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Dextran Sulfate and Stromal Cell Derived Factor-1 Promote CXCR4 Expression and Improve Bone Marrow Homing Efficiency of Infused Hematopoietic Stem Cells

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Abstract

Although the homing of hematopoietic stem cells (HSC) to the bone marrow (BM) is a crucial step in hematopoietic development and BM repopulation, the mechanisms underlying these processes have not been fully clarified. Recent studies suggest that interaction between the chemokine receptor CXCR4 and its ligand, stromal cell-derived factor 1 (SDF-1), plays a critical role in these processes. In addition, dextran sulfate increases plasma SDF-1 levels in mice and nonhuman primates. Thus, we examined the effects of preconditioning with SDF-1 and dextran sulfate on the homing efficiency of HSCs following BM transplantation in mice. We found that the preconditioning of donor mice with either SDF-1 or dextran sulfate enhanced the homing efficiency of infused HSCs in vivo. The greatest effects were obtained with dextran sulfate. Moreover, reverse transcriptase polymerase chain reaction analysis demonstrated that SDF-1 and dextran sulfate increased transcription of a variety of homing-related genes, including those for CXCR4, lymphocyte function associated antigen-1, matrix metalloproteinase-9, very late antigen-4/5, and macrophage inflammatory protein-1. We suggest that whereas SDF-1 directly acts to upregulate CXCR4 expression in HSCs, dextran sulfate acts via multiple pathways involved in the induction of various homing-related molecules, in addition to SDF-1. Thus, preconditioning donors with dextran sulfate offers a novel clinical approach for improving the homing and engraftment of HSCs in the BM.
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Key words: stromal cell-derived factor 1, dextran sulfate, CXCR4, bone marrow transplantation, hematopoietic stem cells

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Introduction

Although transplantation of hematopoietic stem cells (HSCs) is an important strategy in the treatment of severe leukemia, immunodeficiency syndrome, and other malignancies, the precise mechanisms underlying the homing of HSCs following transplantation remain unclear. Following infusion, cells initially roll through the blood vessels before eventually reaching the bone marrow (BM) vessels. The HSCs then adhere to, activate, and pass between the BM vascular endothelial cells, transmigrating into the BM niche, thus reconstituting the recipient BM. Throughout this process, HSC activity is regulated by a variety of chemokines, cytokines, and adhesion molecules. During the initial rolling step, interactions between HSCs and BM vascular endothelial cells that express selectin family proteins play a key role[1]. Thereafter, endothelial cells expressing or presenting stromal cell derived factor-1 (SDF-1), lymphocyte function associated antigen-1 (LFA-1), and very late antigen-4 (VLA-4) are activated by rolling HSCs[2]. The HSCs then extravasate into the BM extracellular matrix via SDF-1 acting in concert with LFA-1 and VLA-4[3]. Finally, the interaction between SDF-1 and its receptor CXC chemokine receptor 4 (CXCR4) appears to mediate translocation of HSCs to their specific niche[4].

The SDF-1 chemokine was originally isolated from a murine BM cell line[5]. SDF-1 binds to CXCR4, a G protein-coupled receptor that was originally identified as a co-receptor for human immunodeficiency virus type 1 but is now known to also be involved in a number of physiological processes during embryogenesis, including hematopoiesis, vascular development, cardiogenesis, and cerebellar development[6]. In addition, it has been suggested that during HSC homing, this SDF-1/CXCR4 interaction may also be involved in the mobilization of CD34+ progenitors to the peripheral blood and in the homing of HSCs to their specific niches[4,7]. In this regard, activation of CXCR4 appears to induce transendothelial migration of HSCs[8].

In addition to SDF-1, cell-surface heparan sulfate proteoglycans (PG) may also play an important role in the adhesion of HSCs to BM endothelial cells[9,10]. Treatment of cells with sulfated polysaccharide, which is similar in structure to dextran sulfate (molecular weight, <10,000), or a sulfuric acid ester of carbohydrate, increases the number of circulating mature white blood cells[11] and plasma SDF-1 levels in mice and nonhuman primates[12]. A semisynthetic PG analog of dextran sulfate inhibits the complement and coagulation cascades and is used clinically as an anticoagulant. Notably, the mechanism underlying this inhibition is thought to be the displacement of heparan sulfate PG on endothelial cell surfaces and in the BM extracellular matrix.

On the basis of these findings, we hypothesized that alterations in the molecular interactions that mediate the homing process may contribute to improved engraftment of intravenously infused donor HSC. We therefore investigated whether preconditioning either donor or recipients with SDF-1 or dextran sulfate before transplantation improves the homing efficiency of HSCs in mice. Here we report that infusion of SDF-1 or dextran sulfate into donor mice increases the expression of CXCR4, and improves the homing efficiency of HSC following BM transplantation (BMT).

Materials and Methods

Experimental Animals

Six- to 8-week-old male C57/BL6J congenic mice (Saitama Experimental Animal Laboratory, Saitama, Japan) and green fluorescent protein (GFP) transgenic mice (same strain as the C57/BL6J) were used in this study. A donor GFP transgenic breeding pair was a generous gift from Dr. Masaru Okabe (Osaka University, Japan)[13]. All experimental mice were bred and maintained under pathogen-free conditions in our animal facility. Neomycin in acidic drinking water was provided for recipients after BMT. All protocols were approved in advance by the Animal Experimental Ethical Review Committee of Nippon Medical School.

