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Biology

Hematopoietic, Mesenchymal, and Immune Cells Are More Enhanced in Bone Marrow than in Peripheral Blood from Granulocyte Colony-Stimulating Factor Primed Healthy Donors

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The use of granulocyte colony-stimulating factor (G-CSF) primed bone marrow (G-BM) has been recently considered as an alternative to mobilized hematopoietic stem cells from peripheral blood (G-PB), especially in the haploidentical transplant setting. The purpose of this study was to compare the effect of in vivo G-CSF priming on BM and PB hematopoietic, mesenchymal (MSC), and immune cells. Forty healthy donors undergoing BM harvest for haploidentical transplant were given subcutaneous recombinant human G-CSF for 7 days. BM and PB samples were harvested on days –7 and 0. The hematopoietic stem/progenitor cells increased significantly after G-CSF priming in both BM and PB with a selective rise of BM $CD34^+CD38^-$ cell subset. A striking enhancement of the mesenchymal progenitors was detected in G-BM. $CD3^+$, $CD4^+$, $CD8^+$, and $CD19^+$ cell fractions; the naive $CD4^+$ and $CD8^+$ subpopulations; and natural killer and regulatory T cells increased in G-BM, whereas only slight changes were detected in PB. Myeloid dendritic cells (DC1) were significantly upregulated in both G-BM and G-PB, whereas DC2 increased only in G-BM. In conclusion, our results show substantial differences in the biologic effects exerted by G-CSF at BM and PB levels on hematopoietic cells and immune cell fractions. Furthermore, the impressive rise of MSC progenitors in G-BM might also be relevant to provide MSCs for several clinical use.

INTRODUCTION

Several hematopoietic stem cell (HSC) sources (steady-state bone marrow [BM], mobilized peripheral blood stem cells [G-PBs], and cord blood stem cells) are today available for allogeneic transplant. Furthermore, several post-transplant outcomes, such as engraftment, graft-versus-host disease (GVHD), and disease-free and overall survival, significantly depend on either intrinsic or cytokine-induced biologic HSC characteristics.

Results from clinical trials have shown that G-PB, although inducing a faster engraftment than steady-state BM, is associated with a higher risk of chronic GVHD (cGVHD), which may adversely affect survival in either HLA-identical sibling transplants [1-3] or unrelated transplants [4]. To fasten the engraftment without increasing the cGVHD rate, granulocyte colony-stimulating factor (G-CSF) primed BM (G-BM) transplant has been explored. Compared with G-PB, the use of G-BM resulted in comparable engraftment, reduced severity of acute GVHD, and reduced incidence of cGVHD [5-7]. It is well known that G-CSF induces significant modifications in both hematopoietic and immunologic compartments. In fact, it has been reported that the mobilization protocols using G-CSF promote immune tolerance through several mechanisms: (1) the promotion of BM-derived regulatory T cells (Tregs) [8,9], (2) the production of a functionally anergic T cell population with impaired cytolytic effector function [5], and (3) the polarization toward Th2 differentiation while inhibiting Th1 development [10]. Moreover, G-CSF mobilizes dendritic cells (DCs), the antigen-presenting cells involved in the immune response able to trigger naive T cell

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differentiation toward Th1 and Th2 cells [11,12]. Several in vitro and in vivo studies have shown that G-CSF induces a decreased release of proinflammatory cytokines while stimulating the production of anti-inflammatory cytokines [13-20]. Clinical studies have suggested that G-CSF may differentially modulate in BM and PB the immune cells responsible for GVHD [21], notwithstanding the similar effect exerted on the cells responsible for hematologic recovery in both the cell sources [22]. However, relatively little is known about the effects of G-CSF on cells released into the PB compared with the cells remaining in the BM.

Published biologic studies, aimed at comparing the effects of G-CSF on the 2 different stem cell sources, have provided controversial results. Several reasons may account for these discrepancies: the small number of donors studied, the use of different donor series to compare the biologic characteristics of cells in steady state and after G-CSF, and the different G-CSF treatment schedule [12,22,23]. An ex vivo biologic study on 40 healthy donors was developed concurrently with a protocol of unmanipulated G-BM haploidentical transplant for patients with high-risk hematologic malignancies [24] to compare the effects of 7 days G-CSF priming on BM and PB hematopoietic and immune cells.

METHODS

Donor Selection, G-CSF Treatment, and Sample Collection

Forty of 80 healthy donors from our previously published clinical study [20] who signed written informed consent were enrolled in the biologic study. The donors (25 men and 15 women; median age, 43 years; range, 26 to 70) were treated with 4 μg/kg/day G-CSF (Filgrastim, Granulokine; Amgen, Milan, Italy) in a single subcutaneous injection for 7 consecutive days. BM and PB cell samples were harvested before the first G-CSF injection (day –7) and on the day of transplant (day 0). The first 2 mL of aspirate were collected for the study at the beginning of the BM harvesting to avoid contamination by the PB cells.

