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Potentiation of Granulocyte Colony-Stimulating Factor–Induced Mobilization of Circulating Progenitor Cells by Seven-Day Pretreatment With Interleukin-3

By Klaus Geissler, Christian Peschel, Dietger Niederwieser, Herbert Strobl, Jutta Goldschmitt, Leopold Öhler, Peter Bettelheim, Peter Kalhs, Christoph Huber, Klaus Lechner, Paul Höcker, and Karin Kolbe

Granulocyte colony-stimulating factor (G-CSF) as a single agent is increasingly used for the mobilization of peripheral blood progenitor cells (PBPCs) for stem cell tranplantation. In patients with perturbed hematopoiesis the mobilizing capacity of G-CSF alone may be inadequate. We have shown in rhesus monkeys that interleukin-3 (IL-3) pretreatment markedly potentiated the increase in PBPC numbers by subsequent administration of granulocyte/macrophage-CSF (GM-CSF). Here we studied the effect of IL-3 pretreatment on G-CSF–induced mobilization of PBPCs in 6 patients with Hodgkin's disease (n = 5) or non-Hodgkin's lymphoma (n = 1) who had low progenitor cell numbers because of previous chemotherapy. Patients were treated in cycle 1 with G-CSF at a dose of 5 μg/kg/d for 5 days and, after a treatment-free interval, received cycle 2 consisting of 5 μg/kg/d of IL-3 for 7 days followed by G-CSF again at a dose of 5 μg/kg/d for 5 days. G-CSF alone increased the the mean number of circulating colony-forming units-GM (CFU-GM) by 21-fold, the number of burst-forming units-erythroid (BFU-E) by 9-fold, and the number of CFU-mix by 24-fold over pretreatment values. Treatment with 5 μg/kg/d of IL-3 for 7 days did not mobilize by itself but significantly potentiated G-CSF–induced mobilization of all progenitor cell types leading to a 56-, 15-, and 46-fold increase over baseline of CFU-GM, BFU-E, and CFU-mix numbers, respectively. In 2 patients in whom leukapheresis was performed after G-CSF alone the target number of 2 × 10^6/kg $CD34^+$ cells was not reached. However, leukapheresis after the IL-3/G-CSF combination obtained ≥2 × 10^6/kg $CD34^+$ cells in 3 of 6 patients, including both patients who had inadequate collection after G-CSF alone. In one patient adequate function of mobilized progenitors could be shown by the demonstration of rapid trilineage engraftment after infusion of progenitors after myeloablative chemotherapy. Seven-day pretreatment with IL-3 may be a useful mean to augment mobilization of circulating progenitors by G-CSF. The combination of IL-3 and G-CSF seems to allow the procurement of sufficient numbers of PBPCs in some patients who cannot be mobilized adequately by G-CSF alone.
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TRANSPLANTATION of peripheral blood progenitor cells (PBPCs) is an established means to protect against hematologic toxicity of myelosuppressive or myeloablative anticancer chemotherapy. The advantages of PBPCs over bone marrow (BM) for autologous transplantation include the ability to collect PBPCs without general anesthesia, the ability to obtain sufficient numbers of progenitors even in the presence of BM damage (due to previous radiotherapy or infiltration with malignant cells), and possibly a lower risk of contamination of the graft with malignant cells. Moreover, infusion of PBPCs alone or as an adjunct to BM results in significantly faster recovery of marrow function than in patients receiving the same treatment without PBPCs.[1-6] Accelerated recovery has been shown to be associated with a number of clinical benefits, including a reduced number of platelet transfusions, a shorter duration of isolation and hospital stay, and a lower requirement for parenteral antibiotics and parenteral nutrition.[3]

Several strategies to mobilize stem cells into the circulation have been designed to facilitate procurement of progenitor cells by leukapheresis. The number of phereses can be decreased if leukapheresis is done at the time of recovery after chemotherapy, especially with the additional stimulus of granulocyte-macrophage colony-stimulating factor (GM-CSF) or granulocyte-CSF (G-CSF).[2,6] Growth factors as single agents have also been successfully used in the mobilization of PB progenitor cells.[7,8] Mobilization of circulating progenitors with G-CSF and their subsequent use for hematopoietic reconstitution is now established.[9,10] Although mobilization by G-CSF alone may allow the harvest of sufficient progenitor cells in patients with unperturbed hematopoiesis, it may be inadequate in patients with low progenitor cell numbers caused by previous chemotherapy.

Combinations of growth factors may represent a strategy to further improve PBPC mobilization without requiring preceding chemotherapy. We have shown in rhesus monkeys that pretreatment with interleukin-3 (IL-3) markedly potentiates the increase in PBPC numbers by subsequent administration of GM-CSF.[11] Here we studied the effect of IL-3 pretreatment on G-CSF–induced mobilization of circulating progenitors in patients with low progenitor cell counts who were potential candidates for autologous stem cell transplantation.

