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The Effect of Granulocyte Colony–Stimulating Factor on Immune-Modulatory Cytokines in the Bone Marrow Microenvironment and Mesenchymal Stem Cells of Healthy Donors

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A B S T R A C T

Granulocyte colony stimulating factor (G-CSF) is sometimes administered to donors before bone marrow (BM) harvest. G-CSF–primed (G-BM) and unprimed BM (U-BM)–derived mesenchymal stem cells (MSC) were obtained from 16 healthy donors and were expanded in vitro. Their proliferative characteristics, morphology, and differentiation capacity were examined. Supernatants of the second passage of MSCs were evaluated for transforming growth factor β1, hepatocyte growth factor, and prostaglandin E2 (PGE2) levels and compared with controls. The analyses of cytokines in the G-BM– and U-BM–derived MSCs supernatants revealed that PGE2 levels were significantly lower in the G-CSF–primed samples. These cytokines were also measured in BM plasma. The level of hepatocyte growth factor in G-BM plasma was significantly increased. The current study is the first to show the effects of G-CSF on the BM microenvironment of healthy human donors. The preliminary data suggest that G-BM– and U-BM–derived MSCs have similar morphologic/phenotypic properties and differentiation capacity but differ in their secretory capacity. Significant changes in cytokine levels of BM plasma in G-CSF–primed donors were also demonstrated. These findings suggest that BM MSCs and changes in the BM microenvironment may contribute to the effects of G-CSF on inflammation and immunomodulation.

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INTRODUCTION

Granulocyte colony–stimulating factor (G-CSF) is the basic hematopoietic growth factor that modulates hematopoiesis and immune system. It is widely used in clinical practice to overcome radiotherapy and chemotherapy-induced myelosuppression, to increase the proliferation and differentiation of hematopoietic cells, and to activate neutrophil functions [1]. In addition, G-CSF is administered to donors to mobilize stem cells from the bone marrow (BM) into peripheral blood before collection of peripheral blood (PB) stem cells. G-CSF is sometimes administered to donors before BM harvest [2-4]. However, if the established number of stem cells needed for the recipient will require a large volume marrow collection (>16 mL/kg) from the donor and if the donor is not eligible for PB collection, G-CSF may be used to increase the stem/progenitor cell content of the product. G-CSF has been shown to affect the immune system by modifying T cell reactivity and antigen-presenting cell function. Some studies have shown that G-CSF stimulates the inflammatory response while suppressing the adaptive immune system. However, there is a paucity of information in the literature regarding the effects of G-CSF–primed stem cells on recipient immune system. Data suggests that a G CSF–induced T helper 1 to T helper 2 shift may play a role in modification of the alloimmune reactions in the recipient [5].

Mesenchymal stem cells (MSCs) were first characterized more than 30 years ago and are described as fibroblast-like

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cells adhering to plastic when they were cultured from BM in vitro. It has been shown that MSCs may be isolated from most mesoderm-derived tissues and have differentiation capacity to mainly connective tissue and different mesoderm tissues [6]. To date, several studies have been performed on the biological properties and function of these cells. MSCs are believed to play a role in supporting other types of cells/stem cells by establishing cellular interactions, providing secretory factors for growth and differentiation and other biological functions, and by contributing to angiogenesis and immune modulation [7]. MSCs have been shown to affect the functions of immune cells mainly by secreting immune-modulatory factors, such as transforming growth factor beta 1 (TGF-β1), hepatocyte growth factor (HGF), prostaglandin E2 (PGE2), indoleamine 2,3 dioxygenase (IDO), and HLA G-5 through cell-cell interactions [8]. In recent years, these cells have aroused increasing attention in regenerative medicine and in the treatment of autoimmune and inflammatory conditions, including steroid-resistant graft-versus-host disease (GVHD) [9].

To our knowledge, the effect of G-CSF on the immunomodulatory functions of MSCs and on the secretion capacity of immune-modulatory cytokines has not been studied. In the present study, it is hypothesized that G-CSF–primed BM (G-BM)–derived MSCs may modulate secretion of immune-modulatory cytokines in the BM microenvironment.

MATERIALS AND METHODS

This study enrolled 16 HLA-identical healthy related donors for children who underwent allogeneic hematopoietic stem cell transplantation (HSCT) at Ankara Children's Hematology Oncology Education and Research Hospital. Eight donors received G-CSF (lenograstim, Granocyte) when the predicted marrow harvest volume was assumed to be more than 16 mL/kg of donor's weight. Eight age-matched donors unexposed to G-CSF (U-BM) were included as the control group.