Clonogenic Assay

The proliferative potential of hematopoietic progenitors was assessed by clonogenic tests carried out with BM and PB samples on days –7 and 0. Briefly, 5×10^4 BM and 10×10^4 PB nucleated cells were suspended in 1 mL methylcellulose medium (Methocult GFH4034; StemCell Technologies, Vancouver, British Columbia, Canada) supplemented with 30% FBS, rhSCF, rhIL-3, granulocyte-macrofage (CFU-GM), recombinant human stem cell factor (rhCSF), and recombinant human erythropoietin (rhEPO) and plated in duplicate in 35-mm tissue culture dishes. Cultures were incubated at 37°C in a humidified atmosphere of 5% CO_2. Each plate was scored for colony-forming unit (CFU) GM, burst-forming unit erythroid, and CFU-granulocyte, erythroid, macrophage, megakaryocyte after 10 and 14 days of incubation using an inverted microscope (Leica DMIL).

CFU-F Assay

The CFU-fibroblast (CFU-F) assay was performed, according to the method of Castro-Malaspina et al. [25], with unmanipulated BM and PB on days –7 and 0. The NC were seeded at 32×10^3 cells/cm^2 (BM) and 120 to 200×10^3 cells/cm^2 (PB) in 25-cm^2 noncoated polystyrene culture flask in complete culture medium consisting of DMEM (Euroclone, Milan, Italy), supplemented with 100 units/mL penicillin, 100 μg/mL streptomycin, 2 mmol/L L-glutamine (Euroclone), 10% FBS (FBS Certified; Gibco, NY).

Cultures were maintained for 14 days at 37°C in a humidified atmosphere 5% CO_2. After 24 to 48 hours, the nonadherent cells were discarded, and the adherent cells were thoroughly washed twice with Dulbecco's PBS (Euroclone). Fresh medium was then added and the flasks cultured for 2 weeks. After incubation the cells were washed with PBS, fixed with methanol, and stained with a Giemsa stain solution (Giemsa; Carlo Erba, Milan, Italy), and colonies consisting of at least 50 cells were counted as CFU-F under an inverted microscope.

Phenotyping Studies

Flow cytometric analysis was performed using whole PB and BM incubated with fluorescent mAbs and using 6-color FACSCanto II (BD, Biosciences) and FACSDiva Software (BD, Biosciences) for acquisition and analysis. Samples were first incubated with mAbs and then processed with the lyse-wash technique (using ammonium chloride solution 1X; BD, Bioscience). HSCs were identified by using CD34-PE, CD38-FITC, and CD33-FITC (BD Biosciences). Phenotype analysis of immune cells was performed by using the following mAbs: anti-CD3-PE, anti-CD4-FITC, anti CD8-PerCP, anti-CD45RA-FITC, anti-CD45RO-PE, anti-CD45-PercPCy5.5, anti-CD19-APC, anti-CD16-FITC, anti-CD56-PE, and anti-CD14-PercPCy5.5 (BD, Biosciences). DCs were identified using the following mAbs: anti-CD45RO-FITC, anti-CD40-PE, anti-HLA-DR-PercP, anti-CD45RA-PE-Cy7.7, anti-CD11c-APC, and anti-CD4-APC H7 for DC1 cells and anti-CD45RO-FITC, anti-CD40-PE, anti-HLA-DR-PerCP, anti-CD45RA-PE-Cy7.7, anti-CD123-APC, and anti-CD4-APC-H7(BD, Biosciences) for DC2 cells.

Flow Cytometric Cytokine Assay

Serum concentrations of IL-2, IL-4, IL-6, IL-10, tumor necrosis factor, and IFN-γ were analyzed in a culture medium of patient cells using a cytometric bead array kit (BD cytometric bead array [CBA]) according to the manufacturer's instructions. Briefly, 50 μL capture beads, 50 μL each unknown sample, and 50 μL human Th1/Th2-PE detection reagent were mixed together in each assay tube and incubated for 3 hours at room temperature. The samples were analyzed by FACSCanto II (Becton Dickinson). Previously established calibration curves were used to detect the concentration of each analyte in the test samples. Data acquisition and analysis of results were performed using DIVA software and FCAP Array (Becton Dickinson, Milan, Italy).

Statistics

All data are shown as the mean ± SEM of total number of cells/μL in BM or PB samples pre– and post–G-CSF or as the mean ± SEM of the ratio between pre– and post–G-CSF values (fold increase [FI]) calculated for each individual donor. Statistical significance was defined as $P < .05$, as calculated by paired Student *t*-test. Analysis of the statistical significance between groups of donors divided by age, sex, and cytomegalovirus (CMV) serology was evaluated on median values by the Kruskal-Wallis test due to the low number of donors in some subgroups.