MATERIALS AND METHODS

Patient selection. Table 1 shows the clinical characteristics of the 6 patients who have entered the study. Eligible patients were those with Hodgkin's disease (HD) or non-Hodgkin's lymphoma (NHL) who were potential candidates for autologous stem cell transplantation. Four patients were in complete remission after initial chemotherapy but had considerable risk of relapse (advanced and/

From the Division of Hematology of the 1st Medical Department, Blood Transfusion Service, Institute of Immunology, University of Vienna, Vienna; the Medical Department, University of Innsbruck, Innsbruck; the Department of Laboratory Medicine, AKH Linz, Austria; and the 3rd Medical Department, Johannes Gutenberg University, Mainz, Germany.

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Address reprint requests to Klaus Geissler, MD, 1st Medical Department, Division of Hematology, University of Vienna, Währinger Gürtel 18-20, A-1090 Vienna, Austria.

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Table 1. Patient Characteristics

Patient No.	Age/Sex	Diagnosis/Stage	Anticancer Therapy	Remission Status	Interval to G-CSF (wk)	Interval Between Cycles (wk)
1	43/F	NHL/IV	4 × CHOP, 3 × IMVP-Dexa	PR	8	8
2	26/M	MH/II bulky	6 × MOPP-ABV Mantle Irr	CR	8	4
3	29/M	MH/III	3 × C-MOPP 3 × ABVD, TNI	PR	28	6
4	25/F	MH/II bulky	6 × MOPP-ABV Mantle Irr	CR	12	4
5	50/M	MH/IV	6 × MOPP-ABV	CR	28	12
6	25/F	MH/III	6 × MOPP-ABV	CR	8	10

Abbreviations: MH, Morbus Hodgkin; NHL, non-Hodgkin's lymphoma; TNI, total nodal irradiation; CR, complete remission; PR, partial remission.

or bulky disease) and 2 had an inadequate response to chemotherapy. All patients have had previous chemotherapy with or without extended field radiotherapy but were free of treatment for at least 8 weeks (8 to 28 weeks) before entering the study. Informed consent was obtained in all patients. The study was approved by the Ethical Comitee at our institution.

Study design. Every patient received two cycles of cytokine treatment. During cycle 1, recombinant human G-CSF (rhG-CSF) was administered by subcutaneous (sc) injection at a daily dose of 5 μg/kg for 5 days. During cycle 2, patients were pretreated with rhIL-3 at a dose of 5 μg/kg/d for 7 days and subsequently received rhG-CSF again at a dose of 5 μg/kg/d for another 5 days. In 2 patients leukapheresis was performed after cycle 1 (G-CSF), and in all 6 patients after cycle 2 (IL-3/G-CSF). In each case, 3 leukapheresis procedures were performed on 3 consecutive days immediately after the end of the respective cycle. G-CSF administration was maintained on days with leukapheresis. Leukaphereses were performed via a central catheter processing approximately 10 L of blood over 3 hours using a Fenwal-CS3000 (Baxter Healthcare Corp, Deerfield, IL).

Recombinant cytokines. rhIL-3 used in this study was produced by Genetics Institute (Cambridge, MA) and provided by Sandoz AG (Basel, Switzerland). The nonglycosylated protein was extracted from *Escherichia coli* cells expressing the IL-3 cDNA from a plasmid vector. rhIL-3 accumulating intracellularly was purified to homogeneity by a series of chromatographic steps, including high-performance liquid chromatography (HPLC). Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) showed the presence of a single Coomassie blue staining band with a purity of greater than 95% and a molecular weight of 14 to 15 kD. The in vitro biologic activity was found to be 4.6×10^6 U/mg protein assayed by thymidine incorporation by chronic myelogenous leukemia (CML) myeloblasts, as described.[12] The endotoxin content was 30 ± 5 pg/mg as determined by the limulus assay (Limulus amebocyte lysate; Whittacker MA Mioproducts, Walkersville, MD).[13]

The rhG-CSF (Neupogen) used was produced by Hoffmann-La Roche (Basel, Switzerland). The recombinant protein was expressed in *E coli* and had a molecular weight of 18.8 kD.[14] Its specific activity was 1×10^8 U/mg protein, and it was free of detectable endotoxin.

Preparation of cells. Ten milliliters of PB was collected into sterile tubes containing 2 mL EDTA. PB mononuclear cells (MNC) were obtained after a Ficoll-Hypaque gradient centrifugation (400*g* for 40 minutes, 1.077 g/mL).

