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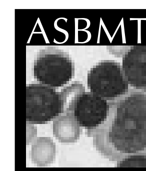

Bone Marrow Mobilized With Granulocyte Colony-Stimulating Factor in Related Allogeneic Transplant Recipients: A Study of 29 Patients

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ABSTRACT

We studied whether a short course of granulocyte colony-stimulating factor (G-CSF) administered to normal donors immediately before bone marrow (BM) harvest would shorten time to neutrophil and platelet engraftment in matched related allogeneic BM recipients. Twenty-nine normal donors received 4 consecutive daily subcutaneous injections of G-CSF (median dose, 12.1 µg/kg per day; range, 9.6-15.7 µg/kg per day) immediately before BM harvest. Donors tolerated G-CSF well, with only mild myalgias and arthralgias, and BM was easy to aspirate. The BM harvest contained a median of 5.3 × 10^8 white blood cells (WBCs)/kg (range, 3.1-11.1 × 10^8 WBCs/kg) and 2.5 × 10^6 $CD34^+$ cells per kg (range, 1.5-7.3 × 10^6 $CD34^+$ cells per kg). Median times to neutrophil (18 days [range, 11-30 days] versus 22 days [range, 16-36 days]; *P* = .05) and platelet (22 days [range, 15-55 days] versus 27 days [range, 18-46 days]; *P* = .04) engraftment were statistically shorter than those of historical control subjects whose donors had not received G-CSF before BM harvest. However, secondary engraftment-dependent outcomes including red blood cell and platelet transfusions, febrile days, days on antibiotics, days from transplant to hospital discharge, and days in hospital during the first 60 days after transplant were not statistically different from historical control subjects. We conclude that G-CSF administered to normal donors immediately before harvest facilitates BM aspiration, increases the WBC content of the harvest, and hastens neutrophil and platelet engraftment compared with historical control subjects.

KEY WORDS

G-CSF • Aspiration • Engraftment

INTRODUCTION

The use of peripheral blood progenitor cells (PBPCs) collected after treatment of patients with chemotherapy or growth factors has become a standard practice for patients undergoing autologous transplantation. The use of autologous PBPCs leads to faster engraftment, less early morbidity, and shorter hospitalization compared with autologous bone marrow (BM) transplantations [1]. More recently, PBPCs collected from normal donors after granulocyte colony-stimulating factor (G-CSF) administration have been used to reduce the early toxicity of allogeneic transplantation. Single-center studies have suggested that neutrophil and platelet engraftment after such allogeneic PBPC transplantations may be faster compared with that of historical control subjects who received allogeneic BM [2-5]. Some reports, however, have indicated that more allogeneic PBPC recipients experience graft-versus-host disease (GVHD), possibly because such PBPC harvests contain approximately 10-fold more T lymphocytes than BM [6,7]. Ongoing randomized studies comparing allogeneic PBPC and BM transplants will more definitively address the relative risk of GVHD associated with these 2 progenitor cell sources [8,9].

Treatment of donors with growth factors such as G-CSF followed by PBPC collection may hasten engraftment

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Table 1. *Preparative Regimens According to Diagnosis and Disease Status*

Diagnosis	Preparative Regimen
Chronic myeloid leukemia (first chronic phase), acute myeloid leukemia (first complete remission)	(1) Intravenous cytarabine 100 mg/m^2 per day by continuous infusion × 5 days followed by intravenous cyclophosphamide 60 mg/kg per day × 2 days and 500 cGy single-fraction total body irradiation (TBI) or (2) busulfan 1 mg/kg by mouth every 6 h × 16 doses followed by intravenous cyclophosphamide 60 mg/kg per day × 2 days
Chronic myeloid leukemia (beyond first chronic phase), acute myeloid leukemia (beyond first complete remission), myelodysplastic syndrome	(1) Intravenous cyclophosphamide 60 mg/kg per day × 2 days followed by 1200 cGy fractionated TBI (200 cGy twice a day × 3 days) or (2) busulfan 1 mg/kg by mouth every 6 h × 16 doses followed by intravenous cyclophosphamide 60 mg/kg per day × 2 days
Acute lymphoid leukemia, non-Hodgkin's lymphoma, multiple myeloma, chronic lymphocytic leukemia	Busulfan 1 mg/kg by mouth every 6 h × 16 doses followed by intravenous cyclophosphamide 60 mg/kg per day × 2 days

through quantitative and qualitative effects of the growth factors on progenitor cells. Faster engraftment may be solely a quantitative effect, since G-CSF–mobilized PBPC harvests contain more CD34$^+$ cells than unstimulated BM harvests. In addition, G-CSF may also activate quiescent progenitors, hasten their maturation, or both, leading to faster engraftment through qualitative changes in the cellular composition of the harvest.

