Stromal cell-derived factor-1 mediates changes of bone marrow stem cells during the bone repair process

Kiyotaka Okada,1 Naoyuki Kawao,1 Masato Yano,1 Yukinori Tamura,1 Shinzi Kurashimo,2 Katsumi Okumoto,2 Kotarou Kojima,1 and Hiroshi Kaji1

1Department of Physiology and Regenerative Medicine, Kinki University Faculty of Medicine, Osakasayama, Osaka, Japan; and 2Life Science Research Institute, Kinki University, Osakasayama, Osaka, Japan

Submitted 3 June 2015; accepted in final form 27 October 2015

Stromal cell-derived factor-1 mediates changes of bone marrow stem cells during the bone repair process. Am J Physiol Endocrinol Metab 310: E15–E23, 2016. First published November 3, 2015; doi:10.1152/ajpendo.00253.2015.—Osteoblasts, osteoclasts, chondrocytes, and macrophages that participate in the bone repair process are derived from hematopoietic stem cells (HSCs) and mesenchymal stem cells (MSCs). However, the roles of these stem cells during the repair of injured bone tissue are still unclear. In the present study, we examined the effects of bone defect on HSCs and MSCs in bone marrow and spleen in 75 mice and its mechanism. We analyzed the HSC and MSC populations in these tissues of a mouse with femoral bone damage by using flow cytometry. The number of HSCs in the bone marrow of mice with damaged femurs was significantly lower than the number of these cells in the bone marrow of the contralateral intact femurs on day 2 after injury. Meanwhile, the number of MSCs in the bone marrow of mice with damaged femurs was significantly higher than that of the contralateral femurs. Both intraperitoneal administration of AMD3100, a C-X-C chemokine receptor 4 (CXCR4) antagonist, and local treatment with an anti-stromal cell-derived factor-1 (SDF-1) antibody blunted the observed decrease in HSC and increase in MSC populations within the bone marrow of injured femurs. In conclusion, the present study revealed that there is a concurrent decrease and increase in the numbers of HSCs and MSCs, respectively, in the bone marrow during repair of mouse femoral bone damage. Furthermore, the SDF-1/CXCR4 system was implicated as contributing to the changes in these stem cell populations upon bone injury.

BONE REPAIR AFTER BONE DESTRUCTION OR FRACTURES is a complex and multistep process that has been suggested to involve a developmental sequence of bone formation at both the tissue and cellular levels (32). The bone repair process is divided into distinct phases, including inflammation, repair, and remodeling (5). Bleeding, local ischemia, recruitment of stem cells, and interaction among inflammatory cells are observed during inflammation phase (18). However, the origin of the cells that participate in callus formation remains unclear. Several reports have suggested that the cells participating in bone repair are derived from the periosteum, muscle, or bone marrow in the area surrounding the injury site (8, 29). Recent studies indicate that bone marrow-derived cells might be the source of endothelial cells, osteoblasts, and osteoclasts, which are responsible for tissue maintenance and repair (38). However, the effect of the bone repair process after bone destruction or fractures on bone marrow cells, particularly bone marrow stem cells (BMSCs), remains unclear.

BMSCs include hematopoietic stem cells (HSCs), mesenchymal stem cells (MSCs), and multipotent adult progenitor cells. HSCs exhibit self-replication competency with pluripotency, which enables these cells to differentiate to all circulating blood cell types, including erythrocytes, granulocytes, monocytes, and thrombocytes. Mononuclear myeloid lineage cells derived from HSCs are attracted to bone surfaces by chemokines and other factors, where they then differentiate into multinucleated bone-resorbing osteoclasts through cell fusion (14). Osteoclasts are thought to play several roles in bone repair, particularly in the remodeling phase. Meanwhile, mononuclear myeloid lineage cells derived from HSCs also differentiate into monocytes. These cells exert specific functions as macrophages in peripheral tissues such as bone tissues. Indeed, several previous studies have suggested that activated macrophages are involved in bone formation and repair (1, 9, 15). Moreover, we revealed previously that macrophages contribute to tissue fibrinolytic system-related bone repair in mice (16).