RESULTS

Effects on CD34⁺ HSC Compartment

In the 40 donors given G-CSF, the absolute number of total BM CD34⁺ cells increased 8.1-fold, from $361.4 \pm 60.6 \times 10^3$/mL to $1347.6 \pm 159.6 \times 10^3$/mL ($P < .001$) (Figure 1A,B). In G-PB the CD34⁺ cell rise was 5.8-fold ($7.1 \pm 1.5 \times 10^3$/mL versus $33.7 \pm 60.6 \times 10^3$/mL) (Figure 1C,D). In G-BM the more primitive CD34⁺CD38⁻ cell fraction was more enhanced (mean, 36.1-fold), with a rise of the total cell number from 154.9 ± 28.8 to $862.8 \pm 134.8 \times 10^3$/mL ($P < .001$), than the late CD34⁺CD33⁺ cells (FI, 19.8; $P = .001$) (Figure 1A,B). Conversely, a comparable increase of both early and late CD34⁺ cells, with a rise of CD34⁺CD38⁻ and CD34⁺CD33⁺ cells, respectively, of 11.1-fold ($P < .001$) and 10.9-fold ($P = .002$) was detected in G-PB (Figure 1C,D).

Effect on Committed Hematopoietic Progenitors

G-CSF priming induced a significant increase ($P < .001$) of the clonogenic progenitors in both BM and PB. The absolute number of the BM CFUs rose from 37700 ± 4700 to 242500 ± 27400 CFU/mL, with a 12.8-fold increase. In PB the number of CFU/mL was 1 log lower than in BM (Table 1).

Effect on Committed Mesenchymal Progenitors

Because of the crucial role of mesenchymal stem cells (MSCs) in hematopoietic transplant, the effect of G-CSF priming on mesenchymal progenitors was evaluated by the in vitro growth of the committed fibroblast progenitors (CFU-F). An impressive increase (FI = 49.9) of BM CFU-Fs was detected after G-CSF priming, with a significant ($P < .001$) increment of the total number of BM CFU-Fs from $170 \pm .5$/mL to 29670 ± 711/mL (Table 1). The PB CFU-Fs were not detectable before or after G-CSF.

Effect on T, B, and Natural Killer Cells

As shown in Table 2, the BM NCs increased 10-fold after G-CSF priming ($P < .001$), whereas in the PB the NCs increased