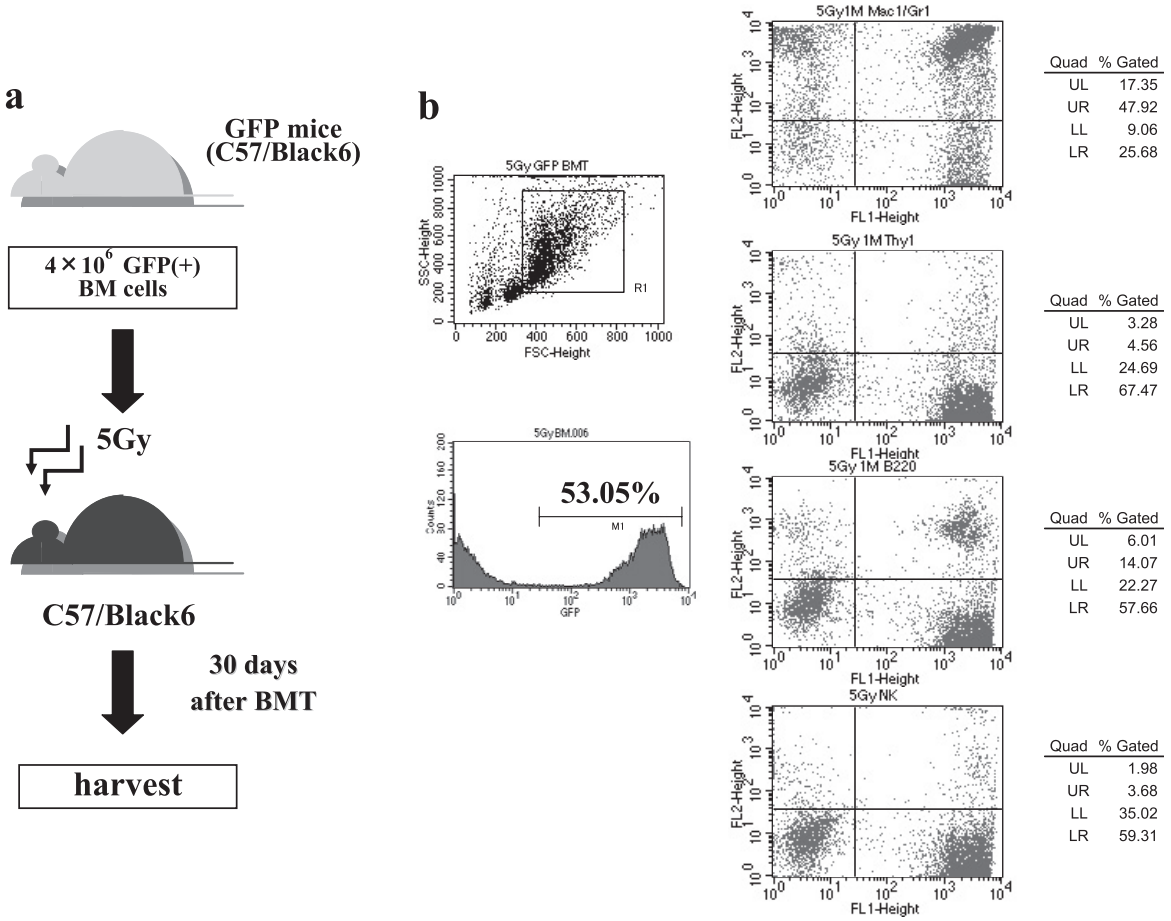

Fig. 1 Optimization of experimental bone marrow transplantation. **a**: The protocol utilized in the current study. GFP transgenic mice served as BM donors and C57/Black6 mice served as recipients. After 5Gy irradiation, recipients were transplanted with donor BM cells and analyzed 30 days post transplant. **b**: The typical flow cytometry panel of recipient mice 30 days post transplant. FACS analysis shows that the rate of donor-derived GFP (+) cells in the recipient mice whole BM mononuclear cells was 53.05%. Specific antibodies directed against Mac1/Gr1 (granulocytes), Thy1 (T cells), B220 (B cells) and NK1.1 (NK cells) labeled GFP (+) and GFP (−) BM cells, and for every linage the rate of donor-derived cells in the recipient mice was approximately the same as the control mice.

SDF-1 and Dextran Sulfate

Dextran sulfate (molecular weight, <10,000) and human SDF-1 were purchased from Sigma-Aldrich (St. Louis, MO, USA). Mouse recombinant SDF-1α and β were purchased from PeproTech (London, UK).

BMT

In our in vivo experiments, mice were divided into 4 groups depending upon their preconditioning regime: 1) Control (phosphate-buffered saline [PBS]), 2) SDF-1 (mouse SDF-1α) + β (100 ng/kg), 3) dextran sulfate 100 (100 mg/kg), and 4) dextran sulfate 500 (500 mg/kg). The cytokines or PBS were injected 4 times (every 24 hours) after transplantation. All agents were dissolved in 0.5 ml of 0.1 M PBS and injected via the tail vein. After preconditioning, BM cells were harvested from the donor mice as previously reported[14]. Recipient mice received whole-body irradiation (137Cs γ rays: 5 Gy at 1.0 Gy/minute) on the day of BMT, and 4×10^6 BM cells were then transplanted via tail vein injection within 4 hour of radiation treatment. All experiments were carried out in triplicate (**Fig. 1a**).