MSCs have multidifferentiation potency and enhance wound healing in a variety of diseases. Recently, MSCs were found to differentiate into tissue-forming cells and to influence their extracellular environment by secreting various cytokines and chemokines that mediate antiapoptotic, immunomodulatory, angiogenic, and cell-mobilizing effects (38). Several studies indicate that MSCs within the bone marrow are recruited to the site of bone fractures (4, 21, 36). Chondrogenic and osteogenic cells are essential for bone repair and for remodeling processes during endochondral ossification. MSCs differentiate into osteoblasts, chondrocytes, and osteocytes in response to multiple stimuli (11). Although “side population” (SP) cells, identified by their unique capacity to efflux fluorescent DNA-binding dyes, have the multilineage potential to differentiate into skeletal myocytes as well as vascular endothelial cells in vivo (13), BMSCs derived from SP cells, regenerated from the hematopoietic compartment, can differentiate into osteoblasts through a mesenchymal intermediate (30). These findings suggest that bone repair processes after bone destruction might influence the population of HSCs and MSCs in the bone marrow.
Recently, we investigated the roles of tissue-fibrinolytic systems and the diabetic state on bone healing using the efficient bone repair model after femoral bone damage in mice (16, 17, 25). Therefore, in the present study, we examined the effects of bone defect on HSCs and MSCs in bone marrow from the damaged femur in mice and tried to clarify their detailed mechanisms using this model. Since the previous study suggested that the spleen serves as a reservoir of osteoclast precursors in mice (28), bone injury might affect distant hematopoietic tissues. Therefore, we analyzed bone marrow cells from the contralateral intact femur as well as spleen cells.

**MATERIALS AND METHODS**

We analyzed flow cytometry, bone repair, and histology using murine bone damage model to examine the effects of bone defect on HSCs and MSCs. Moreover, we employed the experiments using bone marrow transplantation and anti-nestin cell-derived factor-1 (SDF-1) and ADM3100 treatments to clarify the detailed mechanism.

**Materials.** Anti-Osterix, anti-F4/80, anti-alkaline phosphatase (ALP), and anti-green fluorescent protein (GFP) antibodies were obtained from Abcam (Cambridge, UK), AbD Serotec (Raleigh, NC), Abnova (Taipei, Taiwan), and Medical & Biological Laboratories (Nagoya, Japan), respectively. Anti-SDF-1/C-X-C chemokine ligand 12-neutralizing antibody was obtained from R & D Systems (Minneapolis, MN). Violet (V)450/Brilliant Violet (BV)421-conjugated anti-CD29, Alexa 700-conjugated anti-CD34, allogloocyanin (APC)-Cy7-conjugated anti-CD44, V500/BV510-conjugated anti-CD45, APC-conjugated anti-CD73, phycoerythrin (PE)-conjugated anti-CD105, BV711-conjugated anti-c-kit, PE-Cy7-conjugated anti-Sca-1 antibodies, and the peridinin chlorophyll protein complex-Cy5.5-conjugated anti-lineage antibodies cocktail (anti-CD3e, anti-CD11b, anti-B220/CD45R, anti-Gr1, and anti-TER-119 antibodies 1) were obtained from BD Biosciences (San Jose, CA). Finally, the AMD3100 C-X-C chemokine receptor 4 (CXCR4) antagonist was obtained from Sigma-Aldrich (St. Louis, MO).

**Animals.** Six- to 10-week-old male C57BL/6J mice and C57BL/6J-Tg (CAG-EGFP) mice were used for all experiments, and each mouse weighed between 18 and 25 g at the time of the experiment. All experiments were performed according to the guidelines of the National Institutes of Health and the institutional rules for the use and care of laboratory animals at Kinki University. The protocol was approved by the Experimental Animal Welfare Committee of Kinki University (permit no. KAME-27-025).

**Murine bone damage model.** Bone injury was induced in mice according to the method described previously (19). Briefly, under anesthesia induced by pentobarbital sodium (50 mg/kg ip), the anterior skin over the mid-femur of the right leg was incised longitudinally to 5 mm in length. After splitting the muscle, the surface of the femoral bone was exposed. Thereafter, a hole was made using a drill with a diameter of 0.9 mm. The hole was irrigated with saline to prevent thermal necrosis of the margins. The incised skin was then sutured in a sterile manner, and the anesthesia was discontinued.