Informed consent was obtained from all patients, donors, and their legal guardians for HSCT. This study was approved by the local ethical committee.

Harvesting BM

Among 16 donors, 8 received G-CSF at a dosage of 10 μg/kg/day (lenograstim, Granocyte) as a single injection for 3 consecutive days. The other 8 donors had unstimulated BM.

The BM was obtained from the posterior iliac crest of healthy donors under general anesthesia. G-BM and U-BM harvest were performed with a target volume to meet the optimal cell numbers for engraftment and not to exceed 16 mL/kg of donor's body weight. For this study, 2 to 3 mL of BM was separated and frozen.

Collection of Plasma from BM

BM samples were centrifuged at 2000 rpm for 10 minutes. The plasma at the upper part of the tube was collected and stored at −80°C.

Isolation of Human BM–Derived MSCs

Mononucleated cells separation procedure

BM samples were diluted with Dulbecco's PBS (Biochrom, Germany) after the plasma collection and layered on Biocoll (1.077 g/mL) separating solution (Biochrom, Germany) (1:1) and centrifuged at 2200 rpm for 20 minutes.

Mononucleated cell freezing procedure

The buffy coat containing the mononucleated cells (MNCs) was washed with PBS and frozen in DMEM low glucose (DMEM-LG) (Biochrom, Germany) containing 20% FBS (Biochrom, Germany) and 10% DMSO. Cryovials were kept in liquid nitrogen tanks at −196°C.

MNC Thawing procedure

MNC samples in the cryovials were thawed in a 37°C water bath before the seeding procedure. The cells were disaggregated by gentle pipetting several times and centrifuged with PBS at 2000 rpm for 5 minutes to remove freezing solution. The supernatant was discarded, and the pellet was resuspended in 10 mL DMEM-LG, 10% FBS, and 1% penicillin-streptomycin (Biochrom, Germany) and at least 20×10^6 cell were seeded in 75 cm^2 plastic flasks. Flasks were kept at 37°C in a humidified atmosphere containing 5% CO_2 (Galaxy 170R incubator, Eppendorf Company, Hamburg, Germany).

Culture of Human BM–Derived MSCs

After 72 hours, nonadherent cells were removed. The culture medium was changed every 3 days. When 70% to 80% adherent cells were confluent, they were trypsinized (.05% trypsin) at 37°C for 5 minutes (Biochrom). Characterization of MSCs, collection of supernatant for ELISA assay, and MSC differentiation assay were set up within passage 2 of MSC culture.

Characterization and Differentiation Assay of MSCs

In vitro differentiation capacity of MSCs towards adipogenic and osteogenic lineages were tested. Adipogenic Stimulatory Supplements (Human) (MesenCult Adipogenic Differentiation Medium; Stemcell Technologies, Vancouver, Canada) induction was used for adipogenic, and Osteogenic Stimulatory Supplements (Human) (MesenCult Osteogenic Stimulatory Kit, Stemcell Technologies, Vancouver, Canada) was used to induce osteogenic differentiation. Oil red o and alizarin red stains were used to verify, respectively, adipogenic and osteogenic differentiation capacity of MSCs at day 21 of induced cultures. MSCs were also tested for positive staining of HLA ABC, CD90, CD73, CD44, and CD49e (BD Biosciences, Piscataway, NJ, USA) and negative antibody staining for CD34, CD3, CD4, and HLA DR (BD Biosciences, Piscataway, NJ, USA). Flow cytometry with a BD-FACS Aria (BD Biosciences) was used for surface phenotyping of MSCs.

MSC's Coculture with PB MNC

PB MNCs isolation and activation test

MNC were purified from heparinized PB by density-gradient centrifugation using Biocoll (1.077 g/mL) separating solution (as described in MNC separation procedure).

MNCs from PB samples were plated at a density of 3×10^5 cells/cm^2 on T-75 flask (Cellstar, Grenier Bio-one, Kremsmünster, Austria) with a density of 10 μL pythohemagglutinin (PHA)/1×10^6 cells in DMEM supplemented 10% FBS and 1% penicillin-streptomycin at 37°C in a humidified atmosphere containing 5% CO_2 for 72 hours for activation of lymphocytes.