We next examined the effects of preconditioning donor GFP (+) mice with SDF-1 (100 ng/kg) or dextran sulfate (100 mg/kg or 500 mg/kg). An outline of the protocol used is described in **Figure 2**. Donor mice received SDF-1 or dextran sulfate injections on days 3, 2, 1 and at 6 h before

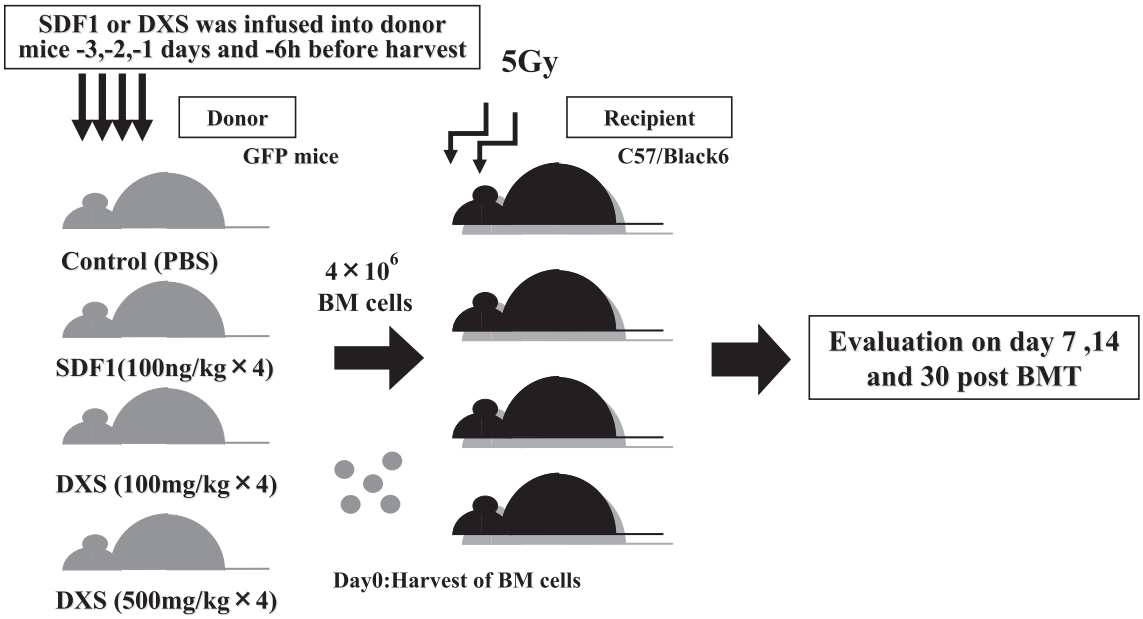

Fig. 2 Protocol for donor preconditioning. Schematic diagram illustrating the protocol used for BM transplantation and preconditioning of donor mice with PBS (Control), SDF-1 (100 ng/kg) or dextran sulfate (100 mg/kg or 500 mg/kg). Donor mice were administered with the indicated agent 3, 2 and 1 day or 6 h prior to harvesting BM cells. Each group of recipient mice was then irradiated with 5 Gy on the day of BMT and transplanted with an equivalent number of BM cells.