Before routinely adopting G-CSF–mobilized PBPCs for allogeneic transplantation, we studied whether G-CSF directly increases the progenitor cell content and engraftment potential of normal BM. We postulated that treatment of normal donors with G-CSF may improve the quantity and quality of progenitor cells harvested from BM, resulting in more rapid engraftment, without the additional T lymphocytes collected in a PBPC harvest. We report the engraftment times, incidence of GVHD, and overall survival of 29 recipients of related allogeneic transplants from donors who received G-CSF before BM donation.

PATIENTS AND METHODS

Patients

Between April 1997 and September 1998, patients who had hematologic malignancies and were eligible for matched related allogeneic transplantation at the Princess Margaret Hospital (PMH) and Queen Elizabeth II Health Sciences Centre (QEII HSC) were invited to participate in this study. Patients eligible for the Canadian Bone Marrow Transplant Group study (CBMTG 96-01) comparing BM and PBPC in patients undergoing allogeneic transplantation for myeloid malignancies [9] were not eligible for this study. The study was approved by the Institutional Review Boards of the University of Toronto and the QEII HSC, and all patients and donors gave informed consent before enrollment.

BM Donors and Harvest

BM donors were first-degree relatives of the recipients, matched at 5 of 6 (2 donors) or all 6 (27 donors) HLA loci. Donors received 4 daily subcutaneous G-CSF injections immediately before BM harvest, according to their actual body weight (<60 kg: 600 µg/day; 60-90 kg: 960 µg/day; >90 kg: 1200 µg/day). This regimen of moderate-dose (median 12.1 µg/kg per day), short-course G-CSF was used to minimize the exposure of normal donors to this growth factor [5]. BM was collected from the posterior iliac crests as described [10]. The BM harvest was completed when a minimum of 3 × 10^8 white blood cells (WBCs)/kg recipient weight, but not more than 22 mL BM per kg donor weight, was collected.

Preparative Regimen and Supportive Care

Recipients were conditioned using chemotherapy with or without radiation according to each recipient's diagnosis and disease status (Table 1) and nursed in single, laminar-flow and/or positive-pressure, high-efficiency particulate air (HEPA)-filtered rooms. At 1 center (PMH), posttransplantation prophylactic antibiotics (trimethoprim-sulfamethoxasole or ciprofloxacin) were administered orally until the patient could no longer swallow. Antifungal prophylaxis and growth factors posttransplantation were not used. Patients who were seropositive for herpes simplex virus received acyclovir (400 mg by mouth twice a day or 80 mg intravenously [IV] twice a day) from day 1 until day 28. Broad-spectrum antibiotics were administered when neutropenic patients (absolute neutrophil count [ANC] <0.5 × 10^9/L) became febrile. Irradiated platelet transfusions were routinely given when the morning platelet count was <10 × 10^9/L, and irradiated red blood cells were transfused when the hemoglobin concentration was <80 g/L.

All patients received cyclosporine (6.25 mg/kg by mouth twice a day or 2.5 mg/kg IV twice a day) from day –1, and the dose was adjusted to maintain a trough whole blood cyclosporine level of 200 to 400 µg/L. Methotrexate was administered IV on day 1 (15 mg/m^2) and days 3, 6, and 11 (10 mg/m^2), with dose reductions for mucositis (Bearman grades 2 and 3), decreased calculated creatinine clearance, and direct hyperbilirubinemia as required. Patients at risk for cytomegalovirus (CMV) disease underwent bronchoscopy with bronchoalveolar lavage (BAL) on day 35 (PMH) or days 35 and 49 (QEII HSC) and were treated with ganciclovir if the BAL shell vial culture was positive for CMV.

Evaluations and Definitions

The WBC count and CD34$^+$, CD3$^+$, and colony-forming unit (CFU) (granulocyte/macrophage [CFU-GM], multipotential [CFU-GEMM], burst-forming unit-erythroid [BFU-E], and megakaryocyte [CFU-Mega]) cell counts of BM harvests were assayed on the day of collection. CFU cell counts of the donors' BM were also assayed before starting treatment with G-CSF. WBC counts were measured using an automated counter (Coulter, Hialeah, FL) and verified visually. CD34$^+$ cells were quantified according to the method of Sutherland et al. [11]. CD3$^+$ cells were measured by flow cytometry using monoclonal antibodies recognizing CD3$^+$ and CD45$^+$ cell surface antigens and a FACSort

