Required Time for Migration of Bone Marrow–derived Cells to Dental Pulp after Bone Marrow Transplantation

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Abstract

Introduction: This study aimed to evaluate the time required for bone marrow–derived cells (BMDCs) from transgenic green fluorescent protein (GFP)+ donor mice (GFP+ mice) to migrate into the dental pulp of wild-type GFP– recipient mice (GFP– mice) by using bone marrow transplantation (BMT) as an *in vivo* model for tracking BMDCs from GFP+ mice (GFP+ BMDCs). **Methods:** GFP+ BMDCs were injected into irradiated GFP– mice. Maxillary arches, tibiae, and femora from GFP– mice were isolated and processed at 24 hours, 48 hours, 4, 7, and 14 days, and 7 weeks after BMT. Confocal laser microscopy analyses were performed to assess the presence of GFP+ BMDCs in the dental pulp, and flow cytometry of BM was performed to confirm the efficiency of engraftment of GFP+ BMDCs. **Results:** Confocal laser microscopy analyses evidenced the presence of GFP+ BMDCs in the dental pulp of GFP– mice from 14 days to 7 weeks after BMT. There was no presence of GFP+ BMDCs at 24 hours, 48 hours, 4 days, and 7 days. Flow cytometry of the BM of GFP– mice demonstrated a constant increase in the presence of GFP+ BMDCs at 24 hours, 48 hours, and 4 days after BMT, which stabilized from 7 days to 7 weeks. **Conclusions:** The study demonstrated the presence of GFP+ BMDCs in the dental pulp from 14 days to 7 weeks after BMT and the feasibility of using GFP+ animals and BMT as an *in vivo* model for tracking GFP+ BMDCs. *(J Endod 2017;■:1–8)*

Key Words

Bone marrow, bone marrow cells, bone marrow transplantation, dental pulp, dental pulp regeneration, dental pulp stem cells, green fluorescent protein, hematopoietic stem cells, mesenchymal stem cells

Significance

BMDCs can contribute to dental pulp healing/repair and regeneration. GFP+ BMDCs can migrate into the dental pulp from 14 days and be present until 7 weeks after transplantation, using GFP+ animals and BMT as an *in vivo* model.

Dental pulp responds to a variety of pathologic injuries by deposition of reparative dentin secreted by odontoblast-like cells in a suitable environment after pulp-capping and restoration (1, 2). The origin, location, and subpopulations of cells giving rise to odontoblast-like cells are not fully understood (3). Available evidence suggests that dental pulp contains different niches of potential resident stem/progenitor cells involved in reparative dentinogenesis, such as periarterial neurovascular niches (4), the cell-rich layer of Höhl (subodontoblastic layer) (3, 5), and stromal dental pulp stem cells (6). In addition to the roles of resident stem/progenitor cell populations, the possible roles and involvement of nonresident cell populations, including progenitor/stem cells from other sites such as peripheral blood (PB) and bone marrow (BM), have been cited (7, 8).

The bone marrow transplant (BMT) model has been used for studying various biological processes, including the behavior of bone marrow–derived cells (BMDCs) migrating to the site of injury from circulating blood in tissue remodeling and repair (9). BMDCs are capable of migrating to injured tissues and differentiating into tissue-specific cells to participate in the repair of pathologic processes such as liver fibrosis (10), acute radiation enteritis (11), and kidney (12) and retinal injury (13).

In the dental pulp, BMDCs have been cited as capable to migrate into normal as well as damaged dental tissues and differentiate into the dental tissue-specific cell type (7, 8, 14). Identifying the time required for the migration of BMDCs into the dental pulp after BMT is important to design strategies for future studies evaluating pulp repair/regeneration and the response of BMDCs to pulp injury.

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In this study, transplantation of green fluorescent protein (GFP)+ BMDCs collected from GFP+ mice and injected into irradiated GFP– mice was used to investigate the required time for the migration of GFP+ BMDCs into the healthy dental pulp. We found that GFP+ BMDCs can migrate to the dental pulp after 14 days and remain therein up to 7 weeks after BMT.

Materials and Methods

Animals and Animal Care

All animal procedures were performed in accordance with the ethical principles of animal research approved by the institutional Animal Research Ethics Committee (CEUA) from the State University of Campinas (UNICAMP, Campinas, SP, Brazil) under protocol no. 3289-1.

C57BL/6-TgN (ACTbEGFP) 10sb/J transgenic GFP+ donor mice and C57BL/6JUnib wild-type GFP– recipient mice were obtained from the Multidisciplinary Center for Biological Investigation into Laboratory Animal Science (CEMIB) from the State University of Campinas (UNICAMP, Campinas, SP, Brazil). All animals used in the present study were housed, supervised, and handled according to the ethics concerns on the use of animals from CEUA guidelines for care and use of laboratory animals.

Transgenic Mice

C57BL/6-TgN (ACTβEGFP) 10sb/J is a transgenic mouse in which GFP expression is under the control of a chicken beta-actin promoter and a cytomegalovirus enhancer. GFP+ is expressed uniformly in all cells except in erythrocytes; therefore, it can be used to track the fate of various cell types including BMDCs that migrate into different tissues (15). To confirm the expression of GFP+ in the dental pulp and BM cells of these transgenic animals, unstained cross sections of maxillary first molars and humeri were analyzed by confocal laser microscopy.