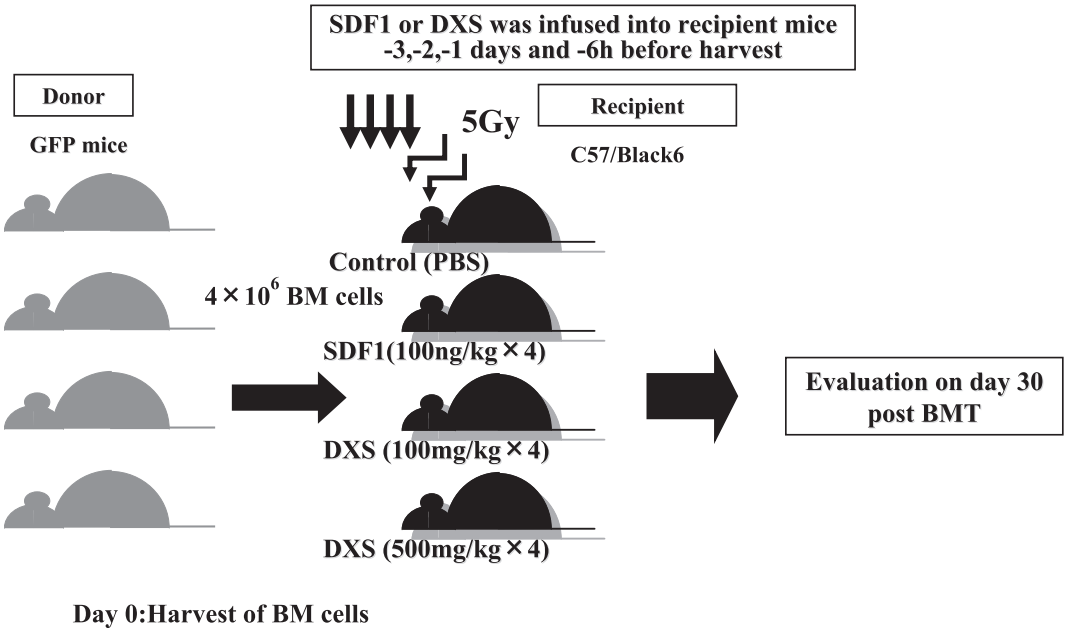

Fig. 3 Effects of preconditioning recipient mice with SDF-1 or dextran sulfate. The protocol used to analyze the effects of preconditioning with SDF-1 or dextran sulfate on recipient mice is described. The results of this experiment demonstrate that the recipient microenvironment was influenced by SDF-1 or dextran sulfate administration in vivo.

transplant, and prior to BM harvesting. As a control, PBS was injected into C57/BL6J at the same time points. Harvested BM cells were then transplanted into C57/BL6J mice as described in the previous section.

Then, in order to test whether preconditioning may also affect the recipient BM microenvironment, we injected SDF-1 or dextran sulfate into recipient

mice using the same treatment regime (**Fig. 3**).

Cell Isolation and Flow Cytometric Analysis

Mice were killed under deep anesthesia by means of intracardiac perfusion via the left ventricle with cold 0.9% NaCl, followed by 4% paraformaldehyde in PBS (pH 7.4). Peripheral blood (PB) cells were then collected via the abdominal aorta, and BM cells were harvested by flushing the BM in the tibias and femurs. Red cells and debris were lysed after density centrifugation using Ficoll-Paque (Amersham Biosciences, Piscataway, NJ). Engraftment of donor-derived BM cells was evaluated with flow cytometry using FACSCalibur (BD Biosciences Immunocytometry Systems, San Jose, CA, USA) based on GFP expression. To confirm whether all cell lineages were differentiated from GFP-positive BM cells, mononuclear cells from PB were stained for 30 minutes on ice with the following cell-specific antibodies (Pharmingen, San Diego, CA, USA): RB6-8C5 (anti-Gr-1) for granulocytes, M1/70 (anti-Mac-1) for monocytes and macrophages, RA3-6B2 (anti-CD 45R/B220) for B lymphocytes, 30-H12 (anti-Thy-1.2) for T lymphocytes, and PK136 (anti-NK1.1) for NK cells. Cell suspensions prepared using the above antibodies were then analyzed with flow cytometry.

BM Cell Expansion Culture

After BM cells had been harvested as described above, the cells were cultured for 72 hours at 37℃ in Dulbecco's modified essential medium (DMEM; GIBCO/Invitrogen, Carlsbad, CA) supplemented with 10% fetal bovine serum (FBS), penicillin (100 mg/mL), streptomycin (100 μg/mL), and glutamine (10 mg/mL). The following cytokines were used in the experimental protocol: recombinant mouse interleukin (IL)-3 (10 ng/mL), IL-6 (10 ng/mL), and stem cell factor (SCF; 100 ng/mL) (kindly provided by Kirin Pharma Co., Ltd., Tokyo). Depending on the experimental group, we also added mouse SDF-1 (1 ng/mL), human SDF-1 (1 ng/mL), or dextran sulfate (100 or 500 ng/mL).

RNA Extraction and Reverse Transcriptase-polymerase Chain Reaction Analysis

Total RNA was extracted from BM mononuclear cells (MNC) using the RNeasy Mini kit (Qiagen, Venlo, the Netherlands) and was treated with RNase-free DNase (Qiagen). The RNA concentration and its purity were checked with a UV spectrophotometer set at 260 nm and 280 nm, respectively. The reverse transcriptase-polymerase chain reaction (RT-PCR) was then performed with 1-μg samples of total RNA with an RNA PCR Kit (AMV) Ver 2.1 (Takara Shuzo Co., Ltd., Kyoto, Japan). PCR was performed with primers designed according to the cDNA sequence (Gene Bank) and were constructed using the Primer3 web site (http://frodo.wi.mit.edu/). The primers were designed to amplify a 200- to 300-bp PCR products and were CXCR4: (GenBank assession no., AB 000803), forward primer, 5′-GCATAGTCGGCAATGG ATTG-3′, and reverse primer, 5′-GCATCATCATCT CCAAGCTG-3′; P-selectin (AK136243): forward primer, 5′-GCCACTGAGAAGATTGCCAC-3′, and reverse primer, 5′-GACAGTCCATTCACTCAGTG-3′; LFA-1 (AK141575): forward primer, 5′-TGCAGCT CATCAGAATGCC-3′, and reverse primer, 5′-GGTC ACTGTATCCGTGAAAC-3′; E-selectin (AK153623): forward primer, 5′-CAGTGTCAGTATGTGGTCCA-3′, and reverse primer, 5′-TGGATGCAGTCCATGGT ACC-3′; VEGFA (AY707864): forward primer, 5′-CAG GCTGCTGTAACGATGAA-3′, and reverse primer, 5′-CTTGCAACGCGAGTCTGTGT-3′; VLA-4 (AK 037794): forward primer, 5′-AGTCAAGTTGTCCTGC ACAG-3′, and reverse primer, 5′-CATTCCGTCTGC ACAGTAGT-3′; VLA-5 (AK291570): forward primer, 5′-CACCTATTCAGTGAATGGCA-3′, and reverse primer, 5′-ACAGTTGTCACGGCACTCTT-3′; MCP-1 (AK132590): forward primer, 5′-CGCCTCCAGCATG AAAGTCT-3′, and reverse primer, 5′-GGGAATGAA GGTGGCTGCTA-3′; MIP-1 (AK029953): forward primer, 5′-CCCACTTCCTGCTGTTTCTC-3′, and reverse primer, 5′-GAGGAGGCCTCTCCTGAAGT-3′; and MMP-9 (004651): forward primer, 5′-ATGGTC GTGGCTCTAAGCCT-3′, and reverse primer, 5′-AG GATTGTCTGCCGGACTCA-3′. The quantity of mRNA was measured and RT-PCR performed simultaneously following the manufacturer's protocol. The PCR cycling protocol included an initial denaturation step at 94℃ for 2 minutes followed by 28 cycles at 94℃ for 30 seconds, 60℃

