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ORIGINAL ARTICLE

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Granulocyte colony-stimulating factor-induced mobilization of peripheral blood stem cells for autologous and allogeneic transplantation

Abstract Peripheral blood stem and progenitor cells (PBSC and PBPC), which circulate at very low levels during steady-state hematopoiesis, show a transient but marked increase during hematologic recovery from marrow-suppressive chemotherapy. To ensure rapid and sustained hematologic engraftment after autologous PBSC transplantation, sufficient PBSC or PBPC must be infused. To confirm the utility of granulocyte colony-stimulating factor (G-CSF) in chemotherapy-induced PBSC mobilization, we investigated the effect of G-CSF on PBSC mobilization in leukemia and lymphoma patients. The study design was such that PBSC mobilization with and without G-CSF was assessed in the same patients. The results indicate that PBSC mobilization can be enhanced significantly when G-CSF is given during the recovery phase postchemotherapy. Interestingly, progenitor cells of different lineages could be mobilized by G-CSF. We subsequently investigated the effect of increasing G-CSF dose on PBSC mobilization during steady-state hematopoiesis in healthy adult donors. The results indicate that not only committed but also primitive progenitor cells are mobilized into the circulation in a dose- and time-dependent manner when G-CSF at 5, 10, or 15 μg/kg was given on each of 5 days and leukapheresis was performed on day 6. From our data we estimate that sufficient PBSC for engraftment after allogeneic PBSC transplantation can be collected on day 5 of administration of G-CSF at 10 μg/kg and by 10-l leukapheresis on days 5 and 6. Furthermore, we found that some G-CSF-mobilized PBSC retained their self-renewal capability. These observations suggest that hematopoietic stem cells for allogeneic PBSC transplantation can be mobilized by short-term administration of relatively high-dose G-CSF.

Key words PBSC · Mobilization · G-CSF · Auto-PBSC transplantation · Allo-PBSC transplantation

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Introduction

The use of peripheral blood stem-cell transplantation (PBSCT) has been expanding as the third type of hematopoietic stem-cell transplantation after allogeneic and autologous bone marrow transplantation (allo-BMT and auto-BMT) in the treatment of hematologic malignancies and solid tumors [10]. Recent clinical evidence indicates that autologous PBSCT is characterized by rapid hematologic recovery and low transplant-related mortality [3, 19]. According to the latest nationwide survey conducted by the Japan Society of Bone Marrow Transplantation, more than 500 autotransplants of PBSC have been performed since 1990. However, for rapid and sustained hematologic engraftment after autologous PBSCT, a sufficient number of PBSC or peripheral blood progenitor cells (PBPC) must be infused.

In a murine model, long-term lymphohematologic reconstitution has been achieved by allotransplantation of granulocyte colony-stimulating factor (G-CSF)-mobilized, blood-derived primitive hematopoietic stem cells (day 11 colony-forming unit-spleen [CFU-S]) [14]. Furthermore, it has been shown that the frequency and self-renewal capacity of murine primitive stem cells in the blood are increased following G-CSF therapy [16]. In humans, long-term culture-initiating cells (LTC-IC), the most primitive hematopoietic progenitors among assayable human progenitor cells, circulate in the peripheral blood, and their proliferation and differentiation capacities are similar to those of

Table 1 Chemotherapy-induced and chemotherapy + G-CSF-induced PBSC mobilization [a]

PBSC harvest	Chemotherapy (n = 10)		Chemotherapy + G-CSF (n = 12)	
	Cycle 1	Cycle 2	Cycle 1	Cycle 2
CFU-GM ($\times 10^4$/kg)	5.39 ± 4.09	3.72 ± 3.78	3.18 ± 3.70	17.5 ± 16.8**
BFU-E ($\times 10^4$/kg)	3.21 ± 2.82	2.77 ± 3.11	3.31 ± 3.11	12.9 ± 9.82**
CFU-GM:10^4 MNC	2.76 ± 2.42	1.51 ± 1.42	0.91 ± 0.88	2.90 ± 3.11**
BFU-E:10^4 MNC	1.24 ± 0.75	0.82 ± 0.90	1.54 ± 1.30	2.49 ± 2.01
CFU-GM:BFU-E	2.91 ± 2.47	3.75 ± 3.31	1.42 ± 2.11	1.43 ± 1.37

* $P < 0.05$; ** $P < 0.01$
[a] Data are expressed as mean values ± SD

bone marrow [25]. Recently we have also reported that G-CSF can mobilize LTC-IC and committed progenitors into the circulation [4]. These findings suggest that allotransplantation of G-CSF-mobilized PBSC may be a feasible alternative to allo-BMT in humans [6, 17].