**Flow cytometric analysis.** Bone marrow stromal cells were obtained from mice as described previously (14). Hanks' balanced salt solution (HBSS) buffer with 2% fetal bovine serum (FBS) was used to flush the bone marrow cells from harvested femurs and the spleen cells from harvested spleens. Bone marrow and spleen cells were then added in an equivalent volume to Ficoll-Paque PLUS (GE Healthcare Bio-Sciences, Uppsala, Sweden) and were harvested by centrifugation for 15 min at 630 g at 4°C. Cells were resuspended in PBS supplemented with 3% FBS.

Bone marrow and spleen cells were analyzed using a FACS Aria II cell sorter (BD Biosciences), as described previously (3). HSCs and MSCs were identified in bone marrow and spleen cell populations using color-conjugated antibodies specific for CD34, c-Kit, Sca-1, and Lin (26 or CD29, CD34, CD44, CD45, CD73, CD105, and Lin) (34). The numbers of HSCs and MSCs harvested from the bone marrow of the contralateral intact and damaged femurs of bone damage on days 0, 1, 2, and 3 after femoral bone injury, as enumerated by flow cytometry. The results represent experiments performed on five mice in each group.

**Bone marrow transplantation.** Bone marrow transplantation was carried out as described previously (37). Bone marrow cells from C57BL/6J-Tg (CAG-EGFP) mice were collected by introducing HBSS containing 2% FBS into the marrow space. Cells were then resuspended in Dulbecco’s modified Eagle’s medium (Wako Pure Chemical, Osaka, Japan) to a density of ~1 × 10^7 cells/0.25 ml. Meanwhile, 6- to 8-wk-old male C57BL/6J recipient mice were subjected to lethal whole body irradiation (10 Gy) and then administered resuspended bone marrow cells by tail vein injection. The recipient mouse bone marrow was then examined by GFP immuno-histochemistry at 4 to 6 wk after transplantation.

**In vivo quantitative computed tomography analysis.** Mice were anesthetized using 2% isoflurane, and femurs were scanned using a Latheta LCT 200 X-ray CT system (Hitachi Aloka Medical, Tokyo, Japan), as described previously (16). The parameters used for the CT scans were as follows: tube voltage, 50 kVp; tube current, 500 μA; integration time, 3.6 ms; and axial field of view, 48 mm, with an isotropic voxel size of 48 μm. Images were generated by integration of two signal averages for each femur. The total scan time was ~5 min/femur. Volume-rendered three-dimensional CT pictures were reconstructed using the VGStudio MAX2.1 software (Nihon Visual Science, Tokyo, Japan). The area of bone damage in each femur was quantified with an image-processing program using Latheta software (Hitachi Aloka Medical). A threshold density of 160 mg/cm³ was selected to distinguish mineralized from unmineralized tissues. The density range was calibrated daily with a manufacturer-supplied phantom.

**Histological analysis.** The mice were anesthetized using pentobarbital sodium (50 mg/kg ip) on day 4, day 7, or day 18 after surgery. Femurs were removed, fixed in 4% paraformaldehyde, demineralized in 22.5% formic acid and 340 mM sodium citrate solution for 24 h, and embedded in paraffin. Thereafter, 4-μm-thick sections were generated and stained with tartrate-resistant acid phosphatase (TRAP) using a TRAP staining kit (Wako Pure Chemical). A blinded evaluation was utilized to measure the number of TRAP-positive multinucleated cells on the bone surface at the site of femur damage or at undamaged trabecular bone regions of damaged femurs. Immunostaining was performed as described previously (16). Briefly, the sections were incubated with the anti-ALP antibody at a dilution of 1:100, anti-Osterix antibody at a dilution of 1:200, anti-F4/80 antibody at a dilution of 1:1,000, and anti-GFP antibody at a dilution of 1:1,000, followed by incubation with the appropriate horseradish peroxidase-conjugated secondary antibody. Positive signals were visualized using the tyramide signal amplification system (PerkinElmer, Waltham, MA), and sections were counterstained with 4',6-diamidino-2-phenylindole and photographed using a fluorescence microscope (E800; Canon, Tokyo, Japan) with a charge-coupled device camera or confocal microscope (C2 Sii; Nikon, Tokyo, Japan).