Table 1 Comparison of the effect of SDF-1 and DXS preconditioning on recipient mice

	Day 7		Day 14		Day 30	
	PB	BM	PB	BM	PB	BM
Control (PBS)	28.7 ± 3.0	33.9 ± 3.5	39.8 ± 3.1	43.7 ± 3.3	45.1 ± 5.9	46.2 ± 2.5
SDF-1 (100 ng/kg)	28.2 ± 2.8	35.8 ± 7.39	48.9 ± 5.0	58.4 ± 1.6 *	56.5 ± 3.3 *	53.8 ± 1.6 *
DEX100 (100 mg/kg)	33.4 ± 3.9	51.8 ± 5.1 *	59.0 ± 2.4 *	62.6 ± 1.3 *	69.4 ± 6.6 *	63.0 ± 2.8 *
DEX500 (500 mg/kg)	29.9 ± 2.7	35.1 ± 2.4	48.3 ± 3.5 *	54.6 ± 4.0 *	56.1 ± 6.1 *	50.5 ± 3.6 *

The rate of donor derived GFP (+) cells in the recipient mice. Data are means (%) ± SEM (n = 6 in each group)

* $p<0.05$, compared to control group

for 30 seconds, and 72℃ for 90 seconds, and a final incubation at 72℃ for 5 minutes. As a cDNA loading control, an aliquot of each sample was also amplified under the same conditions using the β-actin primers (5′-TCACCCACACTGTGCCCATCTACGA-3′ and 5′-CAGCGGAACCGCTCATTGCCAATGG-3′).

DNA Microarray Analysis

After the BM was harvested, total RNA was isolated from control and preconditioned mice using the RNeasy mini kit (Qiagen). Genomic DNA was then removed from the samples using the RNase-free DNase kit (Qiagen). The total RNA was then treated as the starting material for cDNA microarray analysis following the manufacturer's protocol (Mouse Atlas 1.2, BD Biosciences Clontech, Palo Alto, CA, USA). Briefly, aliquots (3–5 μg) of total RNA from each group were labeled with 32P-α-dATP (Perkin Elmer, CITY, Japan) and hybridized overnight at 68℃ to an Atlas Mouse 1.2 Nylon array (BD Biosciences Clontech) that contained 1,176 murine genes. The arrays were then used to expose to x-ray film for 4 days. The films were scanned (ES-2000 scanner, Epson, LOCATION), and the data was analyzed with AtlasImage 1.5 software (BD Biosciences Clontech). The gene expression signals for each of the 3 groups (control, SDF-1, and dextran sulfate) were analyzed in triplicate and were averaged with the software and compared. The differences were calculated as follows: signal intensity of SDF-1 or dextran sulfate-control (PBS). When the differences were greater than or less than 8,000, the expression changes were considered to be significant.

Statistical Analysis

All results are presented as mean ± standard error of the mean (SEM). Statistical analysis was performed with the StatView software program (SAS Institute, Cary, NC, USA). Differences between means were evaluated wit an unpaired Student's t-test. Differences with a $p<0.05$ were considered significant.

Results

Optimization of Experimental Bone Marrow Transplantation

Seven days after BMT, GFP (+) donor-derived cells accounted for 22.58% ± 3.06% of cells in the PB, spleen, and BM of recipient mice (n=6). GFP (+) cells in the recipient BM were 38.07% ± 3.51% (n=6), and 30 days after the BMT, 51.69 ± 4.38% (n=7) of the recipient BM cells had been replaced by GFP (+) donor-derived cells. FACS analysis showed that GFP (+) engrafted cells differentiated into all hematopoietic lineages including T cells, B cells, NK cells and granulocytes (**Fig. 1b**). In addition, the rate of donor derived GFP (+) BM cells from each lineage was similar to the untreated normal controls (data not shown).