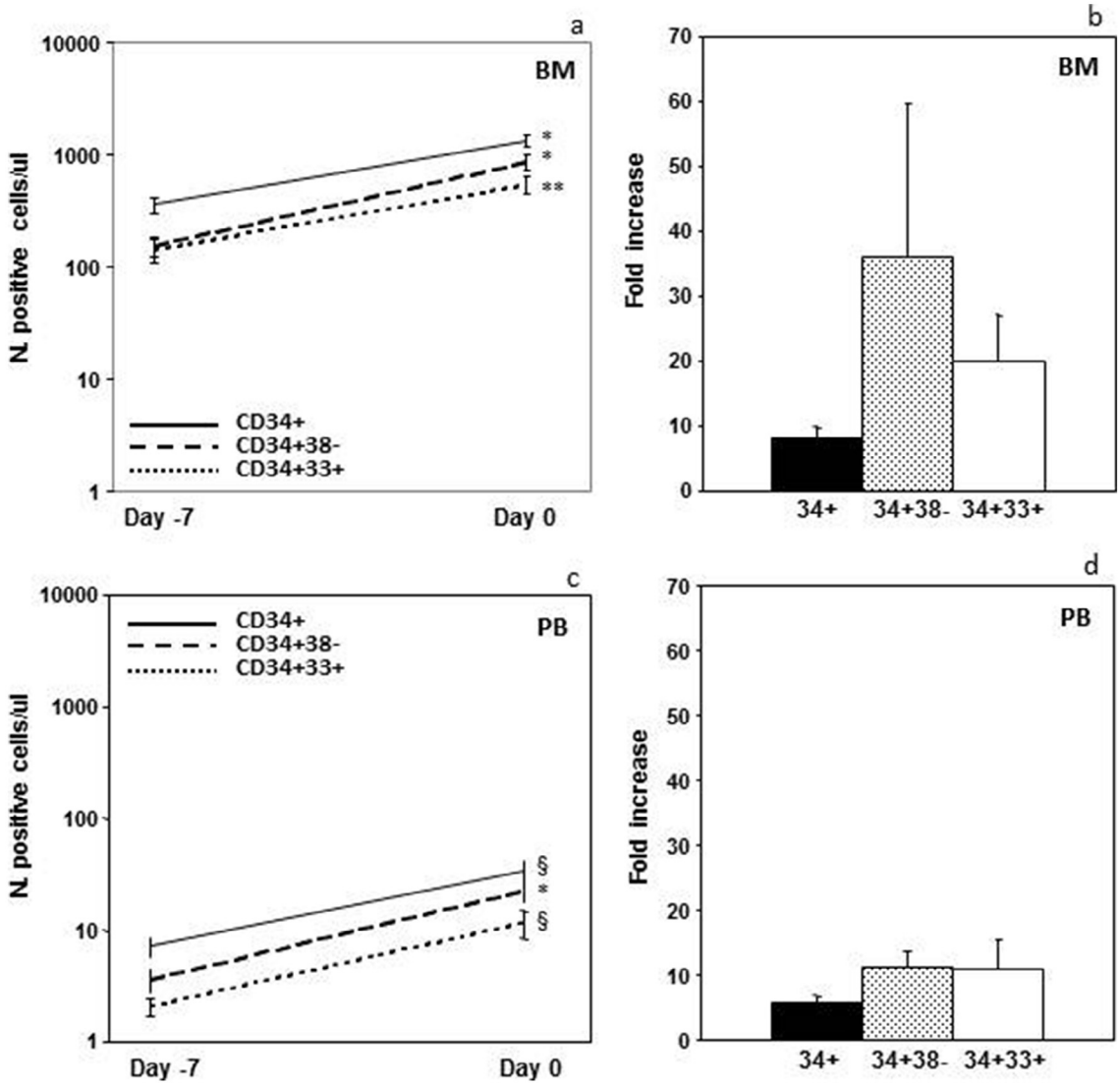

Figure 1. Effect of G-CSF on HSC compartment. The effect of G-CSF priming on the whole $CD34^+$ cells and on the early $CD34^+38^-$ and late $CD34^+33^+$ cell subpopulations was detected in 40 donors in BM (A and B) and PB (C and D) samples collected before and after G-CSF administration. A significant increase of $CD34^+$ cells was detected both in BM, with an 8.1-fold increase of the absolute cell number over the baseline value, and in PB, with a 5.8-fold increase. In BM the early $CD34^+CD38^-$ subpopulation was selectively up-regulated. $^*P < .001$, $^{**}P = .001$, §$P = .002$.

5-fold ($P < .001$). Accordingly, the lymphoid cells increased significantly more in BM ($P = .001$) than in PB ($P = .038$). A different effect of G-CSF on T cells was detected at the BM and PB level. The cells $CD3^+$, $CD4^+$, and $CD8^+$ increased in BM, respectively, 2.3-fold ($P = .007$), 1.8-fold (P = ns), and 3.0-fold ($P < .001$) after G-CSF priming, whereas no significant change was detected in PB. The naive T cell fractions $CD4^+45RA^+$ and $CD8^+45RA^+$ were also evaluated in 22 cases (Figure 2). The naive T helper cells represented about 30% of the total BM $CD4^+$ cells in baseline controls, but after G-CSF priming they increased 2.7-fold (from 576 ± 68 to 1241 ± 269), significantly ($P = .021$) more than the total $CD4^+$ cells. The naive subset of the suppressor T cells represented around 60% of the $CD8^+$ cells both before and after G-CSF priming. The increase of the $CD8^+45RA^+$ cells (FI = 3.2, $P = .042$) was equal to the expansion of the total $CD8^+$ population (FI = 3, $P < .001$). Conversely, no change in number of total and naive $CD4^+$ and $CD8^+$ populations was detected in PB as an effect of G-CSF priming. The B cells increased significantly (3.1-fold, $P = .007$)

Table 1
Effect of G-CSF Priming on BM and PB Hematopoietic and Mesenchymal Progenitors

	BM			
	Pre–G-CSF	Post–G-CSF	FI	*P*
CFU-GM, $\times 10^3$/mL	13.5 ± 1.8	89.6 ± 15.0	11.2	<.001
BFU-E, $\times 10^3$/mL	22.2 ± 3.2	140.2 ± 1.5	18.1	<.001
CFU-GEMM, $\times 10^3$/mL	2.1 ± .3	12.8 ± .3	11.9	<.001
Total CFUs, $\times 10^3$/mL	37.7 ± 4.7	242.5 ± 27.4	12.8	<.001
CFU-F, /mL	170 ± 46	2976 ± 711	49.90	<.001
	PB			
	Pre–G-CSF	Post–G-CSF	FI	*P*
CFU-GM, $\times 10^3$/mL	.3 ± .06	3.6 ± .7	21.3	<.001
BFU-E, $\times 10^3$/mL	.8 ± .1	5.8 ± 1.0	7.5	<.001
CFU-GEMM, $\times 10^3$/mL	.06 ± .02	.8 ± .2	25.9	<.001
Total CFUs, $\times 10^3$/mL	1.1 ± .2	10.4 ± 1.8	9.6	<.001

BFU-E indicates burst-forming unit erythroid; CFU-GEMM, CFU-granulocyte, erythroid, macrophage, megakaryocyte.