Mobilization and collection of PBSC

Very small numbers of hematopoietic stem cells and progenitor cells circulate during steady-state hematopoiesis [4]. These PBSC show a transient but marked increase during hematologic recovery from marrow-suppressive chemotherapy. This mobilization of PBSC from bone marrow into the peripheral blood can be enhanced substantially when a hematopoietic growth factor such as G-CSF is given during the recovery phase after chemotherapy [21, 23]. Recent advances in the PBSC mobilization technique indicate that a sufficient number of PBSC for hematologic reconstitution after marrow-ablative therapy can be collected by leukapheresis using a continuous blood-cell separator during hematologic recovery with or without a hematopoietic growth factor [10, 23]. However, the mechanism for the mobilization of PBSC or PBPC from bone marrow into peripheral blood has not been fully clarified. It is probable that knowledge about the interaction between hematopoietic stem cells or progenitors and bone-marrow stromal cells is important for an understanding of the mobilization mechanism [24].

Chemotherapy versus chemotherapy/G-CSF-induced mobilization

To confirm the utility of G-CSF in chemotherapy-induced PBSC mobilization, we investigated the effect of G-CSF on PBSC mobilization in patients with leukemia or lymphoma. As levels of circulating progenitor cells vary widely from patient to patient [22], the study design was such that PBSC mobilization with and without G-CSF was assessed in individual patients. Two successive cycles of leukapheresis following cytotoxic chemotherapy were performed in 22 patients as follows: chemotherapy-induced mobilization was used after the first cycle of consolidation chemotherapy in all patients; during the second cycle, patients were randomized into 2 groups in which chemotherapy-induced mobilization (n = 10) and chemotherapy + G-CSF-induced mobilization (n = 12) were used.

The results are summarized in Table l. In the group in which repeated chemotherapy-induced mobilization was used, decreased yields of myeloid [colony-forming unit-granulocyte macrophage (CFU-GM)] and erythroid [burst-forming unit-erythroid (BFU-E)] progenitors were obtained after the second mobilization. In contrast, chemotherapy/-G-CSF-induced mobilization produced significantly higher yields of CFU-GM (5.5-fold) and BFU-E (3.9-fold) progenitors than mobilization induced by chemotherapy alone ($P < 0.01$). Interestingly, the ratios of CFU-GM and BFU-E progenitors to 10^4 mononuclear cells harvested and of CFU-GM to BFU-E progenitors were not affected by the addition of G-CSF. This is compatible with a previous report that G-CSF does not selectively mobilize progenitors that express receptors for G-CSF [9] and suggests that G-CSF may induce the release of progenitor cells of different lineages from bone marrow into the peripheral blood. Furthermore, G-CSF produced an earlier peak in CFU-GM progenitors following chemotherapy. Thus, G-CSF was effective in expanding the pool of circulating hematopoietic progenitors. Procedures for PBSC mobilization induced by chemotherapy and by chemotherapy + hematopoietic growth factors are well established [23].

Hematologic recovery after autologous PBSCT

We studied the relationship between the number of CFU-GM progenitors infused and hematopoietic reconstitution after PBSCT in 23 patients with hematologic malignancies who were treated with marrow-ablative chemotherapy and received autologous PBSCT [21]. The results are shown in Fig. l. The median number of days taken for granulocytes, platelets, and reticulocytes to recover to $> 0.5 \times 10^9$/l, 0.50×10^9/l, and $> 1\%$, respectively, was 15 (range 9–23), 54 (range 11–212), and 18 (range 12–42) days, respectively. Logarithmic values for the numbers of CFU-GM progenitors infused were linearly and inversely related to the time required for recovery to the respective cutoff values for granulocytes, platelets, and reticulocytes. Interestingly, linear regression curves generated for chemotherapy-mobilized PBSC and chemotherapy + G-CSF-mobilized PBSC did not differ significantly (data not shown).

Patients receiving a low CFU-GM progenitor dose, particularly $< 10^5$/kg, showed delayed hematologic recovery with markedly delayed recovery of megakaryopoiesis.