Table 2. *Donor and Harvest Characteristics**

	G-CSF-Stimulated BM	Unstimulated BM (Historical Control Subjects)
n	29	20
Donor age, y	48 (18-73)	52 (20-68)
Donor sex, M/F	18/11	12/8
Daily G-CSF dose, μg/kg per day	12.1 (9.6-15.7)	—
BM harvest duration, min	34 (10-80)	67 (50-95)
BM harvest volume, mL	950 (620-1950)	1000 (500-1300)
WBCs/kg recipient weight	5.3×10^8 (3.1-11.1×10^8)	3.13×10^8 (1.6-4.2×10^8)
$CD34^+$/kg recipient weight	2.5×10^6 (1.5-7.3×10^6)	NA
$CD3^+$/kg recipient weight	1.6×10^7 [0.4-3.5×10^7 (n = 21)]	NA
CFU cell content before G-CSF administration†		
CFU-GM/kg recipient weight, $\times 10^4$	18 (2-59)	65 (31-183)
CFU-GEMM/kg recipient weight, $\times 10^4$	0.17 (0.09-0.3)	4.4 (1.5-14.4)
BFU-E/kg recipient weight, $\times 10^4$	21 (3-58)	119 (23.7-331)
CFU-Mega/kg recipient weight, $\times 10^4$	2.9 (0.1-8.4)	14 (1.2-60.3)
CFU cell content after G-CSF administration†		
CFU-GM/kg recipient weight, $\times 10^4$	10 (5-15)	—
CFU-GEMM/kg recipient weight, $\times 10^4$	0.15 (0.07-0.2)	—
BFU-E/kg recipient weight, $\times 10^4$	6 (2-10)	—
CFU-Mega/ kg recipient weight, $\times 10^4$	2.6 (0.7-5.0)	—

*Data are median (range) or n. G-CSF indicates granulocyte colony-stimulating factor; BM, bone marrow; WBC, white blood cell; NA, not available; CFU, colony-forming unit; CFU-GM, colony-forming unit-granulocyte/macrophage; CFU-GEMM, multipotential colony-forming unit; BFU-E, burst-forming unit-erythroid; CFU-Mega, colony-forming unit-megakaryocyte.

†Plating efficiency (number of colonies per cell plated) was measured for cells from the normal donor's bone marrow before G-CSF administration and at the time of BM harvest. For each colony type (CFU-GM, CFU-GEMM, BFU-E and CFU-Mega), the CFU cell content expressed per kg recipient weight was calculated as follows:

$$\frac{\text{colony count}}{\text{number of cells plated}} \times \frac{\text{(WBC concentration of BM harvest)(volume of harvest)}}{\text{weight of recipient}}.$$

instrument (Becton Dickinson, Franklin Lakes, NJ). CFU progenitor cell assays were performed as described [10].

Day of neutrophil engraftment was defined as the second of 2 days with an ANC $>0.5 \times 10^9$/L, and day of platelet engraftment was defined as the second of 2 days with a platelet count $>20 \times 10^9$/L independent of platelet transfusion. Duration of first hospitalization was measured from the day of BM infusion until the day of first hospital discharge. GVHD was graded according to published criteria [12,13]. Patients who experienced neutrophil engraftment and survived until at least day 30 posttransplantation were assessed for acute GVHD. Patients who survived until at least day 100 posttransplantation were assessed for chronic GVHD.

Statistical Methods

Descriptive statistics (median and range) were used to describe donor, recipient, and BM characteristics. Times to neutrophil and platelet engraftment and secondary engraftment-dependent outcomes were compared with historical control subjects using the Kruskal-Wallis rank sum test. Correlation analyses were performed using the Spearman rank test.

RESULTS

BM Donors and Harvest Characteristics

BM donors tolerated G-CSF injections with only mild myalgias and arthralgias. No donor experienced evidence of leukostasis or abnormal bleeding. BM donors who received G-CSF have been followed for at least 2 years and have not had any long-term adverse effects. Donor and BM harvest characteristics are shown in Table 2. BM was consistently very easy to aspirate during the harvest, as indicated by the short median BM harvest duration. There was no significant correlation between donor age or sex and BM characteristics. The CFU cell counts of the BM harvests after G-CSF administration were similar to those before G-CSF administration (Table 2) and lower than those of historical control subjects who had not received G-CSF before BM harvest [5].