**Anti-SDF-1 and ADM3100 treatments.** Either the anti-SDF-1 IgG (25 μg/50 μl in saline) or saline (50 μl) was mixed with the MedGel seat (7.5 × 7.5 mm; MedGel, Kodaira, Japan) and then incubated at 37°C for 30 min. After introduction of femoral bone damage, as described above, anti-SDF-1 IgG or saline with the MedGel seat was added to the damaged site on the femur, and the incised skin was then sutured (7).

The intraperitoneal administration of AMD3100 (5 mg/kg) or saline (control) was performed at 1 h before and 8 h after the bone damage procedure (24).

**Statistical analyses.** All data were expressed as means ± SE. Statistical significance was assessed using unpaired t-tests and one-

---

*AJP-Endocrinol Metab* • doi:10.1152/ajpendo.00253.2015 • www.ajpendo.org
way analysis of variance (ANOVA). Differences with $P < 0.05$ were regarded as significant. All statistical analyses were performed using StatView version 5.0 software (SAS Institute, Cary, NC).

RESULTS

Prevalence of HSCs in bone marrow cells after femoral bone damage. The prevalence of HSCs in the bone marrow from damaged and contralateral intact femurs after femoral bone injury was evaluated by flow cytometric analysis. HSCs were defined as cells that were CD34$^+$, c-Kit$^+$, Sca-1$^+$, and Lin$^-$ (CD34$^-$ KSL) cells, as described in MATERIALS AND METHODS. There were similar expression patterns of HSC markers (CD34$^-$, c-Kit$^+$, Sca-1$^+$, and Lin$^-$) in bone marrow cells harvested from intact right and left femoral bones (data not shown). Likewise, similar numbers of HSCs were observed in the bone marrow cell populations from intact right and left femurs of normal C57BL/6J mice [right: $(3,047 \pm 152)/5 \times 10^6$ cells; left: $(3,097 \pm 74)/5 \times 10^6$ cells]. Flow cytometric analysis data showed HSCs from bone marrow cells from the damaged and contralateral intact femur on day 2 after a femoral bone defect (Fig. 1, A and B). The numbers of HSCs harvested from the bone marrow of damaged femurs were significantly lower than the numbers harvested from contralateral intact femurs on day 2 after femoral bone damage and from intact femurs prior to injury (Figs. 1C and 2A). This change in the number of HSCs in the bone marrow cells from damaged femurs occurred with time, progressing to a significant difference only at the 2-day time point (Fig. 2A).

Prevalence of MSCs in bone marrow cells after a femoral bone damage. Flow cytometric analysis was again utilized to assess the prevalence of MSCs in the bone marrow from both damaged and contralateral intact femurs after femoral bone injury. MSCs were defined as the CD29$^+$, CD44$^+$, CD45$^-$, CD73$^+$, CD105$^+$, and Lin$^-$ cell populations (CD29$^+$CD44$^+$CD45$^-$CD73$^+$CD105$^+$Lin$^-$ cells), as described in MATERIALS AND METHODS. The expression patterns of MSC markers were similar in the bone marrow cell populations harvested from both intact right and left femoral bones (data not shown). Furthermore, as observed with the HSCs, there were similar numbers of MSCs harvested from the bone marrow of both intact right and left femurs of normal C57BL/6J mice [right: $(409 \pm 27)/5 \times 10^6$ cells; left: $(415 \pm 34)/5 \times 10^6$ cells]. Flow cytometric analysis data showed MSCs from bone marrow cells from the damaged and contralateral intact femur on day 2 after a femoral bone defect (Fig. 3, A and B). In contrast to the observations of HSC populations, the numbers of MSCs harvested from the bone marrow of damaged femurs were significantly higher than the numbers of those harvested from contralateral intact femurs on day 2 after femoral bone damage and those harvested from intact femurs before injury (Figs. 2B and 3C). This change in the number of MSCs in the bone marrow cells from damaged femurs occurred with time, progressing to a significant difference only at the 2-day time point (Fig. 2B).