Effect of Preconditioning with SDF-1 and Dextran Sulfate on Donor Mice

We next examined the effects of preconditioning donor GFP (+) mice with SDF-1 or dextran sulfate (**Fig. 2**). After the 4 injections, the PB white cell counts in donor mice (n=6 per group) were induced after treatment with control (PBS), 2,736 ± 329

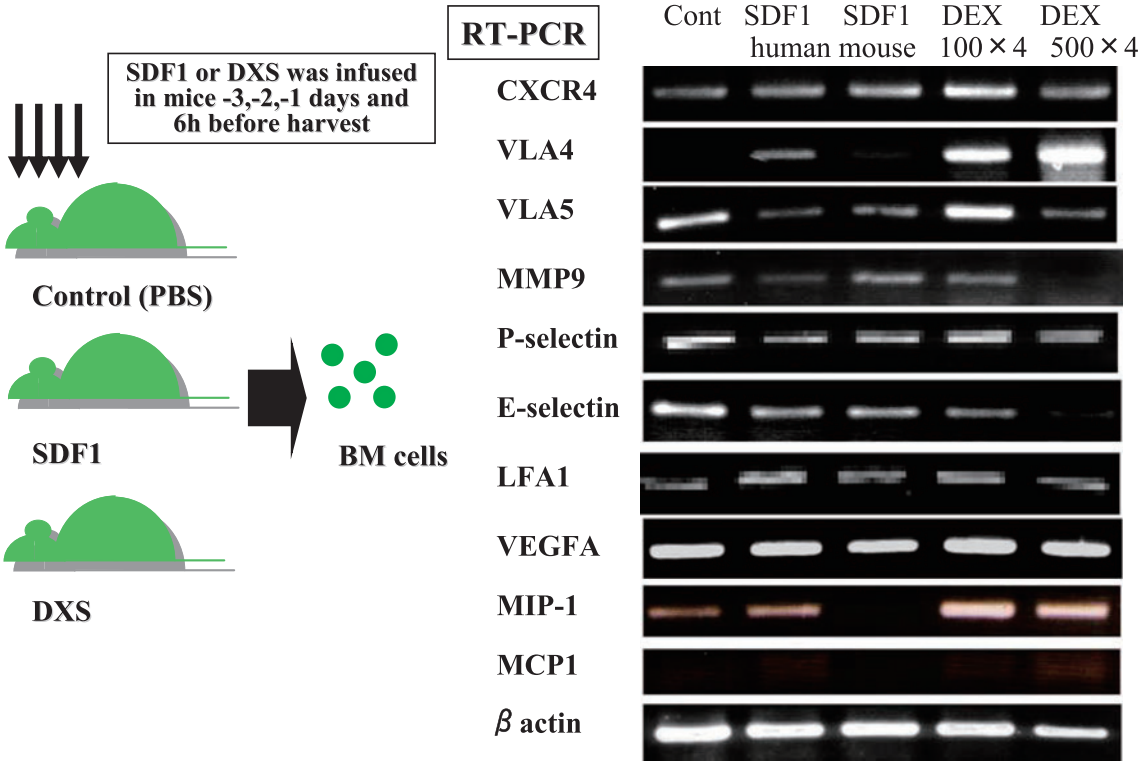

Fig. 4 Effects of SDF-1 and dextran sulfate on the expression of BM homing-related genes in vivo. RT-PCR analysis was used to investigate the expression of 10 genes known to be involved in BM homing. The expression of β-actin was used as a control. This figure outlines the result of the RT-PCR analysis using BM cells from the recipient mice after 4 injections with SDF-1 or dextran sulfate.