Recipients

Twenty-nine patients received allogeneic BM from related donors who were matched for 5 of 6 or 6 of 6 HLA antigens and who had received G-CSF. Patient characteristics are shown in Table 3. The allogeneic BM infusion was well tolerated. Neutrophil engraftment occurred in 27 of 29 (93%) recipients; 2 died before neutrophil engraftment. Platelet engraftment occurred in 24 of 29 (83%) recipients; 5 died before platelet engraftment. Times to neutrophil and platelet engraftment and secondary engraftment-dependent outcomes are shown in Table 3. The times to neutrophil and platelet engraftment were significantly faster compared with those of historical control subjects who received unstimulated BM at our institutions [5]; time to neutrophil engraftment was 18 days (range, 11-30 days) versus 22 days (range, 16-36 days), $P = .05$; time to platelet engraftment was 22 days (range, 15-55 days) versus 27 days (range, 18-46 days), $P = .04$. However, secondary engraftment-dependent

Table 3. *Patient Engraftment and Engraftment-Dependent Characteristics**

	G-CSF–Stimulated BM	Unstimulated BM (Historical Control Subjects)
n	29	20
Recipient age, y	45 (17-61)	46 (20-58)
Recipient sex, M/F	17/12	12/8
Recipient diagnosis		
Chronic myeloid leukemia, first chronic phase	1	4
Chronic myeloid leukemia, relapse after first BMT	2	0
Acute myeloid leukemia, first complete remission	1	2
Acute lymphoid leukemia, first complete remission	7	4
Acute lymphoid leukemia, complete remission 2 or more	2	0
Myelodysplastic syndrome, de novo	4	3
Myelodysplastic syndrome, after autologous BMT	2	0
Non-Hodgkin's lymphoma, chemosensitive	6	3
Multiple myeloma, chemosensitive	2	2
Chronic lymphocytic leukemia, chemosensitive	2	2
Neutrophil engraftment, d	18 (11-30) (n = 27)	22 (16-36)
Platelet engraftment, d	22 (15-55) (n = 24)	27 (18-46)
Platelet transfusions	4 (1-17) (n = 28)	6 (2-16)
Red blood cell transfusions	4 (0-31) (n = 28)	5 (2-21)
Febrile days, day 0 to first discharge	5 (0-19)	2 (0-17)
Days on antibiotics, day 0 to first discharge	16 (2-63)	16 (0-56)
Days to first discharge	29 (16-67)	29 (21-51)
Days in hospital, day 0 to day 60	34 (14-60) (n = 26)	39 (26-60)

*Data are median (range) or n. G-CSF indicates granulocyte colony-stimulating factor; BM, bone marrow.

outcomes, including number of platelet and red blood cell transfusions, number of febrile days, days on antibiotics, days from BMT to first hospital discharge, and days in hospital during the first 60 days posttransplantation were not statistically different from those of historical control subjects at our institutions [5]. In this group of 29 patients, we did not find any significant correlation between characteristics of the BM harvest (WBC, $CD34^+$, and CFU cell content) and times to neutrophil and platelet engraftment.

GVHD and Survival

Patients received a median of 3.75 doses (range, 2-4 doses) of a possible maximum of 4 full doses of methotrexate. Two patients were not assessable for acute GVHD because of early death. The incidence of acute GVHD (grades I to IV) was 19 of 27 (70%), and 11 of 27 (41%) patients experienced grades II to IV acute GVHD. As of May 1999, 19 of 29 (65%) patients were alive and had been followed for at least 6 months. The cumulative incidence of chronic GVHD among these patients was 5 of 11 (45%), with 4 (36%) and 1 (9%) experiencing limited and extensive chronic GVHD, respectively. Most patients (4 of 5) with chronic GVHD required systemic treatment.

At a median follow-up of 6 months (range, 0.5-17 months), the 100-day mortality was 6 of 29 (21%) and the 1-year overall survival of patients followed for at least 1 year was 7 of 17 (41%).

DISCUSSION

Autologous PBPC transplantations result in faster engraftment, fewer transfusions, shorter hospitalizations, and less early transplantation morbidity compared with autologous BM transplantations [1]. Patients also avoid the potential morbidities of anesthesia and BM harvest. In addition, PBPC collections may result in more patients proceeding to transplantation, because growth factor–mobilized PBPC collections contain more progenitors than autologous BM harvests.

The benefits of allogeneic PBPC transplantations are not as clearly established. Several studies have reported that they too hasten engraftment, although the effects reported to date have been modest [2-5]. Furthermore, allogeneic engraftment can also be affected by other factors such as the regimen used for GVHD prophylaxis—in particular, methotrexate. Allogeneic PBPC collections also contain approximately 10-fold more T lymphocytes than BM harvests, which may lead to a greater risk of GVHD [6,7]. For these reasons, we studied whether the engraftment of allogeneic BM could be improved by administering G-CSF to donors before BM harvest. Because a short G-CSF course dramatically increases the number of PBPCs, we postulated that administering a similar course of G-CSF may also either increase the number of BM progenitor cells or activate and mature preexisting BM progenitor cells. Whereas G-CSF administered before BM harvest may not offer any advantage over PBPC collection for patients undergoing autologous transplantation, it may improve allogeneic BM collection, avoiding the more plentiful T lymphocytes present in a PBPC harvest.