Progenitor cell assay. Colony-forming unit-GM (CFU-GM), burst-forming unit-erythroid (BFU-E), and multipotential (CFU-mix) progenitor cells were assayed as previously described.[11,15] Cultures were stimulated with 100 U/mL rhGM-CSF (Sandoz AG) and 10 U/mL rhIL-3 (Sandoz AG). For cultivation of BFU-E and CFU-mix, 1 U/mL erythropoietin (EPO; Toyobo, Osaka, Japan) was added to culture dishes. PB MNC and BM MNC were plated in triplicate at 0.5 to 1.0×10^5/mL. After a culture period of 14 days (37°C, 5% CO_2, full humidity), cultures were examined under an inverted microscope. Aggregates with more than 40 translucent, dispersed cells were counted as CFU-GM. Bursts containing more than 100 red colored cells were scored as BFU-E. CFU-mix were identified by their heterogeneous composition of translucent and hemoglobinized cells. The count of circulating progenitor cells per milliliter blood was determined by multiplying their frequency in culture with the absolute MNC count in the same sample of PB.

Assessment of $CD34^+$ cells. $CD34^+$ cells in the leukapheresis product were assayed as previously described.[16] The fluorescein isothiocyanate (FITC)-labeled CD34 antibody (Anti HPCA-2; Becton Dickinson, Palo Alto, CA) and an isotype-matched control antibody (MOPC-21; Sigma, Deisenhofen, Germany) were used to label cells. Nucleated cells from leukapheresis products were analyzed on a FACSCan (Becton Dickinson) and the data analyzed using a cell analysis program (Lysis II software; Becton Dickinson). From the analysis of forward and sideward light scatter, a gate was established to include all white blood cells (WBCs), excluding platelets (PLTs) and red blood cells (RBCs). The cells in the gate were analyzed for fluorescence of cells labeled with the particular monoclonal antibody (MoAb). The number of $CD34^+$ cells was calculated by multiplying the percentage of $CD34^+$ cells as determined by flow cytometry, and the cell count per microliter as determined by the electronic cell counter. This number was then multiplied by the volume of product collected to give the total number in the product.

Hematologic examinations. For hematologic examinations blood was collected in EDTA-coated tubes. Parameters measured included the total counts of RBCs, WBCs, PLTs, and determination of hemoglobin (Hb), and hematocrit (Sysmex 2000; TOA, Tokyo, Japan). Differential counts were obtained by the examination of 100 cells of a Giemsa-stained blood smear.

Statistical analysis. The Student's *t*-test was used to compare differences with regard to progenitor cell numbers between normal individuals and study patients. The paired *t*-test was used to test for significant differences between data before and during or at the end of G-CSF and IL-3/G-CSF therapy, respectively.

RESULTS

Mobilization of circulating CFU-GM. Baseline CFU-GM numbers in the 6 study patients (mean ± SEM: 52/mL ± 13) were significantly decreased ($P < .05$ by the *t*-test) compared with normal individuals (n = 21; 295 ± 55). In cycle 1, patients received G-CSF alone at a dose of 5 μg/kg/d for 5 days. G-CSF as a single agent markedly mobilized PB CFU-GM in all patients with maximum values at the

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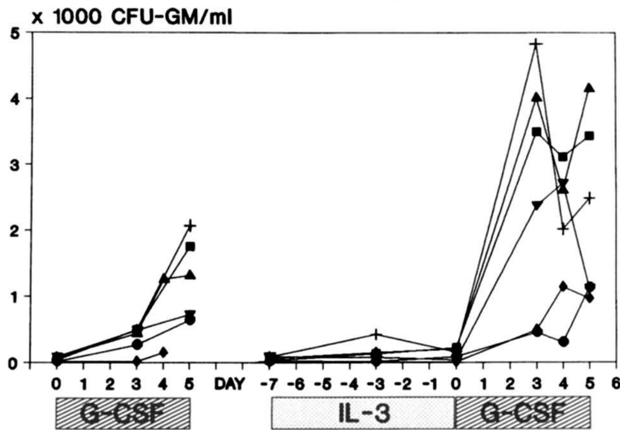

Fig 1. PB CFU-GM numbers in six patients receiving G-CSF (5 μg/kg/d for 5 days) with and without 7-day IL-3 pretreatment (5 μg/kg/d).

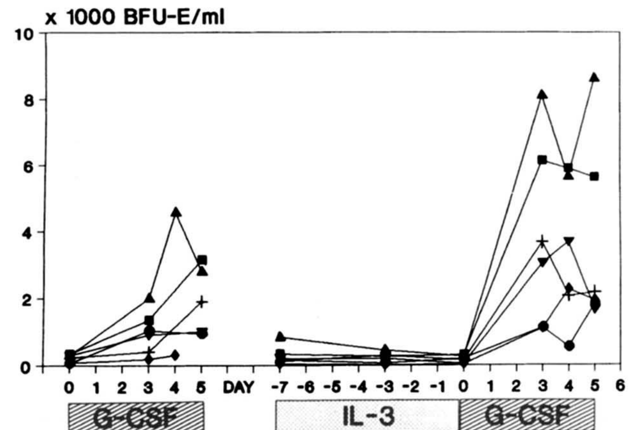

Fig 2. PB BFU-E numbers in six patients receiving G-CSF (5 μg/kg/d for 5 days) with and without 7-day IL-3 pretreatment (5 μg/kg/d).

end of cycle 1 (Fig 1). As shown in Table 2, G-CSF alone increased the mean number of circulating CFU-GM by 21-fold over pretreatment values ($P < .05$ by the paired *t*-test).