Table 2
Changes Induced by G-CSF on BM and PB Lymphocyte Cell Fractions

	BM			
	Pre–G-CSF	Post–G-CSF	FI	*P*
TNCs, $\times 10^6$/mL	25.0 ± 2.7	180.5 ± 15.4	10.0	<.001
Lymphocytes, $\times 10^6$/mL	4.4 ± .6	8.4 ± 1.0	8.4	.001
$CD3^+$, $\times 10^6$/mL	3.1 ± .5	5.3 ± .6	2.3	.007
$CD4^+$, $\times 10^6$/mL	1.8 ± .3	2.3 ± .4	1.8	ns
$CD8^+$, $\times 10^6$/mL	1.3 ± .2	2.7 ± .3	3.0	<.001
$CD19^+$, $\times 10^6$/mL	.7 ± .1	1.3 ± .2	3.1	.007
$CD14^+$, $\times 10^6$/mL	1.0 ± .1	5.0 ± .7	6.4	<.001
	PB			
	Pre–G-CSF	Post–G-CSF	FI	*P*
TNCs, $\times 10^6$/mL	5.5 ± .2	25.1 ± 1.5	5.0	<.001
Lymphocytes, $\times 10^6$/mL	1.7 ± .1	2.0 ± .1	1.3	.038
$CD3^+$, $\times 10^6$/mL	1.3 ± .1	1.5 ± .1	1.3	ns
$CD4^+$, $\times 10^6$/mL	.8 ± .1	1.0 ± .1	1.4	ns
$CD8^+$, $\times 10^6$/mL	.5 ± .04	.5 ± .04	1.3	ns
$CD19^+$, $\times 10^6$/mL	.2 ± .01	.2 ± .02	1.3	ns
$CD14^+$, $\times 10^6$/mL	.3 ± .03	1.0 ± .1	3.7	<.001

TNC indicates total nucleated cells; ns, not significant.

only in BM (Table 2). Regarding the natural killer (NK) cell fractions, a different response to G-CSF was detected in BM and PB, both on the fraction with preponderant cytotoxic activity ($CD3^-CD16^+$) and on the cytokines secreting $CD56^+$ cells. In BM a moderate increase of the 3 subpopulations was observed with the maximum rise in the $CD3^-CD56^+$ cell subset (from $344 \pm 61 \times 10^3$/mL to $476 \pm 79 \times 10^3$/mL). On the contrary, all the NK subpopulations fell by 50% in PB with the most significant decrease ($P = .008$) in the $CD3^-16^+56^+$ cell subset (Figure 3).

Effect on Tregs

Treg fractions were identified in this study as $CD4^+25^+$ and as the highly specific $CD127^-$ cells (Figure 4). The G-CSF induced in BM a 2-fold increase of both the Treg subpopulations with a significant ($P = .014$) rise of the $CD4^+25^+$ cells that increased from 211.9 ± 33.6 to $501.9 \pm 109.4 \times 10^3$/mL in baseline control and after G-CSF. No difference was detected in the number of Treg cell subsets in PB.

