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The bone regeneration using bone marrow-derived mesenchymal stem cell with recombinant human bone morphogenetic protein-2 in allogeneic repair model of femoral segmental defect of rats

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Abstract

Treatment of a large bone defect is a difficult problem especially in modern aging society. Mesenchymal stem cells (MSCs) obtained from bone marrow are a suitable resource for bone regeneration. We performed a transplant of MSCs along with recombinant human bone morphogenetic protein 2 (rhBMP-2) in combination with an allogeneic bone marrow stem cell transplant in a rat model of a femoral segmental defect and observed sufficient bone formation. A single dose or 2-week administration of an immunosuppressive drug was necessary to ensure successful bone formation by the allogeneic stem cell transplant. Equivalent bone formation was attained in all groups 6 weeks after the transplant. As for the persistent administration of the immunosuppressive drug, it was found to be unnecessary because almost all transplanted allogeneic stem cells were absorbed during 8 weeks after the transplant.

Key words : mesenchymal stem cell, MSC, rhBMP-2, bone regeneration, allogeneic transplant, immunosuppressive drug

Introduction

In modern aging society, treatment of a bone defect, such as large skeletal defects due to trauma, tumor-wide resection, infection, or skeletal development, is often required. In many cases, many autologous bone transplants are still frequently performed in clinical practice, but often it is especially difficult to obtain enough bone of sufficient quality for a transplant, especially in the elderly. Furthermore, we cannot ignore the damage to normal bone tissue and the risk of infection at the transplant site. Consequently, different treatment options for bone regeneration are desirable[1)−3)].

Generally, three characteristics are necessary for bone regeneration. First, stem cells or osteoblasts, which can cause bone formation directly, must show osteogenicity. Fresh autologous bone and bone marrow cells fulfill this requirement. Second, artificial bones, such as decalcified bone, hydroxyapatite, and calcium phosphate, play a role in osteoconduction by promoting the growth of bone passively. The third characteristic is osteoinduction, which is needed to differentiate mesenchymal stem cells (MSCs) into bone and cartilage, and cytokines such as bone morphogenetic protein (BMP), fibroblast growth factor (FGF), and vascular endothelial growth factor (VEGF), have effects consistent with osteoinduction[4)−6)].

Stem cells have a strong potential for self-proliferation and multi-differentiation potency. It is

reported that MSCs in bone marrow can differentiate not only into bone tissue but also cartilage, fat, nerve cells, vascular endothelial cells, or hepatocytes[7)−13)]. Recently, embryonic stem cells and induced pluripotent stem (iPS) cells have also been shown to have strong capacity for tissue regeneration. Regenerative-medicine methods involving a self-organizing transplant of iPS cells are now possible; however, because of medical economic problems, it is realistic to prepare some iPS cell clones that cover the variety of HLA by ～80% by means of an existing cell line in an iPS cell bank[14), 15)]. On the other hand, osteogenic capability of MSCs is reported to be equal to that of osteo-induced iPS cells[16)]. Thus, bone marrow-derived MSCs are an attractive resource for clinical bone regeneration owing to their high osteogenic capacity[17)]. Nonetheless, in cases of aged bone, a systemic bone disease, or myelofibrosis, autologous bone marrow is damaged by radiotherapy or chemotherapy; therefore, autologous bone marrow cell culture may become problematic. In this case, an allogeneic MSC transplant is the method that can be used as a substitute for the autologous one. For this approach, an immunosuppressive drug, such as FK506, cyclosporin A, or rapamycin, is necessary to minimize antigenicity of the allograft[18)−20)]. We have previously shown in a rat model of a femoral defect that allogeneic engineered MSCs yield good bone formation after a transplant if immunosuppressant FK506 is used[21)]. FK506 was administered for 3 weeks after the transplant; however, there are few reports regarding sufficient periods and appropriate doses of FK506 for bone regeneration.