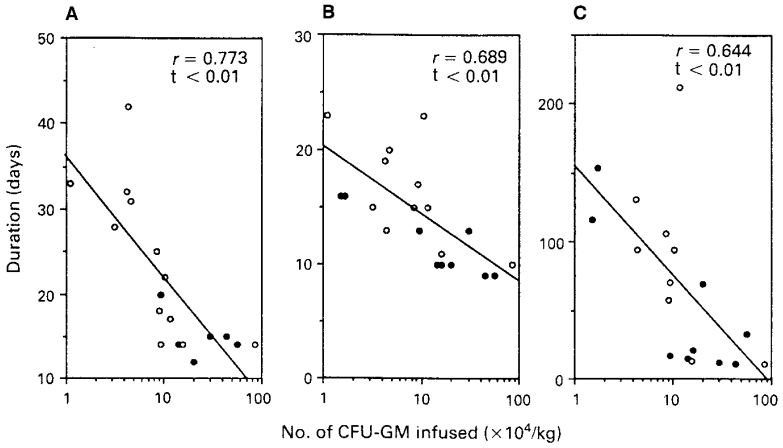

Fig. 1A–C Relationship between the CFU-GM dose and recovery of **A** the reticulocyte count to >1%, **B** the granulocyte count to 0.5×10^9/l, and **C** the platelet count to 50×10^9/l after autologous PBSCT (*white circles* Recovery of chemotherapy-mobilized PBSC, *black circles* Recovery of chemotherapy + G-CSF-mobilized PBSC). Linear regression curves were plotted from the autologous PBSCT data for PBSC collected by both chemotherapy-induced and chemotherapy + G-CSF-induced mobilization. These curves did not differ significantly

In addition, long-term sustained hematopoietic reconstitution was confirmed in most of the patients. Thus, the rate of trilineage hematologic reconstitution after autologous PBSCT showed a significant correlation with the infused dose of CFU-GM progenitors, whether it was assessed after chemotherapy-induced or chemotherapy + G-CSF-induced mobilization. These results suggest that the CFU-GM progenitor yield after chemotherapy + G-CSF-induced and chemotherapy-induced mobilization may predict trilineage hematopoietic reconstitution after autologous PBSCT.

Subsequently we studied hematologic recovery in 27 patients with hematologic malignancies who received marrow-ablative chemotherapy and autologous PBSCT to determine whether the number of infused mononuclear cells (MNC), CFU-GM progenitors, and colony-forming units-megakaryocyte (CFU-Mk) were related to the speed of platelet recovery after autologous PBSCT [20]. Although the number of MNC infused did not show a significant correlation with the time to platelet recovery or to granulocyte or reticulocyte recovery the logarithmic value of the number of CFU-GM progenitors infused did. We also found a significant correlation between the logarithmic value of the number of CFU-Mk progenitors infused and the time to platelet recovery. These findings suggest that the number of CFU-GM progenitors infused is a reliable indicator of hematopoietic recovery and that the number of CFU-Mk progenitors infused is no more reliable than the number of CFU-GM progenitors for predicting platelet recovery after autologous PBSCT.

Leukemic cell contamination in PBSC harvests

One of the potential advantages of autologous PBSCT over auto-BMT is the possibility that PBSC harvests are less likely than bone-marrow mononuclear cells to be contaminated by leukemic cells [10]. We have previously reported that PBSC harvests from patients with acute leukemia, when evaluated using a reverse transcriptase-polymerase chain reaction (RT-PCR) method, are contaminated by leukemia-specific gene products [15]. However, we did not determine the degree of leukemic cell contamination in the PBSC harvests. Recently we have developed a titration assay using a competitive RT-PCR method to estimate the number of leukemia-specific gene transcripts (AML1/ETO) such that minimal residual disease (MRD) can be monitored quantitatively in patients with t(8;21) acute myelogenous leukemia (AML) [13].

Using a qualitative RT-PCR method, AML1/ET0 transcripts could be detected in all samples from 15 PBSC harvests after the first cycle of consolidation chemotherapy and in 11 PBSC harvests after the second cycle of consolidation chemotherapy obtained from 15 patients with t(8;21) AML. Using the competitive RT-PCR assay, the number of AML1/ET0 transcripts was found to be lower in the second PBSC harvest than in the first harvest in every individual. Furthermore, less MRD was found in PBSC harvests than in the corresponding bone marrow obtained on the day of PBSC collection in the individual patients studied. These observations suggest that although PBSC harvests collected after consolidation chemotherapy are contaminated by leukemic cells, the degree of leukemic cell contamination may decrease as chemotherapy is repeated and that PBSC mobilization by repeated chemotherapy may provide a good source of hematopoietic stem cells for autologous PBSCT.