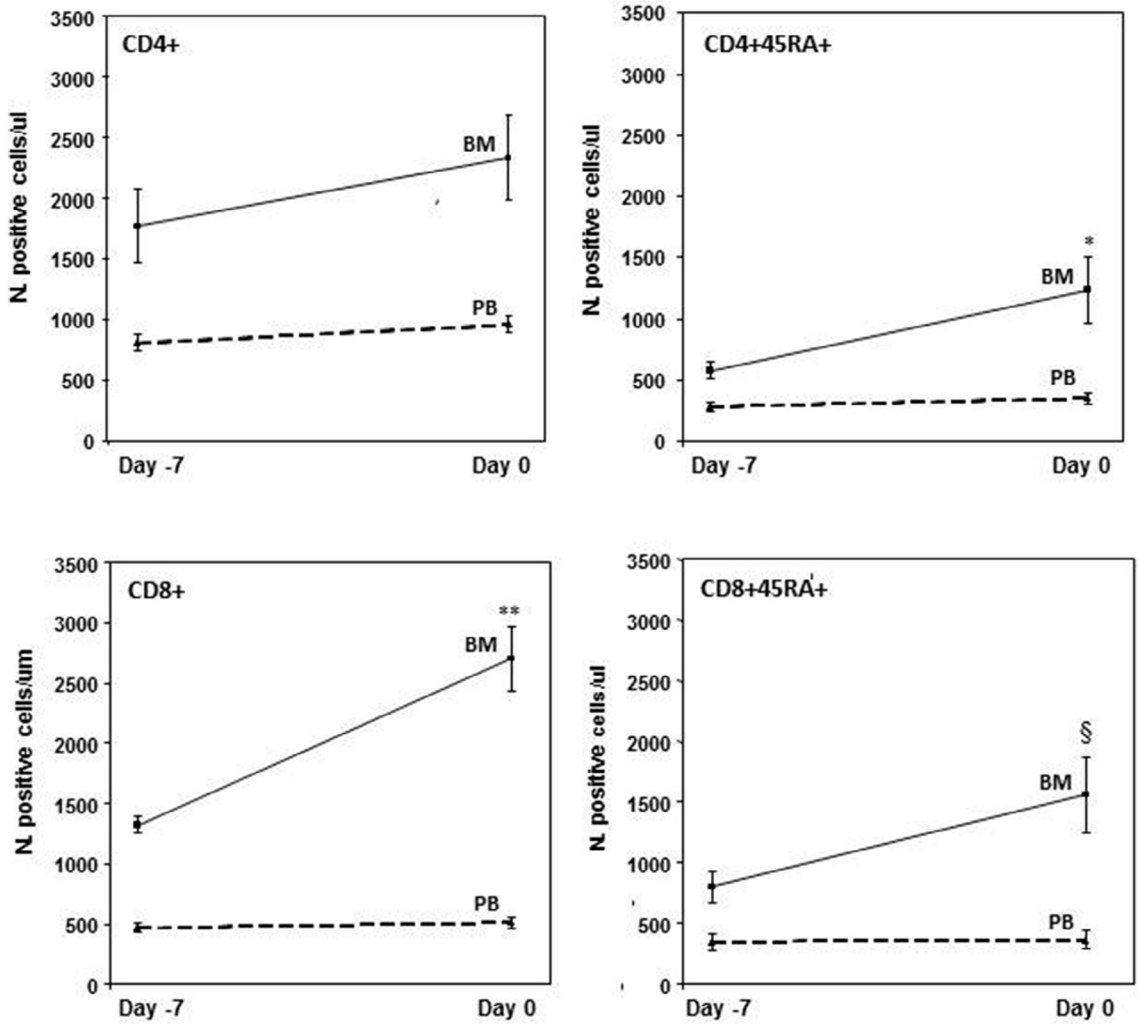

Figure 2. Effect of G-CSF on BM (——) and PB (----) naive $CD4^+$ and $CD8^+$ cells. The BM $CD4^+45RA^+$ subpopulation was selectively affected by G-CSF priming with a significantly higher increase (2.7-fold, $P = .021$) than the total $CD4^+$ cells (1.8-fold, P = ns). Conversely, the increase of the $CD8^+CD45^+$ cell fraction in BM (§$P = .042$) was lower than the rise of the $CD8^+$ cells (**$P < .001$). No significant changes of PB subpopulations were detected after G-CSF exposure.

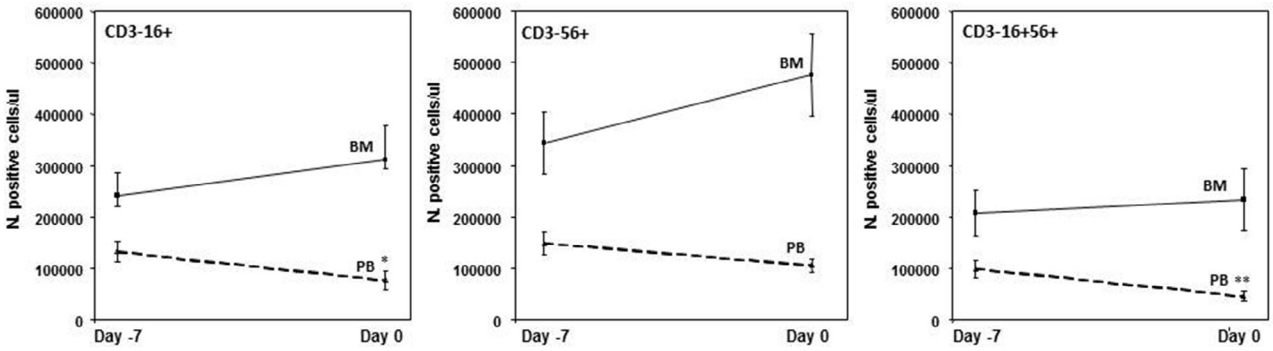

Figure 3. Modifications induced by G-CSF on NK subpopulations. G-CSF affected differently BM (——) and PB (----) NK subpopulations with a slight but not significant increase of all BM NK fractions and a significant decrease of PB cell fractions $CD3^{-}CD16^{+}$ (*$P = .046$) and $CD3^{-}CD16^{+}CD56^{+}$ (**$P = .008$).

Effect on DCs

DCs were evaluated in 15 donors and identified by being positive for HLA-DR and CD40, and the subpopulations of "myeloid" DC1 cells and "plasmocytoid" DC2 cells were identified, respectively, as $CD11^{+}/CD45RO^{+}$ and $CD123^{+}/CD45RA^{+}$. In our study we observed an overall expansion of DC1 and DC2 cells. The total number of DC1 cells increased significantly after G-CSF treatment both in BM (from 8560 ± 3019 to $36{,}221 \pm 10{,}903$, $P = .006$) and PB (from 1332 ± 559 to 6630 ± 2262, $P = .029$). A slight increase was detected in BM and PB for DC2 cell fractions that increased from 4477 ± 2042 to $12{,}163 \pm 3468$ and from 2409 ± 994 to 3276 ± 1500 (Figure 5).

Effect on Cytokines

To determine whether G-CSF priming induced modification of the cytokines released, the concentrations of both Th1 (IFN-γ, IL-2, and tumor necrosis factor-α) and Th2 (IL-4, IL-10, IL-6) cytokines were tested on plasma samples obtained before and after G-CSF administration in 40 donors (data not shown). A significant increase of the IL-10 levels was detected after G-CSF (from 1.85 ± 1.06 to 2.75 ± 1.24 pg/mL; $P < .001$). The IL-6 level also rose after G-CSF exposure (from 7.06 ± 22.85 to 22.98 ± 78.59 pg/mL), but the difference was not statistically significant.