BM MSC isolation

BM MSC isolation was performed as described previously. All assays were performed using MSCs at passage 2.

Coculture

Coculture were carried out in 6-well tissue plates (Cellstar, Grenier Bio-one) with PB MNCs from a healthy donor and allogeneic human MSCs from 2 different sources, G-BM 8 samples and U-BM 8 samples.

Control cultures consisted of MNC in the absence of MSCs, with or without PHA stimulation. Also, another control culture was set that consisted of U-BM MSCs for the evaluation of proliferation capacity of MSCs. For the immunological assays, different sets of cocultures were generated as below: PHA-activated MNCs + G-BM MSCs and PHA-activated MNCs +U-BM MSCs (8 different samples for each condition).

For the coculture experiments, MNCs were washed with PBS at least twice after activation with PHA. Cocultures were performed as in each well, we added 30,000 MNCs, with a ratio of 10:1 (MNC:MSC). The coculture plates were kept at 37°C in a humidified atmosphere containing 5% CO_2 for 4 days. After 4 days, the cultured cells were washed with PBS and centrifuged at 2000 rpm for 5 minutes.

The number of cells and cellular viability were determined.

Flow Cytometry Data Acquisition and Analysis

Cells were acquired on a Beckmann Coulter Navios using Kaluza version 1.2 software. For the immunological assays and to define the different stages of lymphocyte activation, we used mAb against CD3-PC7, CD4-FITC, CD25-PE, CD69-PC5, and HLA-DR-ECD (Beckmann Coulter, Brea, CA, USA).

Quantification of Immune Modulatory Factors

To quantify PGE2, HGF, and TGF-β1, MSCs supernatants were collected at passage 2 and stored at −80°C until measured by ELISA. When MSCs reached 70% to 80% confluency at passage 2, cells were trypsinized and counted before freezing and stored at −80°C. BM plasma was harvested and frozen at −80°C until measured by ELISA. PGE2 ELISA was performed using the Human PGE2 Assay ELISA (R&D Systems, Minneapolis, MN), TGF-β1 ELISA was assayed using the Human TGF-β1 ELISA (R&D Systems), and HGF ELISA was performed using the Human HGF ELISA (R&D Systems). MSC supernatants and BM plasma were analyzed on BioTEK ELx808 Absorbance Microplate Readers (BioTEK, Winooski, VT, USA). Measured ELISA test from supernatant of MSC for standard results were taken as the value for the 10×10^4 per MSCs.

Table 1
G-BM and U-BM–Derived MSCs Expression of CD Markers

Marker	U-BM			G-BM		
	Donor 1	Donor 2	Donor 3	Donor 4	Donor 5	Donor 6
CD90	100	99.5	99.6	99.2	98.9	99.4
CD73	90.3	88.1	93.2	94.3	86.2	80.9
CD29	95.7	89.5	90	97.4	74.5	92.7
CD34	.3	.7	.1	.1	.1	.2
CD45	.2	.4	.2	.2	.3	.2

Data shown are percentages.

Statistics

The statistical significance was assessed by Mann Whitney U-test. A P value < .05 was considered significant. Statistical analyses were performed using the SPSS 16,0 software program (SPSS Inc, Chicago, IL).

RESULTS

Demographic Features of Donors

The median age was 9.5 years (range, 4.5 to 17 years) in U-BM group and 9 years (range, 3.5 to 17 years) in G-BM group. There were 4 girls and 4 boys in U-BM group and 3 girls and 5 boys in G-BM group. There was no significant difference in sex or age of donors between the 2 groups.

There was no immediate or subacute complication in donors after G-CSF injections. The median harvested BM volume was 585 ± 211 mL in the G-BM group and 513 ± 163 mL in the U-BM group. There were no complications during the harvesting BM.

Expansion and Characterization of Human MSCs from G-BM and U-BM

Both types of MSCs were expanded and used in the experiments at passage 2. The immunophenotype analysis showed that both types of MSCs were positive for HLA ABC, CD90, CD73, CD44, and CD49e and negative for CD34, CD3, CD4, and HLA DR. G-BM– and U-BM–derived MSCs expression levels of CD markers are summarized in Table 1.

The adipogenic and osteogenic differentiation capacity of both study and control groups of MSCs were confirmed by differentiation assays. Images from a representative sample are shown in Figure 1 and Figure 2.