After a treatment-free interval, all 6 patients received cycle 2 consisting of a 7-day pretreatment with IL-3 at a dose of 5 μg/kg/d immediately followed by G-CSF at a dose of 5 μg/kg/d for 5 days. CFU-GM levels before cycle 2 were not significantly different from that before cycle 1 (Table 2). There was no clear increase in the number of circulating CFU-GM during the IL-3 administration (Fig 1). Although IL-3 did not mobilize itself, 7-day pretreatment with IL-3 consistently potentiated mobilization of PB CFU-GM by G-CSF in all 6 patients (Fig 1). Peak values were reached before the end of cycle 2 in 4 of 6 patients. As shown in Table 2, the mean increase in maximum CFU-GM numbers over baseline values was 56-fold by the IL-3/G-CSF combination ($P < .01$). As compared with the maximum CFU-GM levels achieved by G-CSF alone, maximum values by the IL-3/G-CSF were significantly higher ($P < .01$) (Table 2). If the x-fold increase in maximum values by the IL-3/G-CSF combination compared with G-CSF alone was calculated in individual patients, a mean potentiation of 3.4-fold (range: 1.8 to 7.6) by the 7-day IL-3 pretreatment was found (Table 2).

Mobilization of circulating BFU-E and CFU-mix. Circulating BFU-E and CFU-mix in study patients before cycle 1 were also significantly decreased compared with normal individuals (n = 21) (BFU-E: 222/mL ± 49 *v* 749 ± 102, $P < .05$; CFU-mix: 3.8/mL ± 2.8 *v* 33 ± 5, $P < .05$). Mobilization of circulating progenitor cells by G-CSF alone and by the IL-3/G-CSF combination was not restricted to CFU-GM but was also observed for BFU-E and CFU-mix (Figs 2 and 3; Tables 3 and 4). After G-CSF alone the mean number of BFU-E increased by 9-fold and that of CFU-mix by 24-fold over baseline, respectively. Again, 7-day IL-3 treatment did not mobilize by itself but enhanced maximum numbers of BFU-E and CFU-mix, respectively, by the subsequent administration of G-CSF, leading to a 15-

Table 2. Maximum Values of PB CFU-GM/ML Achieved by G-CSF Alone and G-CSF After Pretreatment With IL-3 for 7 Days

Patient No.	Cycle 1 Pre	Cycle 1 G-CSF	Cycle 2 Pre	Cycle 2 (IL-3)/G-CSF	Potentiation by IL-3
1	64	2,064	87	(158)/4,826	2.3
2	88	727	86	(39)/2,704	3.7
3	44	1,748	34	(221)/3,490	2.0
4	20	635	27	(88)/1,122	1.8
5	79	1,312	67	(208)/4,149	3.2
6	14	148	10	(11)/1,128	7.6
Mean ± SEM	52 ± 13	1,106 ± 298	52 ± 13	2,903 ± 631	3.4 ± 0.9

Cycle 1 Pre vs G-CSF: $P < .05$; Cycle 2 Pre vs (IL-3)/G-CSF: $P < .01$; G-CSF vs (IL-3)/G-CSF: $P < .01$

Progenitor cell numbers were determined as described in Materials and Methods. Numbers in brackets indicate progenitor cell numbers at the end of the IL-3 pretreatment. Potentiation by IL-3 was defined as the x-fold increase in maximum values by the IL-3/G-CSF combination compared with G-CSF alone. The paired *t*-test was used to test for significant differences.