Impact of Age, Sex, and CMV Infection on G-CSF–Induced Modifications

The effects of G-CSF were also evaluated on categories of donors divided by median age (43 years), sex (56.5% male and 54.5% female), and CMV serology (28% negative, 72% positive). The G-CSF effect on the hematopoietic compartment was not significantly correlated with these variables except for a higher increase of $CD34^{+}$ cells in younger donors.

Donor age had a significant impact on the increase induced by G-CSF on PB $CD3^{+}$ and $CD4^{+}$ cells and on BM $CD4^{+}RA^{+}$, $CD19^{+}$ and Treg $CD4^{+}CD25^{+}$, which was significantly higher only in younger donors. Conversely, BM DC1 and DC2 and PB DC1 increased significantly only in older donors ($P = .0017$, $P = .04$, and $P = .03$, respectively). As to the donor sex, BM Tregs increased only in female donors ($P = .046$), whereas the increase of BM $CD19^{+}$ and DC1 was significant ($P = .0065$ and $P = .005$, respectively) only in male donors. Finally, BM $CD4^{+}$, $CD19^{+}$, DC1, and PB DC1 increased only in CMV-positive donors ($P = .03$, $P = .014$, $P = .003$, and $P = .012$, respectively). Surprisingly, the CD4 and CD8 naive T cells increased

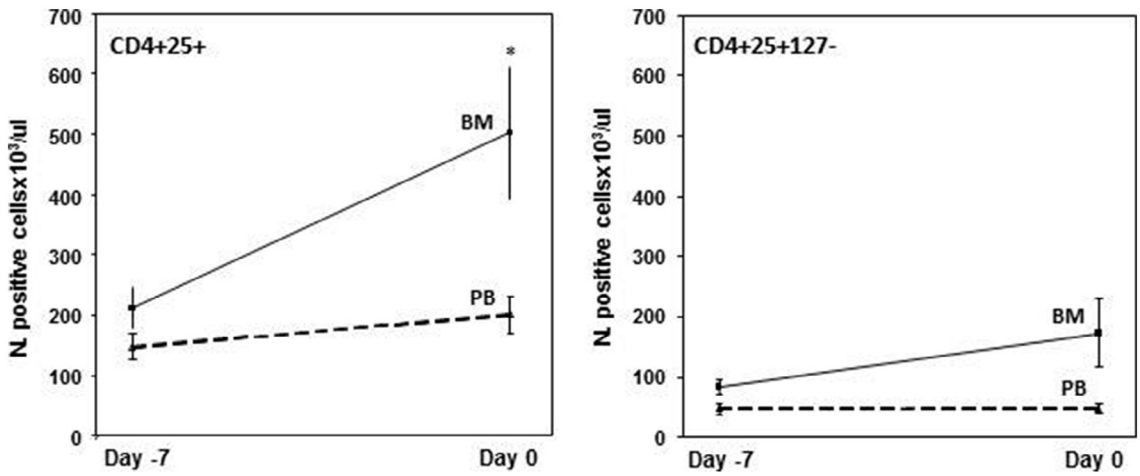

Figure 4. Changes induced by G-CSF on Treg lymphocytes in BM (——) and PB (----). The BM $CD4^{+}25^{+}$ subpopulation increased significantly after G-CSF priming ($P = .014$). A less marked increase was detected for the $CD4^{+}25^{+}127^{-}$ BM subpopulation. No significant change was detected at PB level.