ELISA assays

The levels of immunosuppressive/immune modulatory cytokines (HGF, PGE2, and TGF- β1) were determined by ELISA assay in the BM plasma and MSC supernatants of G-CSF–primed and unprimed donors. There was a significant increase in the level of HGF (P < .05) in BM plasma of G-CSF–primed donors when compared with that of controls.

The investigation of the MSC supernatants for the immunomodulatory cytokines revealed a statistically significant decrease in PGE2 levels in G-CSF–primed samples (P < .05).

Results of ELISA assays are summarized in Table 2 and Figure 3. HSCT patients' outcomes summarized in Table 3.

Immunological Assays

Lymphocytes undergo clonal division after mitogenic stimulation. To investigate the kinetics of lymphocyte activation, in the presence of PHA and the effects of MSCs derived from G-BM and U-BM on activated lymphocytes, we analyzed activation markers of lymphocytes with flow cytometry. We analyzed subpopulations of lymphocytes, especially T lymphocytes phenotypically defined as $CD3^+CD69^+$, $CD3^+HLADR^+$, and $CD4^+CD25^+$ in different culture conditions. The percentage of $CD3^+CD69^+$ cells was decreased in both the PHA-activated MNCs + G-BM MSCs group and PHA-activated MNCs + U-BM MSCs group when compared with MNC + PHA. Also the percentage of $CD3^+HLA\text{-}DR^+$ cells was increased in both PHA-activated

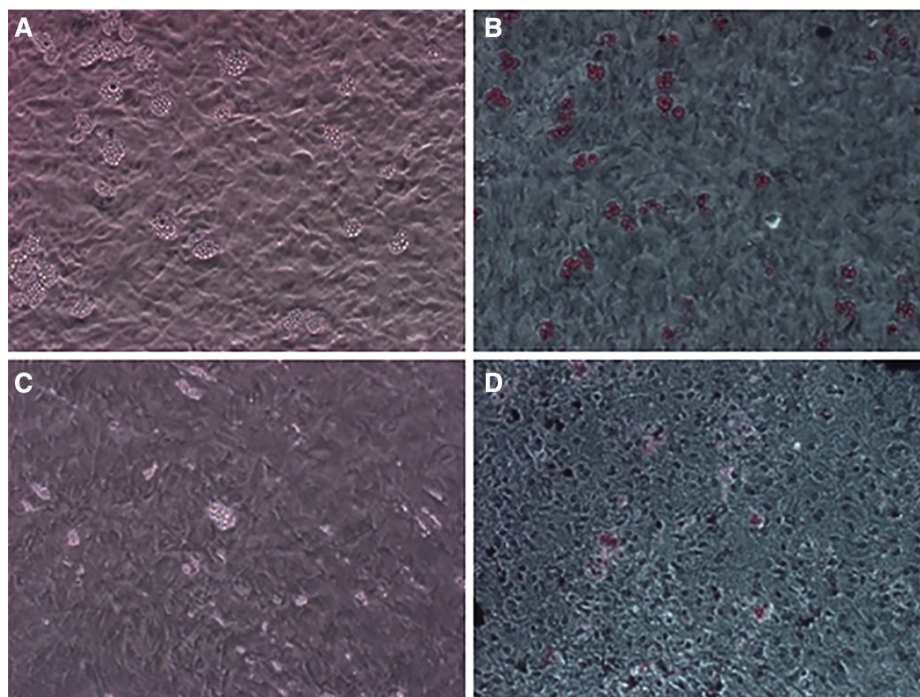

Figure 1. In vitro differentiation of G-BM–derived MSCs into adipocytes (×4 magnification). (A) Unstained, (B) oil o red staining, (C) unstained, (D) oil o red staining. (This figure is available in color online at www.bbmt.org).