Johnson et al. [14] studied 22 patients with hematologic malignancies who received G-CSF, granulocyte-macrophage CSF (GM-CSF), or interleukin (IL)-3 before autologous BM harvest and found that each cytokine increased the number of light-density cells isolated from BM. G-CSF and GM-CSF also increased the total number of CFU-GM cells. Engraftment times were not reported. In a similar study, Slowman et al. [15] administered GM-CSF or G-CSF before BM harvest to 11 patients with nonhematologic

malignancies and found higher nucleated cell and CD34+ cell concentrations compared with concurrent control subjects. Although neutrophil engraftment was faster in the cytokine-treated group (12 versus 24 days), this group also received cytokines after infusion, confounding the effect of cytokines administered before harvest. Lowenthal et al. [16] administered G-CSF before BM harvest in 15 heavily pretreated patients and reported prompt engraftment, but there was no control group for comparison.

Other investigators have not found faster autologous engraftment with this strategy. Hansen et al. [17] administered G-CSF, GM-CSF, or IL-3 before BM harvest to 37 patients with hematologic diseases and found increased marrow cellularity, a higher myeloid:erythroid ratio, and more myeloid progenitors, but engraftment was not faster compared with that of historical control subjects. Sosman et al. [18] administered IL-3 before BM harvest to 19 patients with nonhematologic cancers, and engraftment was not improved, although the CFU-GM content of the BM was increased.

Because patients undergoing autologous transplantation have often received prior chemotherapy, which may have damaged hematopoietic stem cells in the BM, or have tumor involvement of the BM, their BM may not respond well to cytokine-mediated stimulation. Surprisingly, there have been few reports of cytokines administered to normal donors before allogeneic BM harvest. Meisenberg et al. [19] administered G-CSF to 8 normal donors before BM harvest and found prompt neutrophil and platelet engraftment, but G-CSF was also administered after infusion. Isola et al. [20] described 5 patients whose donors received G-CSF before BM harvest and reported median neutrophil and platelet engraftment at 14 and 16 days, respectively, which was faster than in concurrent control subjects. Finally, in 12 patients also treated with G-CSF [21], a median of 1.6×10^6 CD34+ cells per kg was collected from the stimulated BM, but engraftment times were not reported and the collections were T-cell–depleted, making comparison with non–T-cell–depleted engraftment difficult.

Our study of 29 patients is the largest reported series to examine the effect of G-CSF administration to normal donors before BM harvest. We found that this strategy did modestly improve times to neutrophil and platelet engraftment compared with those of historical control subjects who had received unstimulated BM. Although our patient cohort was similar to the historical control subjects in terms of age and disease and they received identical GVHD prophylaxis and supportive care, this was not a randomized comparison of G-CSF–stimulated BM and unstimulated BM, and there may have been other differences between the 2 groups that have not been accounted for.

In this study, G-CSF was well tolerated, BM was easy to aspirate, and the median WBC per kg recipient weight of the harvest was high. However, we did not find an improvement in secondary engraftment-dependent outcomes compared with those of historical control subjects. This may be explained by the small sample size, which limited our ability to detect differences in secondary outcomes if they were present. The small size of this study also precluded us from establishing whether the use of G-CSF–stimulated BM affects the likelihood of developing GVHD. The incidence of acute and chronic GVHD was similar to that of recipients of unstimulated BM at our centers [5,10].

This study indicates the need for further investigation of the strategy of administering a growth factor before allogeneic BM harvest. Administering G-CSF for 4 days immediately before a BM harvest may not be the best way to collect BM progenitor cells. Although G-CSF is administered in this way before PBPC collection, the direct effect of G-CSF on BM may be greater after a different G-CSF dose or a different time interval between G-CSF administration and BM harvest. In a murine model, Bodine et al. [22] found that the repopulating ability of BM cells was low immediately after treatment with G-CSF and stem cell factor but increased more than 10-fold 14 days later. Whereas G-CSF effectively facilitates PBPC collections and has been shown in this study to modestly hasten engraftment after BM transplantation, BM progenitor cells may be more effectively stimulated by other cytokines or cytokine combinations. Further studies of other cytokines, cytokine combinations [23,24], and BM harvest schedules may be indicated, particularly if allogeneic PBPC transplantations are shown to lead to unacceptably high rates of GVHD.

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