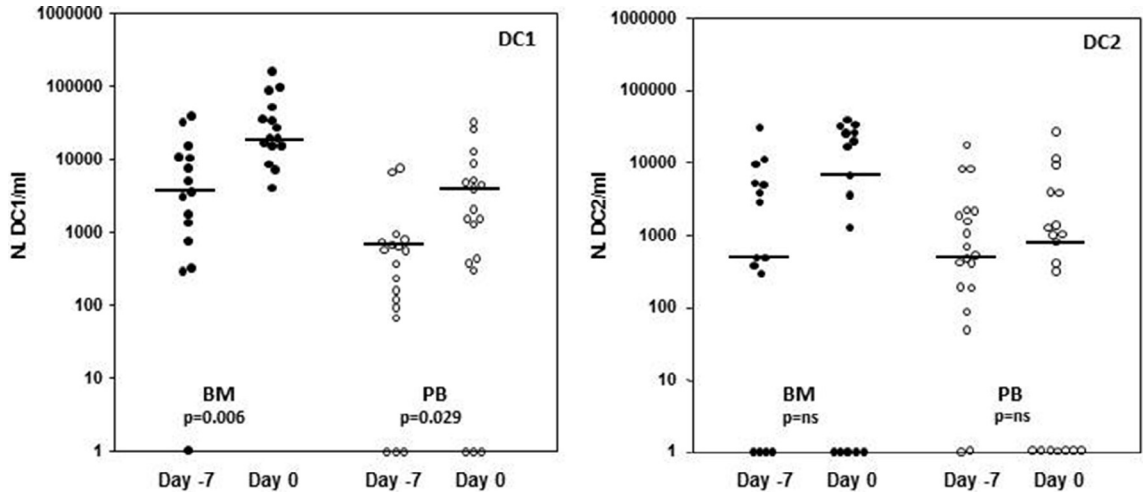

Figure 5. Modulation of DC1 and DC2 by G-CSF in BM and PB. The frequency of DCs was detected in 15 BM and 19 PB samples collected from the same donor before and after G-CSF administration. The DC1 cell fraction ($CD40^+CD45RO^+CD4^-HLA\text{-}DR^+CD11c^+CD123^-$) increased significantly after G-CSF priming both in BM (8.7-fold over the baseline values, $P = .006$) and in PB (2.6-fold, $P = .029$). A less marked rise was detected for the DC2 cell fraction ($CD40^+CD45RA^+CD4^-HLA\text{-}DR^+CD11c\text{-}CD123^+$) that increased 3.2-fold in BM ($P = .066$) and only 1.4-fold at the PB level.

significantly ($P = .047$ and $P = .04$) in CMV-negative donors (n = 5), whereas in CMV-positive donors (n = 17) the increase was not significant.

DISCUSSION

Our study compared the effect ex vivo of G-CSF priming on both BM and PB hematopoietic and immune cells in a large number of healthy donors for haploidentical BM transplant [24]. Donors were primed with G-CSF at 4 mg/kg/day for 7 consecutive days according to previously described clinical protocols [26,27]. It is now generally accepted that G-CSF mobilization promotes cell cycling of multipotent HSCs and modulates the immune system; nevertheless, the kinetics of G-CSF mobilization is still unclear. Experimental studies have suggested that low doses of G-CSF induce a higher recruitment of $CD34^+$ cells, but more time is needed to reach the maximum effect [22].

Our data show that G-CSF induced a more significant rise of $CD34^+$ cells in BM than in PB, and the more primitive cell fraction ($CD34^+38^-$) was selectively enhanced in BM. Our results are in agreement with Chiang et al. [22], who found a higher increase in BM, whereas Shier et al. [23] found a higher increase in PB, probably because of the different schedule of G-CSF priming.

A very interesting effect of G-CSF was the impressive rise of the BM mesenchymal progenitors (CFU-F) that increased 50-fold. Similar results were obtained in the murine model by Brouard et al. [28] that showed a time-dependent increase of BM CFU-F peaking at day 7. The authors hypothesized that the CFU-F increase detected in G-CSF–treated mice could be linked to the bone remodeling induced by the cytokine. It is known that MSCs play a pivotal role in physiologic HSC survival and differentiation; after transplant, MSCs enhance hematopoietic and immune reconstitution [29,30]. Furthermore, as shown by experimental clinical results [31-37], MSCs may affect the function of cells involved in the immune response and reduce the incidence and severity of GHVD because of their immunomodulatory properties. Because the MSC progenitors were undetectable in PB before and after G-CSF, G-BM could be a more useful HSC source for transplant as well as an MSC source in other clinical settings.

It is well known that G-CSF induces immune tolerance. Our study shows that the immune cells were affected differently by G-CSF in BM and PB. T and B cells were significantly increased in BM and not in PB, and, interestingly, the BM naive T cell fractions were selectively increased. This finding could be significant because clinical analysis has shown a better overall survival and less grades III to IV acute GVHD in patients receiving a higher number of BM-naive $CD4^+$ and $CD8^+$ cells [38].

In contrast with previously reported data showing no significant effect of G-CSF on NK subpopulations [23,39], our results show that although in PB the NK cell fractions were significantly down-regulated by G-CSF, in BM a modest increase of both $CD3^-CD16^+$ and $CD3^-CD56^+$ was detected. This activity of G-CSF on BM NK cells might be of particular importance in G-BM haploidentical transplant because NK cells are important effector cells in mediating the graft-versus-leukemia effect. The significant rise of Tregs observed only in G-BM could be interesting because although the protective role of Tregs against aGVHD is still controversial, recent clinical results show a remarkable effect in GVHD prevention when Tregs are used as part of the conditioning regimen for the haploidentical transplant [40].

In BM, G-CSF induced a significant increase of the DC1 cell fraction and a slightly lower increase of the DC2 cells, whereas in PB only the DC1 cells were expanded. These results are not in agreement with most of the data previously reported [11,12,41,42], where G-CSF has been shown to selectively enhance the DC2 cell fraction. The variability between single samples, the reduced number of cases tested for DC content, and the different characterization used in our studies, including the positivity of CD40, which identifies mature DC subpopulations, may account for the difference.

To date, the role of DCs in allogeneic transplant is not clear; however, some clinical observational and in vitro functional studies suggest that large numbers of DC2 have adverse effects on survival and are associated with increased post-transplant relapse and decreased cGVHD [41-43]. By contrast, Waller et al. [38] reported decreased treatment-related mortality and graft rejection after transplant of a high number of donor BM DC2. In conclusion, our in vitro study performed

on a large number of donors shows that after G-CSF priming the hematopoietic, mesenchymal, and immune cell fractions acquire different profiles depending on BM or PB. This findings could be helpful to direct the clinician toward the best graft source according to clinical requirements.

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