Many cytokines are known to induce MSCs to differentiate into osteocytes, chondrocytes, and other lineages. BMPs perform multiple functions during development and tissue homeostasis, including regulation of bone homeostasis[22)]. It has been well documented that BMPs can promote osteoblastic differentiation of MSCs. Several reports have revealed that recombinant human BMP-2 (rhBMP-2) enhances bone regeneration in laboratory animals[23)−27)], and clinical application to humans has been reported[28)]. Despite the low efficiency of production of rhBMP-2, a large amount of expensive rhBMP-2 is necessary to ensure sufficient bone formation. It is important to achieve sufficient bone formation using a small amount of rhBMP-2[29), 30)].

Table 1. Experimental design.
Allogeneic recipients were subdivided into two groups (A and B); group C is a syngeneic model.

	Donor rat (male)	Recipient rat (female)	FK506	
			Dose	Period
Group A (n=20)	Lewis	Brown Norway	1 mg/kg body weight	every day for 1 week and every other day for 1 week
Group B (n=20)	Lewis	Brown Norway	10 mg/kg body weight	single injection (operative day)
Group C (n=8)	Lewis	Lewis	(-)	(-)

Herein, we performed a transplant of MSCs with rhBMP-2 for bone repair in a rat model of a femoral segmental defect, to demonstrate the contribution of MSCs during bone regeneration with a small amount of rhBMP-2. Furthermore, we examined the difference in bone regeneration between the allogeneic group and syngeneic group by changing the regimen of administration of FK506.

Materials and Methods

A rat model of a femoral segmental defect was used in this study. This study's protocol was approved by the Animal Experiment Committee of Yamagata University Faculty of Medicine, and rats were maintained in a laboratory at the Animal Facility of Yamagata University in accordance with the "Guideline for Experiments Using Laboratory Animals at Yamagata University."

Experimental design

Inbred Lewis ($RT1^l$) and Brown Norway ($RT1^n$) rats served as donors or recipients. These strains strongly differ in histocompatibility antigens[31)]. Lewis rats (males, 4 weeks old, Charles River, Japan) were used as donors of bone marrow-derived MSCs. Brown Norway rats (females, 15 weeks old, Charles River) served as recipients (allogeneic model). The allogeneic recipients were subdivided into two groups (Table 1). Group A was allogeneic recipients with FK506 treatment (Astellas Pharma Inc., Japan) for 2 weeks, and group B comprised allogeneic recipients with a single dose of FK506. As a control (group C), Lewis

rats (females, 15 weeks old, Charles River) served as recipients (syngeneic model). Intramuscular injection of FK506 (1 mg/[kg body weight]) was performed every day after the surgical procedure in group A for 1 week followed by administration on alternate days for the next 1 week. In group B, a single dose (10 mg/[kg body weight]) was administered immediately after the operation. FK506 was injected into a nonsurgical site of the rats in groups A and B and was not given to group C.

MSC isolation and culture

Male Lewis rats ($RT1^l$) were euthanized by pentobarbital overdose. MSCs were harvested from bone marrow of bilateral femurs. The femoral bone marrow tissue was flushed out using 10 ml of the Minimum Essential Medium Eagle, alpha modification (α-MEM, Gibco, Gaithersburg, MD, USA) containing 10% of heat-inactivated fetal bovine serum (FBS; Gibco) and antibiotics (penicillin 100 U/ml, streptomycin 100 μg/ml). After elimination of soft tissue and bone tips, cells were seeded in a 100-mm dish and cultured at 5% CO_2 and 37°C for 2 weeks. After cell density reached 70% confluence, we harvested the cells with 0.05% trypsin (Gibco) and 0.02% EDTA and subcultured them[21), 32)]. A total of 8 x 10^6 cells obtained after the second passage of culture were collected and mixed with 2 ml of 3.0 mg/ml type I collagen gel (Vitrogen 100, Collagen Corp., Alto, CA, USA). After three-dimensional (3D) culturing in 12-well plates, we added rhBMP-2 into the culture medium (6 μg per well) and prepared an MSC-collagen mixture. We conducted 3D culture under conditions of 5% CO_2 at 37°C overnight, and the MSC-collagen mixture shrunk and was used for the surgical procedure the next day.