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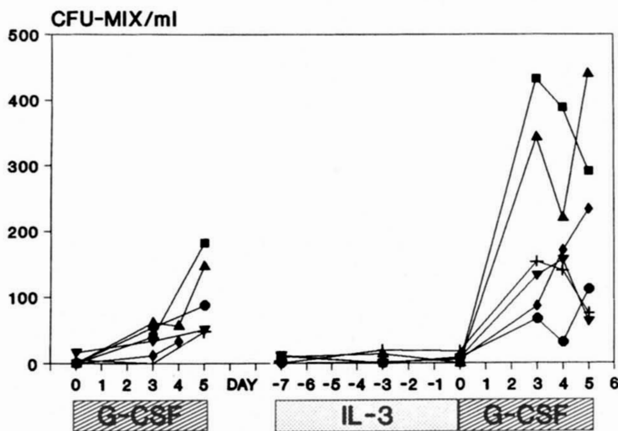

Fig 3. PB CFU-mix numbers in six patients receiving G-CSF (5 μg/kg/d for 5 days) with and without 7-day IL-3 pretreatment (5 μg/kg/d).

fold (BFU-E) and 46-fold (CFU-mix) increase over baseline by the IL-3/G-CSF combination. Maximum levels of BFU-E and CFU-mix achieved by the IL-3/G-CSF combination were significantly higher than by G-CSF alone (BFU-E: $P < .005$; CFU-mix: $P < .05$, respectively) Potentiation by IL-3 was observed in all patients for BFU-E (mean 3.1-fold, 1.9 to 7.0) and for CFU-mix (mean 3.4-fold, 1.3 to 7.1).

Effects of cytokine therapy on leukapheresis products. In 2 patients leukapheresis was performed after G-CSF alone and in all 6 patients after the IL-3/G-CSF combination. In each case 3 leukapheresis procedures were performed on 3 consecutive days immediately after the 5 days of G-CSF treatment. G-CSF administration was maintained on days with leukapheresis. In both patients in whom leukapheresis was performed after G-CSF alone the target number of 2×10^6/kg CD34$^+$ cells was not reached. Leukapheresis that was performed in all 6 patients after the IL-3/G-CSF combination obtained a mean number of 7.3×10^8/kg $\pm$ 0.6 NC, 6.0×10^8/kg $\pm$ 0.7 MNC, 18.8×10^4/kg $\pm$ 3.8 CFU-GM, and 2.0 $\times 10^6$/kg $\pm$ 0.3 CD34$^+$ cells. The target number of $\geq 2 \times 10^6$/kg CD34$^+$ cells was obtained in 3 of 6 patients, including both patients who had inadequate collection after G-CSF alone (Table 5, nos. 5 and 6).

Effects of cytokine therapy on blood cell counts. As shown in Table 6 WBC counts at the end of cycle 1 were markedly increased (17,018/μL $\pm$ 4,170) ($P < .05$) compared with baseline values (4,352 $\pm$ 581). IL-3 pretreatment raised leukocyte counts also significantly (7,757 $\pm$ 2,047), but less than G-CSF. There was a clear potentiation of G-CSF–induced leukocytosis by IL-3 pretreatment leading to significant higher WBC counts at the end of the IL-3/G-CSF therapy (27,170 $\pm$ 4,708) compared with leukocytosis by G-CSF alone ($P < .01$). Leukocytosis by G-CSF alone and by G-CSF after IL-3 pretreatment was in both cases caused by increases in neutrophils. There was no significant difference in the percentage of neutrophils at the end of G-CSF (81% $\pm$ 5%) and at the end of the IL-3/G-CSF combination (83% $\pm$ 3%), respectively. Platelet counts were not significantly changed by G-CSF or IL-3, respectively. However, platelet counts at the end of the IL-3/G-CSF application were slightly but significantly ($P < .05$) higher compared with pretreatment levels. Hb values were not significantly changed by G-CSF alone. Although Hb levels were significantly decreased at the end of IL-3 and of the IL-3/G-CSF combination, they remained within normal limits (>12 g/dL).

Side effects. The most commonly observed side effect was headache, which was observed in all 6 patients during IL-3 (Table 7). Other side effects observed under IL-3 were fever (3 of 6 patients), myalgia (3 of 6), and conjunctivitis (1 of 6). Under G-CSF therapy, only bone pain (in 2 of 6 patients) was reported. All the side effects listed above could be easily ameliorated with oral paracetamol. IL-3 pretreatment did not enhance side effects observed under subsequent G-CSF administration. In no patients did side effects exceeding grade II toxicity according to the World Health Organization (WHO) scale occur.

Engraftment by IL-3/G-CSF mobilized progenitors. One patient (no. 5) relapsed 24 months after having achieved

Table 3. Maximum Values of PB BFU-E/ML Achieved by G-CSF Alone and G-CSF After Pretreatment With IL-3 for 7 Days

	Cycle 1		Cycle 2		
Patient No.	Pre	G-CSF	Pre	(IL-3)/G-CSF	Potentiation by IL-3
1	238	1,896	135	(167)/3,677	1.9
2	304	1,010	212	(48)/3,692	3.7
3	343	3,149	353	(320)/6,134	1.9
4	70	948	178	(202)/1,825	1.9
5	301	4,574	848	(266)/8,612	1.9
6	78	324	45	(33)/2,256	7.0
Mean ± SEM	222 ± 49	1,984 ± 654	295 ± 118	4,366 ± 1,049	3.1 ± 0.8
	$P < .05$ (Pre vs G-CSF)		$P < .01$ (Pre vs (IL-3)/G-CSF)		
		$P < .005$ (G-CSF vs (IL-3)/G-CSF)			

Progenitor cell numbers were determined as described in Materials and Methods. Numbers in brackets indicate progenitor cell numbers at the end of IL-3 treatment. Potentiation by IL-3 was defined as the x-fold increase in maximum values by the IL-3/G-CSF combination compared with G-CSF alone. The paired *t*-test was used to test for significant differences.