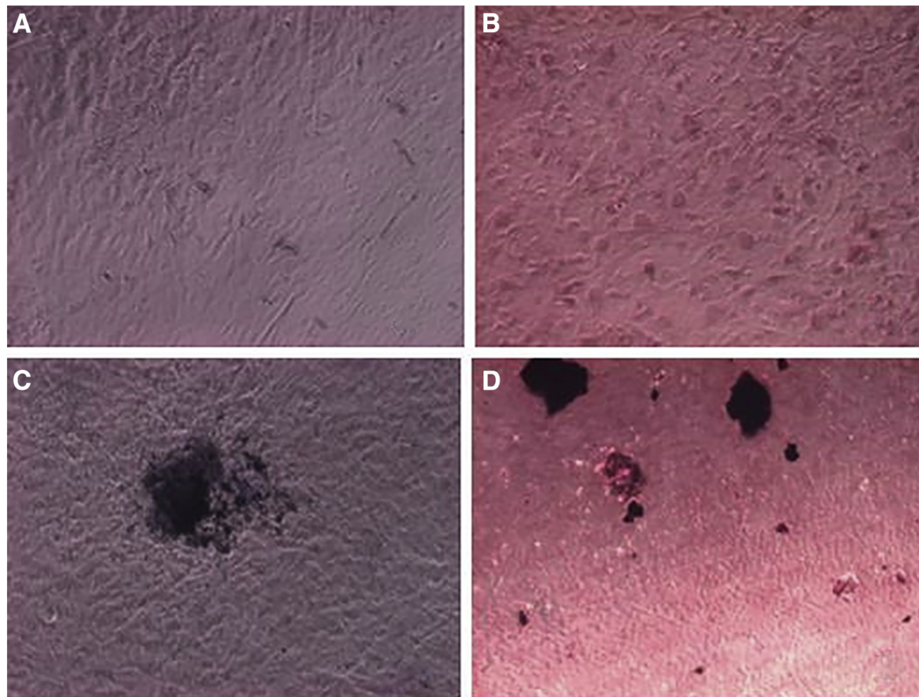

Figure 2. In vitro differentiation of G-BM–derived MSCs into osteocytes (×4 magnification). (A) Unstained, (B) alizarin red staining, (C) unstained, (D) alizarin red staining. (This figure is available in color online at www.bbmt.org).

MNCs + G-BM MSCs group and PHA-activated MNCs + U-BM MSCs group when compared with MNC + PHA. Therefore, the results were not statistically significant. Results of these immunological assays are summarized in Table 4.

DISCUSSION

This study evaluates the relationship between G-BM–derived MSCs and the immunosuppressive and immune modulatory cytokines of MSCs. This is the first report showing that G-BM– and U-BM–derived MSCs have similar morphologic/phenotypic properties and differentiation capacity but they differ in their secretory capacity, an important feature of MSCs. Additionally, significant changes in cytokine levels of BM plasma of G-CSF–primed donors were also demonstrated in this study.

In the present study, the post-transplantation immunologic effects (incidence of GVHD, immune reconstitution, etc.) are presented in a table without comparison because of the limited number of patients, different diagnosis, and conditioning regimens used in this patient group. Instead, immune-modulatory properties of the G-BM microenvironment were obtained in this study.

Table 2
ELISA Assays Levels of Cytokines in the G-BM– and U-BM–Derived MSCs Supernatants and BM Plasma

Cytokines	U-BM	G-BM	*P*
MSCs supernatant (10 × 10^4 cells)			
HGF, pg/mL	125.91 ± 135	121.60 ± 68	.24
TGF- β1, pg/mL	86.30 ± 49.39	53.98 ± 47.48	.41
PGE2, pg/mL	314.66 ± 97.18	143.19 ± 31.69	.001*
BM plasma			
HGF, pg/mL	2340.56 ± 843.97	6699.92 ± 4125.69	.027*
TGF- β1, pg/mL	4956.93 ± 2158.45	14757 ± 24,415.12	1
PGE2, pg/mL	2254.68 ± 794.15	3350.06 ± 1654.91	.11

Data presented are mean ± SD.
* $P < .05$ statistical significance.

In the literature, the mechanisms for immunosuppressive potential of MSCs include both cell-cell contact and the release of soluble mediators. These immune modulatory factors include TGF-β1, HGF, PGE2, indoleamine 2,3 dioxygenase, and HLA-G5 [10-13]. These properties make them promising tools in the treatment of steroid-resistant GVHD and autoimmune diseases [9].