Implantation of MSCs into the femoral-segmental-defect site

The model of a femoral bone defect was surgically created in rat right femurs. Briefly, rats were anesthetized with ketamine (6 mg per 100 g of body weight) and medetomidine hydrochloride (0.04 mg per 100 g of body weight). A 23-mm high-density polyethylene fixture plate (Hospital for Special Surgery, New York, USA) was placed onto the anterior side of the thigh bone, fastened with a screw, and fixed with a wire. A 6-mm defect was made on the femoral diaphysis of recipient rats, and the MSC-collagen mix (8 x 10^6 cells) was transplanted into the defect, after a high-density polyethylene plate was attached to the lateral aspect of a recipient's femur. We sutured muscles thoroughly with 4-0 nylon so that the MSC-collagen mixture did not leak.

Radiographic examination

Serial radiographs of a rat femur (five rats from groups A and B and two rats from group C) were examined 2, 4, 6, and 8 weeks after the cell implantation. Each of these rats was anesthetized by intraperitoneal administration of ketamine hydrochloride (6 mg per 100 g of body weight) and hydrochloric acid medetomidine (0.04 mg per 100 g of body weight), and we fixed a hind leg in an externally rotated position and imaged it under conditions of 60 kV, 3 mA, for 30 seconds (Softex CMB-2 type, Softex Co., Ltd., Kanagawa). The magnitude of new bone formation was scored on a 6-point scale. This scale evaluates the size of a bone shadow in the bone defect area as follows[32), 33)]: no bone shadow was detected, 0 points; under 25%, 1 point; 26-50%, 2 points; 51-75%, 3 points; 76-99%, 4 points; and 100%, 5 points. Moreover, bone union was defined as at least 25% osseous bridging of two ends of the defect.

Histological examination

On days 2, 4, and 6 and 8 weeks after the transplant, the operated femur was excised and decalcified. Briefly, the femurs of five rats from groups A and B and two rats from group C were fixed by perfusion of 4% paraformaldehyde for 48 hours, after removal of the plate and metal after fixation, followed by decalcification with 14% EDTA (pH 7.2) for 2 weeks. Microscopic evaluation was performed on a paraffin-embedded section stained with hematoxylin and eosin and with Safranin-O.

Fluorescence in situ hybridization (FISH)

FISH analysis was performed on paraffin-embedded histological sections of the whole operated femur. A rat Y chromosome probe (Y-probe) in a plasmid was kindly provided by Dr. Barbara Hoebee (National