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Table 4. Maximum Values of PB CFU-MIX/ML Achieved by G-CSF Alone and G-CSF After Pretreatment With IL-3 for 7 Days

	Cycle 1		Cycle 2		
Patient No.	Pre	G-CSF	Pre	(IL-3)/G-CSF	Potentiation by IL-3
1	6	48	0	(18)/153	3.2
2	17	51	12	(8)/156	3.1
3	0	182	11	(0)/432	2.4
4	0	87	0	(9)/111	1.3
5	0	147	10	(0)/440	3.0
6	0	33	0	(6)/233	7.1
Mean ± SEM	3.8 ± 2.8	91 ± 25	5.5 ± 2.5	254 ± 60	3.4 ± 0.8

Cycle 1 Pre vs G-CSF: $P < .05$; Cycle 2 Pre vs (IL-3)/G-CSF: $P < .01$; G-CSF vs (IL-3)/G-CSF: $P < .05$

Progenitor cell numbers were determined as described in Materials and Methods. Numbers in brackets indicate progenitor cell numbers at the end of IL-3 treatment. Potentiation by IL-3 was defined as the x-fold increase in maximum values by the IL-3/G-CSF combination compared with G-CSF alone. The paired *t*-test was used to test for significant differences.

complete remission (CR). He went into CR again after 2 cycles of mini-BEAM[17] and subsequently received high-dose chemotherapy followed by infusion of IL-3/G-CSF mobilized progenitors. The myeloablative regimen consisted of a single dose of carmustine (BCNU) 600 mg, etoposide 400 mg for 4 days, cytarabine 400 mg every 12 hours for 10 doses, and dexamethasone 20 mg for 3 days. A total number of 3.0×10^6/kg $CD34^+$ cells was infused 24 hours after the end of chemotherapy. To accelerate reconstitution of neutropoiesis the patient received 5 μg/kg/d filgrastim starting 1 day after progenitor cell infusion. He rapidly achieved trilineage engraftment as documented by recovery of PB (Fig 4), and by marrow examination (not shown). The time to reach a granulocyte count over 500/μL in the presence of G-CSF was 10 days. Until hematopoietic reconstitution the patient required 2 erythrocyte and 3 platelet transfusions.

DISCUSSION

PB normally contains very few hematopoietic progenitors. Both GM-CSF and G-CSF markedly enhance rebound mobilization of PBSCs that occurs after chemotherapy.[2,6] The mobilizing capacity of growth factors alone is less pronounced.[7] In this study we examined a strategy to enhance progenitor cell mobilization by G-CSF without the need of preceding chemotherapy. To minimize the impact of interpatient variability we chose an intraindividual comparison that has been shown to have good reproducibility with respect to progenitor cell mobilization.[18] We show that a 7-day pretreatment with IL-3 reproducibly potentiates the mobilizing capacity of G-CSF in patients who had low progenitor cell numbers because of previous chemotherapy.

Table 5. Effects of Cytokine Treatment on Leukapheresis Products

Patient No.	Therapy	NC $\times 10^8$/kg	MNC $\times 10^8$/kg	CFU-GM $\times 10^4$/kg	$CD34^+$ $\times 10^6$/kg
1	IL-3/G-CSF	6.0	5.4	17.3	1.5
2	IL-3/G-CSF	5.2	4.6	11.3	1.2
3	IL-3/G-CSF	8.1	4.4	ND	2.1
4	IL-3/G-CSF	9.0	8.7	ND	1.2
5	G-CSF	6.0	5.9	12.8	1.4
5	IL-3/G-CSF	8.1	6.0	17.4	3.0
6	G-CSF	3.6	3.5	3.1	0.7
6	IL-3/G-CSF	7.2	6.6	29.3	2.8

In each case leukapheresis procedures were performed on 3 consecutive days immediately after the end of cytokine treatment.

Abbreviations: NC, nucleated cells; MNC, mononuclear cells; CFU-GM, colony-forming unit-granulocyte-macrophage; ND, not done.