G-CSF-induced mobilization of PBSC for allotransplantation

Recent studies have shown that G-CSF alone can mobilize large numbers of hematopoietic progenitor cells during steady-state hemopoiesis in patients and healthy adult donors [12, 19, 26]. We investigated the effect of increasing G-CSF dose on mobilization of committed and primitive hematopoietic progenitor cells, including CFU-GM, BFU-E, and LTC-IC, in addition to $CD34^+$ cells, and yields of progenitor cells in PBSC harvests obtained by leukapheresis in healthy adult donors [7]. G-CSF at 5, 10, or 15 μg/kg was given to the donors subcutaneously for 5 days and 5-l leukapheresis was performed on day 6 of G-CSF administration.

The results indicate that not only committed (CFU-GM and BFU-E) but also primitive (LTC-IC) progenitor cells, including $CD34^+$ cells, were mobilized into the circulation in a dose-dependent and time-dependent manner. Table 2

Table 2 Total numbers of progenitor cells collected in PBSC harvests

Case	G-CSF dose (μg/kg)	MNC ($\times 10^9$)	CFU-GM ($\times 10^5$)	BFU-E ($\times 10^5$)	CD34+ ($\times 10^6$)	LTC-IC ($\times 10^4$)
1	5	11.2	5.9	16.9	51.5	10.1
2	5	8.5	14.7	37.7	42.5	4.8
3	5	8.2	11.1	50.0	62.3	8.7
Mean ± SD		9.3±1.4	10.5±3.6	34.8±13.7	52.1±8.1	7.9±2.2
4	10	12.3	19.7	45.1	65.2	21.1
5	10	13.8	17.9	67.2	122.8	19.3
6	10	10.9	19.9	71.1	139.5	17.1
Mean ± SD		12.3±1.2	18.9±0.7	61.3±11.4	109.0±31.8	19.2±1.7
7	15	15.8	52.6	123.7	109.0	28.7
8	15	12.3	20.5	38.5	54.1	15.9
9	15	14.8	39.2	100.5	186.7	36.7
Mean ± SD		13.3±1.6	37.7±13.2	87.5±36.0	117.0±54.4	23.2±7.3

shows the total number of progenitor cells collected in PBSC harvests. The data show that the numbers of CFU-GM, BFU-E, and CD34+ cells and LTC-IC collectable are dependent on the G-CSF dose given for PBSC mobilization. Despite the limited number of healthy adult donors studied, we estimate that a sufficient number of $CD34^+$ cells for engraftment after allogeneic PBSCT could be collected on day 5 of administration of G-CSF at 10 μg/kg to the donors and by 10-l leukapheresis on 2 consecutive days (days 5 and 6) when a target or threshold dose of PBSC for engraftment after allogeneic PBSCT is proposed to be 2- to 3-fold that needed for autografting ($4-6\times10^6$/kg $CD34^+$ cells) [5] or $>5\times10^6$/kg CD34+ cells [1]. Adverse effects included general fatigue and bone pain in most of the donors and fever and headache in some; these symptoms were tolerated in most instances. Laboratory test abnormalities, including transient thrombocytopenia, increased platelet aggregation, and increased levels of some liver enzymes, were induced by G-CSF administration, but all were reversible within a short time.

We have recently investigated the self-renewal capacity of G-CSF-mobilized progenitor cells by replating them for secondary colony formation [4]. The results indicate that some G-CSF-mobilized committed and primitive progenitor cells retain their self-renewal capacity. These observations suggest that hematopoietic stem cells for allogeneic PBSCT can be mobilized by short-term administration of relatively high-dose G-CSF. In our clinical trial of allogeneic PBSCT, in which G-CSF-mobilized PBSC from HLA-identical donors were transplanted, rapid hematologic recovery was observed [2, 8, 11, 18]. Interestingly, although the numbers of T-cells present in PBSC harvests are 10-fold those present in bone marrow grafts, the incidence and severity of acute graft-versus-host disease are not increased. These early experiences with allogeneic PBSCT suggest that allotransplantation of G-CSF-mobilized PBSC may be feasible as an alternative to allo-BMT.

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