Bone Marrow Transplantation and Irradiation Dose

GFP+ mice were euthanized by deep anesthesia, the tibiae and femora were collected, the epiphysis and diaphysis of each bone were cut off, and GFP+ BM cells were obtained by flushing BM from the medullary cavities by using a 3-mL syringe and a 26-gauge needle containing Dulbecco modified Eagle medium (DMEM) supplemented with 1% penicillin and streptomycin. The erythrocytes were removed by treatment with 2 mL erythrocyte lysis buffer (eBioscience, San Diego, CA) in 6 mL DMEM containing GFP+ BMDCs. Lysis buffer–treated GFP+ BMDCs were resuspended in 6 mL fresh DMEM and washed twice. Then, 3×10^6 cells/mL GFP+ BMDCs were suspended in 2 mL DMEM.

GFP– mice were exposed to 12-gray (Gy) whole-body irradiation split into 2 doses within a 3-hour interval to minimize gastrointestinal toxicity, and the animals experienced no pain (16). Eight to 12 hours after irradiation, GFP– mice were anesthetized, and 100 μL 3×10^6 cells/mL GFP+ BMDCs suspension was injected into the retro-orbital plexus of the animals. To analyze morphologic changes in the BM (to confirm BM ablation) and in the dental pulp, 24 and 48 hours after BMT, hematoxylin-eosin (H&E)–stained cross sections of maxillary molar and humerus were analyzed by light microscopy.

Twelve female (12- to 16-weeks-old) GFP+ mice and 36 age-matched female GFP– mice were used (2 GFP+ mice were used as BM donors for 6 GFP– mice at each time point). Six GFP– transplanted mice were used at each time point of analysis (24 hours, 48 hours, 4, 7, and 14 days, and 7 weeks).

Flow Cytometry Analysis

To confirm the success of BMT and to quantify the presence of GFP+ BMDCs in the BM of GFP– mice, flow cytometry analysis of the BM was performed at 24 hours, 48 hours, 4, 7, and 14 days, and 7 weeks after the irradiation dose and BMT. GFP– mice were anesthetized and killed by cervical dislocation; the tibiae and femora were immediately removed and trimmed of excessive muscle tissue. BM was flushed from the medullary cavities of bones with 5 mL phosphate-buffered saline (PBS) (Life Technologies, Carlsbad, CA) by using a 3-mL syringe with a 26-gauge needle.

The BM cell suspension was filtered through a cell strainer (100 μm) to remove debris, followed by centrifugation at 3000 rpm for 5 minutes. After centrifugation, the supernatant was removed, and cells were resuspended in 2 mL ammonium chloride (ACK Lysing Buffer; Invitrogen, Grand Island, NY) and placed for 5 minutes on ice to lyse red cells. Five milliliters of PBS was added immediately, and the suspension was centrifuged at 3000 rpm for 5 minutes. After centrifugation, the supernatant was removed, and the cells were resuspended in 5 mL PBS. Then 3×10^6 cells/mL were used for analysis with a BD-LSR II multicolor analyzer (Becton Dickinson, San Jose, CA). The data were processed by using FlowJo 7.6 software (Tree Star Inc, Ashland, OR).

Tissue Isolation and Confocal Analysis

To analyze the presence of GFP+ BMDCs into the dental pulp of GFP– mice, the animals were anesthetized and euthanized by intracardiac perfusion with 10% buffered formalin 24 hours, 48 hours, 4, 7, and 14 days, and 7 weeks after BMT. The maxillary arches were isolated, cleaned from soft tissue, trimmed, and fixed in 10% formalin solution for additional 24 hours. The samples were decalcified for 7 days in 15% EDTA (pH, 7.5) at 4°C, dehydrated through ascending grades of ethanol and xylene, and embedded in paraffin. Serial cross sections of 6 μm were placed onto ProbeOn Plus slides (Fisher Scientific, Pittsburgh, PA), deparaffinized, rehydrated, washed in distilled water, and mounted with glycerol/PBS (50%:50%). The fluorescence signal was examined in at least 20 sections from each tooth by using a Zeiss LSM 780-NLO confocal scan head under an Axio Observer Z.1 microscope (Carl Zeiss AG, Oberkochen, Germany). Images were collected by using 488-nm to 543-nm laser lines for excitation and 498- to 542-nm and 647- to 690-nm emission filters for GFP fluorophores and autofluorescence, respectively; the images were overlaid to eliminate tissue autofluorescence. The pinholes were set to 1 airy unit for each channel and to a 1024 $\times$ 1024 image format. After confocal analysis, the same sections of dental pulp were washed in PBS, processed for H&E staining by using standard protocols, and analyzed by light microscopy for assessment of the morphology of the analyzed tissue. Six mice at each time point were analyzed after 24 hours, 48 hours, 4, 7, and 14 days, and 7 weeks after BMT.

Results

GFP Expression in Transgenic Mice

Confocal analysis of the unstained section of molars and humeri from C57BL/6TgN (ACTβEGFP) 10sb/J GFP+ transgenic donor mice showed high GFP+ expression in the dental pulp cells and in the entire layer of a fully differentiated odontoblast extending to the odontoblastic process (Fig. 1*A*). GFP+ signal was also expressed in several mononuclear and polymorphonuclear cells and in megakaryocytes in the BM (Fig. 1*B*). These observations confirm the feasibility of using C57BL/6-TgN (ACTbEGFP) 10sb/J transgenic GFP+ mice as donors for tracking GFP+ BMDCs in the dental pulp. C57BL/6JUnib wild-type