The rationale for the sequential mode of application in our study was based on the data from primate models. Simultaneous administration of IL-3 and GM-CSF was less effective in increasing WBC counts in rhesus monkeys than the sequential IL-3/GM-CSF treatment.[19] This could be explained by competition for the same receptor subunit by both ligands.[20] In patients we have recently reported that 3-day pretreatment with IL-3 at doses of 2.5, 5, and 10 μg/kg/d, respectively, did not show consistent effects on GM-CSF induced stem cell mobilization.[21] However, pretreatment with 5 μg/kg/d of IL-3 for 7 days potentiated the increase in progenitor cell numbers by subsequent administration of GM-CSF in all 4 patients tested.[21] Although IL-3 alone increased circulating CFU-GM by about 10-fold in rhesus monkeys,[11] it did not mobilize progenitor cells in patients. Data from others suggest that IL-3 can increase the absolute number of progenitor cells in BM[22] and thus enhance their release by subsequent administration of G-CSF. We don't think that prolonging the time of G-CSF administration will further enhance the mobilizing capacity of the IL-3/G-CSF combination. Circulating progenitors have been shown to peak at day 4 or 5 during G-CSF administration[3] and after IL-3 pretreatment we saw peak values even earlier occurring before day 5 in the majority of patients.

The mobilizing potential of the sequential IL-3/G-CSF therapy, in comparison with other strategies to mobilize PBSCs, remains to be established. Differences in the progenitor cell assay technique, differences in dose and duration of cytokine treatment, and differences in the hematopoietic

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Table 6. Effects of Cytokine Treatment on Blood Cell Counts

	Cycle 1		Cycle 2		
	Pre	G-CSF	Pre	IL-3	G-CSF
			$P < .05$ (Pre v IL-3)		
WBC (/μL)					
Group mean	4,352	17,018	4,617	7,757	27,170
±SEM	±581	±4,170	±1,073	±2,047	±4,708
	$P < .05$ (Pre v G-CSF)		$P < .005$ (Pre v G-CSF)		
		$P < .01$ (Cycle 1 G-CSF v Cycle 2 G-CSF)			
			NS (Pre v IL-3)		
PLT ($\times 10^3/\mu$L)					
Group mean	213	190	212	227	247
±SEM	±48	±28	±44	±38	±41
	NS (Pre v G-CSF)		$P < .05$ (Pre v G-CSF)		
			$P < .05$ (Pre v IL-3)		
Hb (g/dL)					
Group mean	13.1	13.1	13.2	12.4	12.5
±SEM	±0.5	±0.2	±0.4	±0.3	±0.3
	NS (Pre v G-CSF)		$P < .05$ (Pre v G-CSF)		

Numbers indicate blood cell counts before (pre) and at the end of cytokine treatment, respectively. The paired *t*-test was used to test for significant differences.

Abbreviations: WBC, white blood cells; PLT, platelets; Hb, hemoglobin; NS, not significant.

reserve after previous myelotoxic pretreatment make it extremely difficult to compare results from different studies. The 21-fold mobilization of circulating CFU-GM by G-CSF alone in our study was lower than reported by Sheridan et al,[3] who observed a median of 58-fold increase of CFU-GM over pretreatment values. Although the duration of G-CSF aministration was similar in both studies (5 days in our study *v* 6 days in the report of Sheridan et al) the G-CSF dose of 12 μg/kg/d in the study of Sheridan et al was considerably higher than in ours. Recently, the CFU-GM increment by 5 and 10 μg/kg G-CSF therapy, respectively, has been directly compared and a better mobilization in patients receiving 10 μg/kg been observed.[23] Therefore, the mobilizing capacity of the IL-3/G-CSF combination might be even more pronounced, if higher doses of G-CSF are used and if the potentiating effect of IL-3 is maintained. In fact there is some evidence that IL-3 pretreatment can still enhance G-CSF–induced effects if higher G-CSF doses are used.[24]

Although chemotherapy administered to enhance CSF-induced PBPC mobilization may be of benefit in some patients because of its antineoplastic activity, it may be associated with considerable hematologic and nonhematologic toxicity. IL-3 treatment was well tolerated in our patients and lacked any relevant hematological toxicity. Therefore, IL-3 as a mean to augment CSF-induced stem cell mobilization may be preferred over chemotherapy in some patients, particularly in those with compromised hematopoiesis. However, if IL-3 can replace chemotherapy in such patients remains to be shown. Data about the

Table 7. Side Effects

	Cycle 1				Cycle 2							
	G-CSF				IL-3				G-CSF			
Symptom	I	II	III	IV	I	II	III	IV	I	II	III	IV
Headache	—	—	—	—	6	—	—	—	—	—	—	—
Fever	—	—	—	—	—	3	—	—	—	—	—	—
Myalgia	—	—	—	—	2	1	—	—	—	—	—	—
Bone pain	2	—	—	—	—	—	—	—	2	—	—	—
Conjunctivitis	—	—	—	—	1	—	—	—	—	—	—	—

Severity of side effects was classified according to WHO grading I-IV.