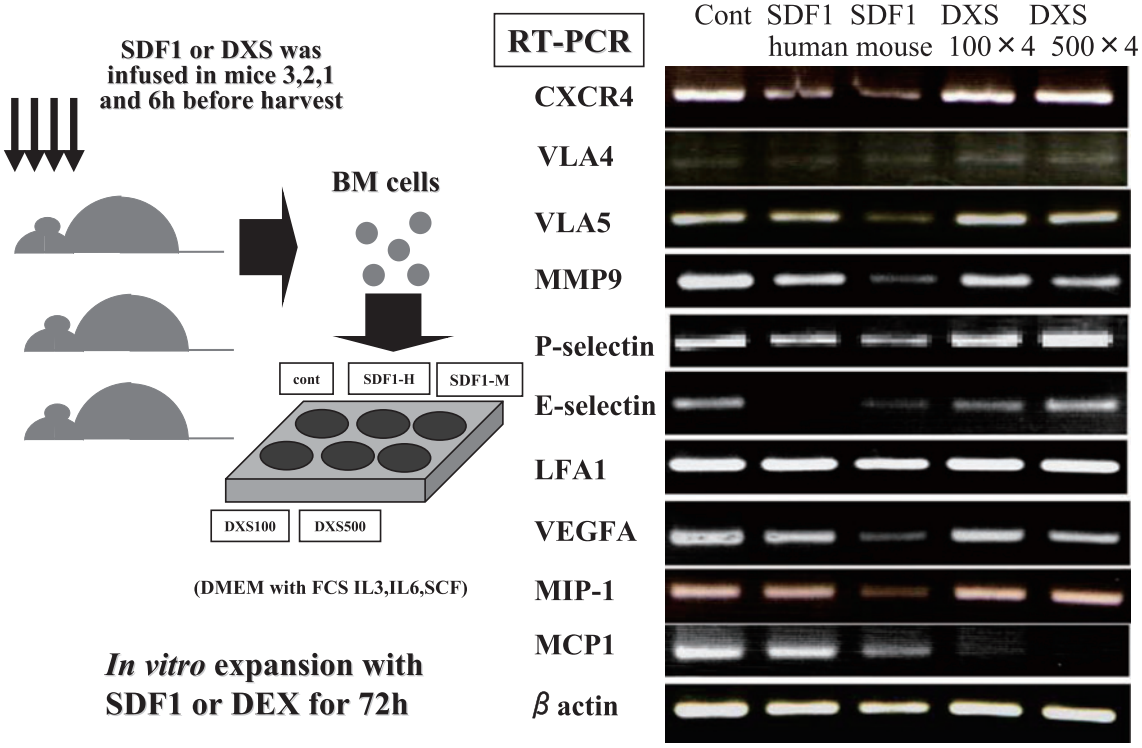

Fig. 5 Effects of SDF-1 and dextran sulfate on the expression of BM homing-related genes in vitro. Cells were incubated with SDF-1 or dextran sulfate for 72h. To maintain the BM cell phenotype, the culture medium also contained the indicated cytokines. Note the difference between the expression profiles obtained in vivo and in vitro.

Table 2 Effect of SDF-1 and DXS on the expression of homing related-genes in BM cells

	Mouse SDF-1		DXS 100	
	Up	Down	Up	Down
In vivo	CXCR4 MMP-9 LFA-1	VLA-5 E-selectin P-selectin MIP-1	CXCR4 VLA-4 VLA-5 MIP-1	E-selectin
In vitro		CXCR4 VLA-5 MMP-9 E-selectin VEGFA MIP-1		E-selectin MCP-1

(mean ± SEM per μL); SDF-1, 3,849 ± 136; dextran sulfate (100 mg/kg), 9,797 ± 1,242; and dextran sulfate (500 mg/kg), 20,760 ± 3,709. Among the donor mice, administration of SDF-1 ($p<0.05$) and dextran sulfate (100 mg/kg, $p<0.001$; 500 mg/kg, $p<0.001$) induced the number of PB white blood cell counts compared to control mice.

FACS analyses of recipient PB and BM cells harvested on day 7, 14 or 30 days following BMT (**Table 1**) revealed a time-dependent cell replacement of the donor-derived GFP (+) BM cells in each treatment group. Moreover, significantly increased rates of replacement were obtained in the BM and PB 30 days after transplantation when comparing non pre-conditioned with preconditioned donors treated with SDF-1 (46.2 ± 6.2% vs. 53.8 ± 4.0%, $p<0.05$) and dextran sulfate 100 mg/kg (46.2 ± 6.2% vs. 63.0 ± 6.8%, $p<0.001$). By 7 days after the BMT, donor-derived GFP (+) cells were found in every PB and BM lineage, and by 30 days there were no significant differences in all lineages between transplanted and control animals (data not shown).

The Effect of Pre-conditioning on the Microenvironment of the Recipient Bone Marrow

Pre-conditioning with SDF-1 and dextran sulfate affected donor BM cells. In this experiment, affect of preconditioning to the recipient BM microenvironment was studied (**Fig. 3**). We found that this treatment did not significantly affect the number of GFP (+) cells present in the BM or PB of recipient mice on days 7, 14 or 30 after BMT (data not shown). Thus, the rate of GFP (+) chimerism was unaffected following treatment of the recipient microenvironment.

SDF-1 and Dextran Sulfate Induce the Expression of Homing-related Genes

We next analyzed the mRNA levels of 10 genes that are thought to be related to HSC engraftment by RT-PCR (**Fig. 4**, **Table 2**). Compared to the control group, the levels of CXCR4, VLA-4, VLA-5, P-selectin and MIP1 mRNA were all upregulated in the dextran sulfate group, while preconditioning with SDF-1 upregulated the expression of CXCR4, MMP-9 and LFA-1. In contrast, dextran sulfate injected was found to result in downregulation of E-selectin expression while SDF-1 was found to downregulate expression of VLA-5, E-selectin, P-selectin and MIP-1.

To evaluate the differences between in vitro and in vivo stimulation of BM cells, we also cultured BM cells for 72h in the presence of SDF-1 or dextran sulfate at approximately equivalent doses as used in the in vivo experiments (**Fig. 5**). We found that IL-3, IL-6, and SCF were also present at appropriate concentrations in this in vitro experiment. However, subsequent RT-PCR analysis of the cultured BM cells revealed a gene expression profile that differed substantially from that seen in vivo (**Table 2**). Notably, levels of both CXCR4 and MMP-9 mRNA were downregulated by SDF-1 treatment. In contrast, dextran sulfate administration

Table 3 Microarray analysis of BM cells harvested from mice preconditioned with SDF-1 or DXS

Control/SDF-1 Up-regulated after SDF-1 infusion		Control/DXS 100 Up-regulated after DXS infusion	
Difference	Gene	Difference	Gene
11,739	LEF-1	9,604	Gap junction beta 4
18,589	MMI-alpha	10,196	IL1 receptor
18,974	LAT ZAP70	10,239	Fas antigen ligand
19,569	Patched homolog 1	10,623	Gap junction beta 1
19,969	LPS receptor	11,604	CD40 Ligand
24,105	Cytokeratin 15	12,409	Gap junction alpha 1
38,115	Prothymosin alpha	18,935	MCT-1
38,645	Cytoplasmicbeta-actin	20,426	PMCA
		23,367	BK channel subunit
		35,252	LPS receptor

Atlas Mouse 1.2 arrays (Clontech inc.)