Stem cells in spleen cells after a femoral bone defect. Both HSCs and MSCs were present in the spleen tissues harvested from normal C57BL/6J mice. Furthermore, both the expression patterns of HSC markers and the numbers of HSCs were similar in the spleen tissues harvested from the healthy mice and those with bone damage on day 2 after the procedure (Fig. 4, A–C). Likewise, although the number of MSCs was very low

Fig. 1. Hematopoietic stem cells (HSCs) in bone marrow cells from the damaged and contralateral intact femur on day 2 after a femoral bone defect. A: flow cytometric analysis was used to identify HSCs from damaged femurs on day 2 after femoral bone damage. B: flow cytometric analysis was used to identify HSCs from damaged femurs on day 2 after femoral bone injury. C: nos. of HSCs harvested from the bone marrow of the contralateral intact (intact) and damaged (defect) femurs 2 days after femoral bone damage, as enumerated by flow cytometry. Data represent means ± SE. **$P < 0.01$. These results were obtained from experiments performed on 5 mice in each group. SSC-A, side scatter area.
in spleen cells, they were similar between the mice with bone defect and intact mice on day 2 (Fig. 4, D–F).

Transplanted GFP-positive bone marrow cells localize to osteoclasts and macrophages after femoral bone damage. Bone marrow-derived cells were traced during bone repair using the GFP-labeled bone marrow transplantation model, as described in MATERIALS AND METHODS. Bone marrow cells from C57BL/6-Tg (CAG-EGFP) mice were transplanted into C57BL/6J wild-type mice. After transplantation, femoral bone damage was induced. Bone repair was then assessed histologically on days 4 and 7 postoperation. GFP-positive hematopoietic cells were observed in bone marrow vessels at noninjury sites (data not shown), suggesting that the donor cells successfully replaced the native hematopoietic cells. Immunohistochemical staining using a GFP-specific antibody indicated that the bone marrow transplantation successfully replaced the hematopoietic cells with GFP-positive donor cells (Fig. 5). TRAP and GFP double-positive cells were observed at the damaged site on day 18 after femoral bone damage (Fig. 5A). Moreover, F4/80 and GFP double-positive cells were observed at the damaged site on day 7 after femoral bone damage (Fig. 5D). However, ALP- or Osterix-positive cells were GFP negative at the damaged site on the day 7 after femoral bone injury (Fig. 5, B and C).

Effects of AMD3100 or anti-SDF-1 antibody treatment on the numbers of stem cells present in the bone marrow after femoral bone damage. To investigate the role of SDF-1 during the bone repair process, the effects of AMD3100 and local anti-SDF-1 antibody treatments on stem cells in the bone marrow of damaged and contralateral intact femurs were assessed by flow cytometry. Intraperitoneal administration of AMD3100, a CXCR4 antagonist, blunted the observed decrease in HSC populations and increase in MSC populations in the bone marrow harvested from damaged femurs (Fig. 6, A and B). Similar results were obtained upon local treatment with an anti-SDF-1 antibody at the damaged site (Fig. 7, A and B). Moreover, the antibody treatment resulted in a significant delay in bone repair, as quantitated by quantitative computed tomography (Fig. 7, C and D).
In the present study, the numbers of HSCs harvested from the bone marrow of damaged femurs by day 2 after femoral bone damage were lower than the numbers of cells harvested from the contralateral intact femurs or from the femurs prior to injury. These data suggest that bone damage induces a decrease in the number of HSCs within the bone marrow by mobilizing these cells and recruiting them to the damaged site to facilitate bone repair. In the bone marrow, special microenvironments, known as niches, control the proliferation and differentiation of hematopoietic stem cells and progenitor cells (27). During the bone repair process, HSCs are likely mobilized from these niches to the site of injury, resulting in a decrease in the number of HSCs within the bone marrow. Since matrix-embedded osteocytes are required for the mobilization of HSCs in response to granulocyte colony-stimulating factor in mice, which involves a sympathetic nerve-related mechanism (2), various bone cells may regulate mobilization of HSCs through certain soluble factors or neurological signals.