HGF was first identified and partially characterized as mitogenic protein for hepatocytes in 1984 [14]. It has also been shown to function in placenta, kidney, lung, muscle, salivary gland, and hematopoietic tissue [15]. Many studies have demonstrated that embryogenesis, angiogenesis, hematopoiesis, as well as organ regeneration, are controlled by HGF [16,17]. A previous study demonstrated that HGF levels are altered by various factors, including age and sex. Serum HGF levels were reported as (mean ± SD) 360 ± 160 pg/mL in females and 350 ± 250 pg/mL in males at 10 to 19 years of age [18]. In our study, HGF levels are 6700 ± 4125 pg/mL and 2340 ± 843 pg/mL in the G-BM and U-BM plasma, respectively. These levels are very high compared with reported blood levels. There was a statistically significant difference in the HGF levels of G-BM and U-BM plasma ($P = .027$). The results of the present study suggesting that G-CSF induces HGF release from the BM microenvironment may have implications for MSCs, as they are the major cells of BM microenvironment [19]. For this purpose, we analyzed levels of HGF in the MSCs supernatants. HGF levels were 121 ± 68 pg/mL and 125 ± 135 pg/mL in the G-BM and U-BM–derived MSCs supernatants, respectively. The difference was not statistically different. In the present study, relatively early passage cells (passage 2) were used to maintain the in vivo functional properties as much as possible during ex vivo expansion. It has been demonstrated that

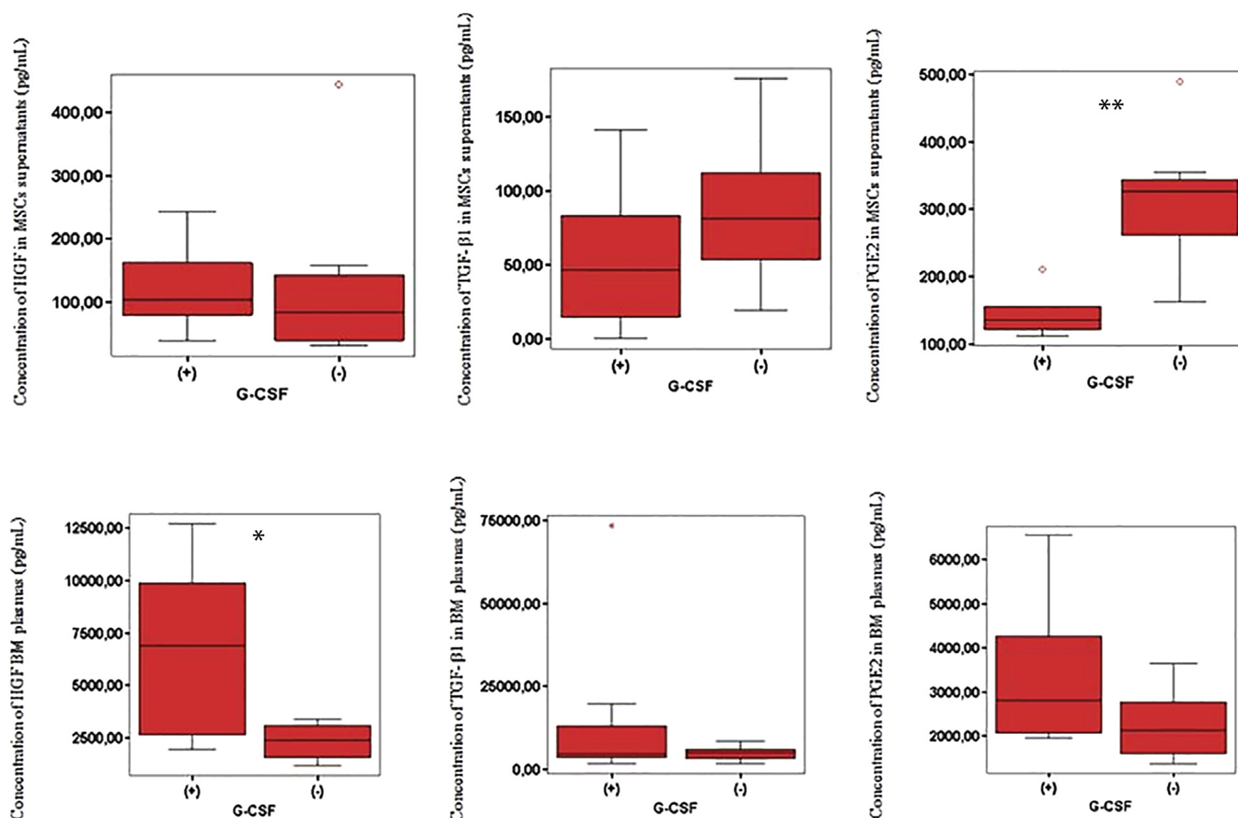

Figure 3. Concentration of HGF, TGFβ1, and PGE2 in MSCs supernatants and BM plasma. *In the BM plasmas, HGF with G-CSF increase. **In the MSCs supernatants, PGE2 with G-CSF decrease.

immunosuppressive factors are released upon exposure to cytokines, including IFN-γ, IL-1β, in MSCs medium [20]. Unlike in vivo, MSCs has no contact with other cells in culture medium, so lack of the alerts could the change secretory functions of MSCs. In further studies, experiments can be repeated with priming with cytokines and coculture with hematopoietic cells.

