A.W.R., unpublished data) peak in the blood on the same day. In humans, the CD34+38- fraction of PBPC is highly enriched for LTC-IC,¹⁸ and large numbers of these cells were present in the leukapheresis products from the normal volunteers. Consistent with these observations, LTC-IC in comparable numbers (per mononuclear cells) as present in the bone marrow have been documented in the blood of patients with breast cancer receiving G-CSF.¹⁹

One of the major concerns about PBPC allografting has been a higher incidence and severity of acute GVHD due to large numbers of T cells in the infusion product.^{8,14,15} Our data confirm the high number of T cells in a 200-mL leu-

kapheresis product. However, preliminary evidence from clinical studies indicates that the risk of acute GVHD may not be increased after PBPC allografts.⁵⁻⁷ This suggests that above a particular T-cell threshold, it is the specificity of T cells for genetic disparities between the donor and recipient, rather than the absolute number, that determines the risk for GVHD. If further experience confirms these findings, then strategies such as selection for CD34+ cells or T-cell depletion may not be required.

The cost implications of using allogeneic G-CSF-mobilized PBPC remain to be established. It is likely that daily outpatient injections of G-CSF followed by a leukapheresis will be no more expensive and will be better tolerated than a bone marrow harvest under general anesthesia. The relative costs of different dose regimens of G-CSF will depend not only on the cost of the drug itself, but on factors such as extra staff time, consumables, and laboratory expenses required if more than one collection is necessary. However, the decision to routinely use G-CSF-mobilized PBPC rather than bone marrow for allotransplantation may ultimately depend on whether there are benefits for recipients in terms of accelerated engraftment and, perhaps, an augmented graft-versus-leukemia effect. Optimized mobilization and collection regimens minimizing both donor exposure to G-CSF and the number of leukaphereses should be used in future studies addressing this important question.

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Optimizing dose and scheduling of filgrastim (granulocyte colony-stimulating factor) for mobilization and collection of peripheral blood progenitor cells in normal volunteers [see comments]

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