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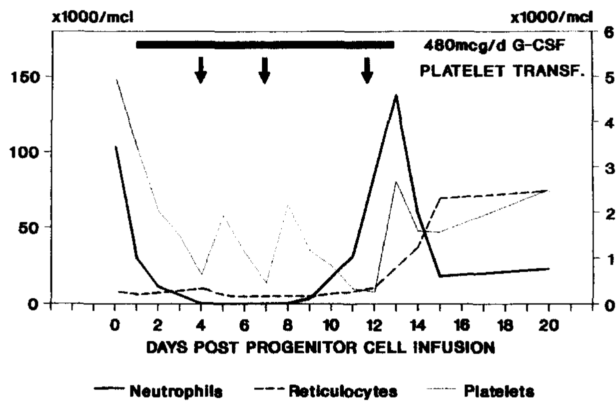

Fig 4. Hematopoietic engraftment in patient 5 after infusion of IL-3/G-CSF mobilized autologous progenitor cells after myeloablative chemotherapy (reticulocyte and platelet count, Y1 axis; neutrophil count, Y2 axis). The myeloablative regimen consisted of a single dose of carmustine (BCNU) 600 mg, etoposide 400 mg for 4 days, cytarabine 400 mg every 12 hours for 10 doses, and dexamethasone 20 mg for 3 days. A total number of 3.0 × 10^6/kg CD34$^+$ cells was infused 24 hours after the last chemotherapy.

impact of preceding chemotherapy on CSF induced mobilization of CFU-GM are limited. Socinski et al[7] have reported an 18-fold increase in PB CFU-GM numbers by GM-CSF alone and an approximately 60-fold increase if GM-CSF was given during the period of leukocyte recovery chemotherapy. Considering the 21-fold increase in CFU-GM numbers by G-CSF alone and the 56-fold increase by the IL-3/G-CSF combination in our study, one may anticipate that chemotherapy and IL-3 treatment may be similar effective in improving stem cell mobilization by CSFs. Certainly, studies that compare both strategies directly are warranted.

This study provides further evidence that combinations of hematopoietic growth factors may be more powerful in achieving hematopoietic effects in vivo than either factor alone. Synergistic in vivo effects on the production of mature blood cells have been reported with the combinations of IL-3 and GM-CSF on myelopoiesis,[19,25] of IL-3 and EPO on erythropoiesis,[26] and of IL-3 and IL-6 on thrombopoiesis,[27] respectively, in the preclinical monkey model. Recent observations indicate that similar synergistic effects on blood cell production can be obtained in humans by administering IL-3 before GM-CSF.[28] Our observation that IL-3 potentiated G-CSF–induced leukocytosis suggests that the IL-3/G-CSF combination may be clinically useful in granulocytopenic patients who cannot be sufficiently stimulated by G-CSF alone. With respect to progenitor cell mobilization, early acting cytokines other than IL-3 could be equally or even more effective in combination with G-CSF. Considering the marked effects of stem cell factor (SCF) alone and in combination with G-CSF on the number of progenitor cells in monkeys,[29,30] this combination seems to be a very promising strategy to maximally mobilize stem cells in patients.

We have reinfused PB stem cells mobilized by the IL-3/G-CSF combination in one patient after myeloablative chemotherapy. Thus, the functional quality of the progenitor cell compartment expanded by this combination could be documented by the rapid trilineage engraftment observed in this patient. The minimal number of progenitors required for rapid and save engraftment is still unclear. The predictive value of infused CFU-GM numbers with respect to hematopoietic recovery is markedly hampered by the lack of standardized CFU-GM culture techniques. CD34$^+$ numbers are probably a more reliable parameter for prediction of hematopoietic reconstitution.[31] A number of at least 2 × 10^6/kg of CD34$^+$ cells is generally considered as a dose that provides a rapid engraftment of neutrophils and platelets.[32] Our study suggests that some patients with low progenitor cell numbers don't achieve this target cell number if leukapheresis is performed after G-CSF therapy alone. In some of these patients the IL-3/G-CSF combination may offer the possibility to obtain sufficient numbers of PB progenitors.

Sustained hematopoietic reconstitution results from a population of pluripotent stem cells that cannot at present be assayed in humans. In animal studies cytokine mobilized PBSCs transplanted into syngeneic irradiated recipients produce long-term lymphomyelopoietic regeneration, as confirmed by the presence of genetic markers in mature cells in blood.[33] Persistence of normal blood counts for long periods after severely myelotoxic regimens in patients receiving cytokine mobilized stem cells alone still needs to be shown. The observation that increases in progenitor cell numbers were not restricted to CFU-GM but were also seen for more primitive progenitors (CFU-mix) suggests, but does not prove, that progenitor cells being able to restore hematopoiesis more durably have been mobilized as well.

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