Various cell types that are essential for bone repair, such as chondroblasts, osteoblasts, and endothelial cells, are derived from MSCs (21, 40); however, although it is assumed that the MSCs that differentiate into these cell types come from either the bone tissue, the bone marrow, or the surrounding tissues, the exact origin of these MSCs is poorly understood. In the present study, compared with the numbers of MSCs harvested from the contralateral intact femurs or from the femurs prior to femoral bone injury, the numbers of MSCs in the bone marrow samples harvested from damaged femurs by day 2 after induction of femoral bone injury were significantly higher. The onset of the bone repair process might induce the differentiation of MSCs within the bone marrow through the production of certain chemokines, cytokines, and growth factors, resulting in the observed increase in the number of MSCs within the bone marrow.
Fig. 5. Localization of green fluorescent protein (GFP)-positive and tartrate-resistant acid phosphatase (TRAP)-, alkaline phosphatase (ALP)-, Osterix-, or F4/80-positive cells at the site of femoral bone injury in C57BL/6J mice subjected to bone marrow cell transplant from C57BL/6J-Tg (CAG-EGFP) mice. 

A: microphotographs of TRAP and GFP-double positive cells at the damaged site on day 18 after femoral bone damage in C57BL/6J mice. Arrowheads indicate TRAP and GFP double-positive cells.

B: microphotographs of ALP- or GFP-positive cells at the damaged site on day 7 after femoral bone damage in C57BL/6J mice.

C: microphotographs of Osterix- or GFP-positive cells at the damaged site on day 7 after femoral bone damage in C57BL/6J mice.

D: microphotographs of F4/80 and GFP double-positive cells at the damaged site on the day 7 after femoral bone damage in C57BL/6J mice. Arrowheads indicate F4/80 and GFP double-positive cells. Scale bars, 20 μm (A–D). DAPI, 4',6-diamidino-2-phenylindole.
marrow. These findings suggest that bone damage might enhance the proportion of MSCs that commit to osteoblastic progenitor lineage within the bone marrow.

Several studies indicate that stem cells and osteoblast precursors exist in circulation (31). It is possible that bone marrow disruption due to bone injury may induce egress of MSCs and HSCs into circulation (10). Furthermore, Kumagai et al. (22) reported that circulating BMSCs are recruited to fracture calluses, although their contribution to fracture repair is limited. Meanwhile, extrinsic bone damage induces the production of certain chemokines, cytokines, and growth factors, which influence the surrounding BMSCs and niches within the endosteal region. In this study, there was a respective decrease and increase in the numbers of HSCs and MSCs in the bone marrow samples harvested from the sites of bone damage. In contrast, there were no changes in the HSC and MSC populations in the bone marrow of the contralateral undamaged sites or in the spleen cells after femoral bone injury. These data suggest that bone damage affects the number of stem cells in the neighboring bone marrow but not in the bone marrow or hematogenous tissues that are anatomically distant.

Bone marrow cells include HSCs, MSCs, and multipotent adult progenitor cells. It is generally considered that only HSCs can colonize after bone marrow transplantation. The possible flexibility of HSCs, which allows them to act as pluripotent cells, has been debated. Several studies suggest that this phe-

![Fig. 6. Effects of AMD3100 treatment on the no. of stem cells within the bone marrow after artificially induced femoral bone damage.](http://ajpendo.physiology.org/)

**A**: no. of HSCs harvested from the bone marrow of the contralateral intact (intact) and damaged femurs on day 2 after femoral bone damage (defect), with or without intraperitoneal administration of AMD3100 (5 mg/kg), as enumerated by flow cytometry. Data represent means ± SE. **P < 0.01. Results were obtained from experiments performed on 5 mice in each group.**

**B**: no. of MSCs harvested from the contralateral intact and damaged femurs on day 2 after femoral bone injury, with or without AMD3100 treatment, as counted by flow cytometry. Data represent means ± SE. *P < 0.05. These results were obtained from experiments performed on 5 mice in each group.