TGF-β is a multipotent cytokine, modulator of cell proliferation, differentiation, apoptosis, adhesion, inflammation, and matrix synthesis. Most cell types, including immature hematopoietic cells, lymphocytes, neutrophils, macrophages, and dendritic cells, produce TGF-β and are sensitive to its effects [21]. Kulkarni et al. demonstrated that TGF-β plays a pivotal role in maintenance of immune cell homeostasis in TGF-β1 knockout mice experiments [22]. In vitro studies have shown that TGF-β affects B and T cells at all stages of development, inhibits proliferation, and stimulates apoptosis, thus acting as an immune-suppressive molecule [23]. Several studies in the literature have demonstrated that release of TGF-β1 contributes to immunosuppressive effects

Table 3
Characteristics and Outcomes of HSCT Patients

Patient No.	G-BM	Disease	MNC count, $\times 10^8$/kg	$CD34^+$ Cell count, $\times 10^6$/kg	Neutrophil Engraftment	Platelet Engraftment	Acute GVHD	Chronic GVHD	Graft Failure
1	No	BTM	4.26	3.88	+20 day	-	No	No	+30 day
2	No	HES	4.6	6.77	+20 day	+25 day	Grade III	No	
3	No	BTM	6.72	4.99	+16 day	+23 day	No	No	
4	No	CHS	9.67	5.18	+16 day	+19 day	Grade III	No	
5	No	BTM	5.14	7.76	+13 day	+24 day	No	No	
6	No	AML		4.21				No	
7	No	BTM	5.5	14.1	+18 day	+31 day	No	No	
8	No	FA	4.71	5.62	+11 day	+15 day	No	No	
9	Yes	HL	6.33	2.17	+16 day	+24 day	No	Liver	
10	Yes	AML	1.31	4.87	+6 day			No	
11	Yes	ALL	9.33	1.67	+20 day	+23 day	Grade II	No	
12	Yes	BTM			+15 day	+29 day	No	No	
13	Yes	ALL					Grade IV	Skin, liver	
14	Yes	ALL	4	3.72	+16 day	+23 day	Grade II	No	
15	Yes	ALL	3.24	2.12	+17 day	+32 day	No	No	
16	Yes	AML	4.58	2.2	+13 day	+17 day	No	No	

BTM indicates beta thalassemia major; HES, hyper-immunoglobulin E syndrome; CHS, Chediak-Higashi syndrome; AML, acute myeloid leukemia; FA, Fanconi aplastic anemia; HL, Hodgkin lymphoma; ALL, acute lymphoblastic leukemia.

Table 4
Immunological Assay by Determination of Activation Markers after Coculture of PHA-Activated MNCs with G-BM MSCs and U-BM MSCs

Activation markers	MNC + PHA + G-BM MCSs, median (min-max) (n:8)	MNC + PHA + U-BM MCSs, median (min-max) (n:8)	MNC + PHA, (n:1)	P (control versus G1*)	P (control versus G2*)	P (G1 versus G2)
$CD3^+$-$CD69^+$	25.71 (21.49-36.51)	26.30 (15.70-29.03)	34.59	.75	.22	.53
$CD3^+$-$HLADR^+$	49.09 (36.21-57.37)	50.14 (42.35-57.76)	36.10	.25	.22	.90
$CD4^+$-$CD25^+$	50.00 (44.48-53.57)	50.27 (45.39-53.46)	49.94	1.00	.88	.90

Data presented are percentages. Statistical significance is $P < .05$.
* G1 (group 1): MNC + PHA + G-BM MCSs; G2 (group 2): MNC + PHA + U-BM MCSs; and Control: MNC + PHA.