Difference: The signal difference of SDF-1 or DXS compared with Control (PBS). When the difference of the signal was over 8,000, the gene expression were considered obviously prevalent and listed above.

demonstrated a less profound negative regulatory effect, and did not result in the upregulation of any genes.

Microarray Analysis of BM Cells Treated with SDF-1 and Dextran Sulfate

Finally, we used microarray analysis to investigate the expression levels of additional genes that have not previously been associated with BM homing. Using this approach, we identified a number of genes to be significantly upregulated following preconditioning with SDF-1 or dextran sulfate (n=6 per group), (**Table 3**). These upregulated genes were found to encode molecules previously reported to be involved in cell adhesion and activation.

Discussion

When transplanting HSC it is essential that physicians choose an adequate source for the patient. However, this selection is often limited by either the quantity of HSC available or the availability of fully matched human leukocyte antigen (HLA) donors. In practical terms, the difficulty of adapting cord blood (CB) for transplantation in adults is due to an insufficient number of HSC present. To address this problem, more than 2 independent donor CB samples have been combined in some cases[15]. An additional approach to improving the outcome of HSC transplantation is to expand the total number of HSC showing sustained functionality after harvest via methods including in vitro expansion in the presence of various cytokine combinations[16]. While these approaches may contribute to the improvement of HSC transplantation outcomes, numerous problems still remain with respect to efficacy, safety and convenience.

In the present study, we evaluated the effects of HSC stimulation in vivo on the outcomes of HSC transplantation, and on the expression of homing-related genes with the aim of determining whether modification of the expression of these homing-related genes could affect HSC trafficking to the BM. In our in vivo experiment, we found that pre-treating donors with SDF-1 or dextran sulfate prior to BMT enhanced the expression of CXCR4 in BM cells and improved their homing efficiency. Other research groups have also reported that increasing plasma SDF-1 levels enhances BM cell mobilization[17]. We have extended these findings by showing that administration of SDF-1 to donor mice influences HSC homing directly via upregulation of CXCR4 and, indirectly by upregulating MMP-9 and LFA-1, proteins that are also thought to be involved in BM homing events.

Low-molecular weight dextran sulfate is a sulfated polysaccharide that belongs to the glycosaminoglycan family, that also includes heparin, heparan sulfate and dermatan sulfate[18]. dextran sulfate has been reported to result in platelet dysfunction and is currently used clinically as an anticoagulant agent[19]. Thus, treatment with dextran sulfate was more effective than treatment with SDF-1, which was consistent with the previously reported finding that plasma SDF-1 levels are upregulated by dextran sulfate[12]. However, the finding that BM cells home to BM niches[7,8] was not sufficient to explain the improved outcome in our dextran sulfate group. On the other hand, our findings may suggeste that dextran sulfate upregulates additional important genes to a greater extent than SDF-1. For instance, enhanced integrin interaction via upregulation of VLA-4 and VLA-5 may also contribute greatly to not only HSC mobilization[20], but also to the homing of HSC. Such a response would be consistent with the increased homing rate elicited by dextran sulfate pre-conditioning. In contrast, neither SDF-1 or dextran sulfate treatment upregulated the expression of homing-related genes in vitro, suggesting that although SDF-1 and dextran sulfate contribute to HSC homing, the process involves complex interactions and cooperative signaling with other pathways that may be lost when the cells are removed from their BM microenvironment.

To reveal additional genes that are involved in HSC homing, we used a DNA microarray chip that enabled us to investigate 1,176 murine genes. Using this approach, we found that a number of genes related to cell adhesion and activation were significantly upregulated, compared to controls. For instance, the LPS receptor that was originally identified on macrophages and monocytes was identified in the preconditioned animals, especially in the dextran sulfate group. In the dextran sulfate group, the expression of ion channel genes including plasma membrane calcium ATPase (PMCA) and the BK channel subunit were also significantly increased. The transporter gene MCT1 and the cell adhesion-related Gap junction alpha 1 gene were also altered following preconditioning treatment. Although homing-related genes such as CXCR4, VLA-4 and VLA-5 play important roles in HSC homing, additional cascades involving the combined activities of ion channels, transporters and cell adhesion molecules, may also contribute to the increase in homing efficiency elicited by SDF-1 or dextran sulfate treatment.

In summary, we have demonstrated that treatment of donor mice with SDF-1 or dextran sulfate prior to BMT enhances the expression of CXCR4 on BM cells and increases HSC homing efficiency in vivo. As dextran sulfate increases homing efficiency to a greater degree than SDF-1, dextran sulfate might exert its effects directly via the CXCR4/SDF-1 pathway, and indirectly via additional cytokines and chemokines including P-selectin, MMP-9 and VLA-5. Thus, preconditioning patients with dextran sulfate may offer a novel clinical approach in improving the homing and engraftment of HSC.

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