Fig. 7. Effects of treatment with an anti-stromal cell-derived factor-1 (SDF-1) antibody on the no. of BMCs after femoral bone damage. **A**: no. of HSCs within the BMC populations harvested from the contralateral intact (intact) and damaged femurs on day 2 after femoral bone damage (defect), with or without local treatment with an anti-SDF-1 antibody, as enumerated by flow cytometry. Data represent means ± SE. These results were obtained from experiments performed on 5 mice in each group. **B**: no. of MSCs within the BMC populations harvested from the contralateral intact and damaged femurs on day 2 after femoral bone injury, with or without local treatment with an anti-SDF-1 antibody, as counted by flow cytometry. Data represent means ± SE. These results were obtained from experiments performed on 5 mice in each group. **C**: 3-dimensional images of an injury site on days 1, 4, 7, 10, and 14 after femoral bone injury in the presence or absence of local anti-SDF-1 antibody treatment, as assessed by quantitative computed tomography (qCT). Arrowheads indicate the damaged site. **D**: quantification of the area of bone damage on days 1, 4, 7, 10, and 14 after femoral bone injury in the presence (solid line) or absence (dashed line) of local anti-SDF-1 antibody treatment, as assessed by qCT. Data represent SE. *P < 0.05; **P < 0.01. These results were obtained from experiments performed on 7 mice in each group.
nomenon might be the result of cellular fusion or microchimerism (35, 40), which negates the perception of HSC flexibility. Results obtained by Tsujigawa et al. (37) indicated that bone marrow-derived osteoclasts are involved in remodeling of the tibial epiphysis and that bone marrow-derived cells participate in maintaining the bone tissues by differentiating into hematopoietic cells and osteoblasts. In the present study, GFP-positive bone marrow cells were double stained using an osteoclast-specific TRAP stain at the injured site after femoral bone damage. These results were consistent with previous evidence that osteoclasts and hematopoietic cells were GFP positive during the bone repair process in a GFP mouse bone marrow transplantation model; however, osteoblasts or osteocytes were GFP negative in that study (37). These data suggest that bone marrow HSCs are mobilized and recruited to the site of bone damage, where they differentiate into osteoclasts and contribute to the bone-remodeling phase during bone repair.

The stem cell factor, thrombopoietin and the chemokine SDF-1 (also known as CXCL-12) are crucial for the maintenance of the HSC niche. Chemokines are chemoattractant cytokines that form chemical gradients for cell migration. The chemokine SDF-1 and its receptor CXCR4 are involved in the regulation of migration, survival, and development of multiple cell types, including bone stromal and bone marrow cells (33). SDF-1 is thought to play an important role in cell migration at fracture sites (6). In addition, SDF-1/CXCR4 signal transduction has been reported to be important for bone repair after fractures (39). Indeed, the expression level of SDF-1 is elevated within fracture sites (19, 20, 23). The present study revealed that the intraperitoneal administration of AMD3100, a CXCR4 antagonist, or local treatment with an anti-SDF-1 antibody counteracted the observed bone damage-induced decrease and increase in the prevalence of HSCs and MSCs, respectively, within the mouse bone marrow. Therefore, these results suggest that the SDF-1/CXCR4 system is involved in mediating the observed changes in mouse bone marrow stem cell populations induced during bone repair after femoral bone damage. We propose that bone damage might induce the expression of SDF-1 at the damaged site, leading to the migration of HSCs from the bone marrow and induction of MSCs within the bone marrow.

In conclusion, we detected a respective decrease and increase in the numbers of HSCs and MSCs within the bone marrow samples harvested from damaged femurs during bone repair. Furthermore, our results indicate that the SDF-1/CXCR4 system contributes to the changes in the bone marrow populations of these stem cells induced by bone injury.

GRANTS
This study was supported partly by a grant from the Nakatomi Research Foundation to N. Kawao and a Grant-in-Aid for Scientific Research (C: 24590289 and 15K08195) to H. Kaji and K. Okada from the Ministry of Education, Culture, Sports, Science, and Technology of Japan.

DISCLOSURES
The authors declare no conflicts of interest, financial or otherwise.

AUTHOR CONTRIBUTIONS

REFERENCES
SDF-1 MEDIATES CHANGES OF BMSCs IN BONE REPAIR