of MSCs [10,13]. In the present study, we showed TGF-β1 was secreted by G-BM– and U-BM–derived MSCs. TGF-β1 levels are 54 ± 47 pg/mL and 86 ± 49 pg/mL in the G-BM and U-BM MSCs supernatants, respectively. There was no statistically significant difference between these TGF-β1 levels ($P = .41$). Here again, the cell culture medium and the absence of dynamic cell-cell interactions that change the properties of secretory cells comes to mind. We examined BM plasma that would reflect dynamic cell-cell interactions. TGF-β1 levels were found to be 14,757 ± 24,415 pg/mL and 4956 ± 2158 pg/mL in the G-BM and U-BM plasma, respectively, and there was no statistically significant difference between these TGF-β1 levels ($P = 1.00$). However, in some cases, the level of TGF-β1 in the plasma of G-BM was found to be very high (73,540 pg/mL), whereas the levels in the supernatants of MSCs remained low (6 pg/mL). Additionally, there was heterogeneity between different donors particularly in G-CSF–primed samples. Mesenchymal stem cells interact with other cells in BM microenvironment, so when they lose the signals during ex vivo expansion their functional properties are not maintained. Signals by macrophages, natural killer cells, and damaged tissue induce MSCs to produce more TGF-β1 [20].

Prostaglandins are small-molecule derivatives of arachidonic acid, produced by cyclooxygenases. PGE2 can be produced by all cell types of the body. PGE2 is relatively stable in vitro, although its decay is accelerated by albumin. In contrast, PGE2 has very rapid turnover rate in vivo and is rapidly eliminated from tissues. Aggarwal et al. showed that MSCs, when cocultured with immune cells, such as T cells, resulted in increased PGE2. They observed that MSCs exhibit a bell-shaped time-dependent curve of PGE2 secretion (after an initial increase, there is a decrease in levels of PGE2 after 4 to 5 days) [10]. Spaggiari et al. demonstrated that MSCs strongly inhibited the maturation and functioning of monocyte-derived dendritic cells by interfering selectively with the generation of immature via inhibitory mediator of MSC-derived PGE2 [23]. In our study, we determined that level of PGE2 was 143 ± 31 pg/mL and 314 ± 94 pg/mL in the G-BM and U-BM–derived MSCs supernatants, respectively. These results reflect the secretion of PGE2 from MSCs. We showed that MSCs isolated from G-BM were significantly decreased for releasing PGE2 compared with the control. The difference was statistically significant ($P = .001$). The BM plasma PGE2 levels were 3350 ± 1654 pg/mL and 2254 ± 794 pg/mL in the G-BM and U-BM plasmas, respectively, and there was no statistically significant difference ($P = .11$). Although G-CSF didn't lead to any change in PGE2 levels in BM plasma, there was a statistically significant difference in MSC supernatant levels. However, further studies under activating conditions are needed to delineate MSC secretory functions.

PHA is a lectin with the ability to bind and crosslink different cell membrane glycoproteins, leading to the polyclonal activation of lymphocytes [24]. The early activation marker CD69 is expressed on natural killer, B, and T cell surfaces 4 hours after activation and is implicated on the transcription of interleukin 2 (IL-2) and TNF-α; 12 to 24 hours after cell activation, the α subunit of IL-2 receptor (CD25) expression is upregulated. Between 48 and 60 hours, T cells initiate HLA-DR expression [25]. Although many studies have been completed to determine the immune suppressive function of MSCs, to our knowledge, there is not any study concerning the immunological properties of G-BM and U-BM–derived MSCs. Based on this, we evaluated cytokines and immunological assays with G-BM compared with U-BM. Immunological assays that we performed in this study revealed that there is no significant difference between HLA-DR expression of MNCs when cocultured, even with G-BM–derived or U-BM–derived MSCs. Therefore, HLA-DR expression of MNCs are different in both the PHA-activated MNCs + G-BM MSCs group and PHA-activated MNCs + U-BM MSCs group when compared with MNC + PHA. That expression pattern indicated that coculture of MNCs with MSCs could possibly facilitate activation of T lymphocytes.

In summary, the present study investigating the G-CSF–primed BM plasma and MSCs revealed that in vivo G-CSF exposure affects the release of immune modulatory cytokines/growth factors from the BM microenvironment and in cultured MSCs. The changes in HGF and PGE2 levels were statistically significant. In addition, BM-derived MSCs were shown to secrete cytokines, including HGF, TGF-β1, and PGE2, in unstimulated culture conditions. These preliminary results suggest a possible role for these secretory factors in immunomodulation. However, coculture experiments, cytokine array analysis, and extensive further studies of stimulation with soluble factors would be useful to show the effect of G-CSF.

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