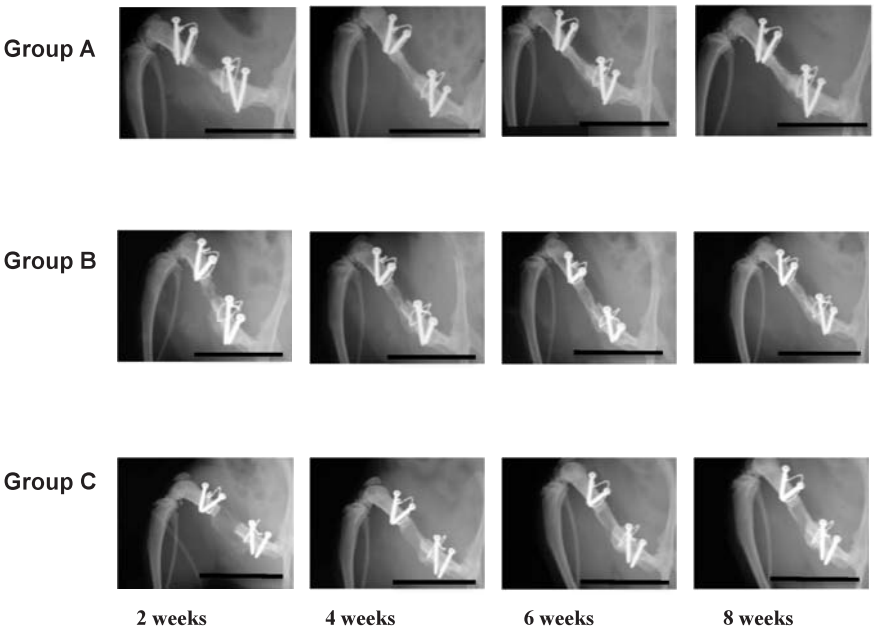

Fig. 1. Radiographic findings.
A rat model of a femoral segmental defect was set up, and then each rat received an implant of bone marrow-derived MSCs as described in Table 1: Groups A and B received allogeneic transplants; Group C received a syngeneic transplant. Group A was injected with FK506 (1 mg/kg) every day for 1 week and then every other day for 1 week, whereas group B was injected with FK506 only once (10 mg/kg) on the day of the operation. Two, 4, 6, and 8 weeks after the cell implantation, radiographs of rat femur were obtained (scale bar = 20 mm).

Institute of Public Health and Environment Protection, Netherlands). The probe was labeled with digoxigenin (dig) by nick translation, then incubated with the pretreated bone samples[34]. Probe hybridization was allowed to proceed overnight at 37°C. Hybridized slides were stained with a rhodamine-labeled anti-dig antibody and counterstained with 4',6-diamine-2-phenylindole dihydrochloride (DAPI, Fisher Scientific Company, Pittsburgh, PA, USA). The hybridization signals in 100 non-overlapping nuclei were counted under a fluorescence microscope. As a positive control, FISH was performed on a femur specimen of a Lewis male rat that did not undergo the surgical procedure, and we determined a proportion (labeling efficiency) of FISH-positive cells. The labeling efficiency in the positive control was 68.0% on average. The proportion of FISH-positive cells in each section (transplanted cells' survival rate) was adjusted using the labeling efficiency.

Statistics

Data are presented as mean ± standard deviation (SD). Differences among groups were subjected to one-way analysis of variance (ANOVA) or unpaired Student's t test. Data analysis was performed in the R commander software (version 2.3-0). Differences with $P < 0.05$ were considered significant.

Results

Radiographic findings

Bone formation was observed in all three groups (Fig. 1). In the bone defect, a shadow equivalent to a callus around the proximal femur appeared after the transplant in three groups within 2 weeks. The bone defect site showed continuity 4 weeks after the transplant, whereas bone did so 6–8 weeks after the transplant. All three groups developed clear bridging with cortical bone. The 6-point scale evaluation showed that less pronounced bone formation occurred in group B (3.4, 3.6) than in group A (3.8, 4.2) or group C (4.0, 4.5) 2 and 4 weeks after the transplant, but 8 weeks after the operation, approximately similar bone formation was observed (group A: 4.6,

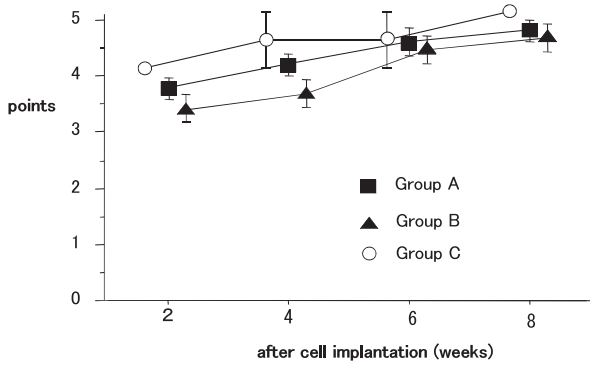

Fig. 2. Radiographic analysis on a 6-point scale.
The rat model of a femoral segmental defect was implemented, and then each rat received an implant of the bone marrow-derived MSCs as described in Table 1: Groups A and B received allogeneic transplants; Group C received a syngeneic transplant. Group A was injected with FK506 (1 mg/kg) every day for 1 week and then every other day for 1 week, whereas group B was injected with FK506 only once (10 mg/kg) on the day of the operation. Bone regeneration in the rats was examined by radiography and evaluated on the 6-point scale. There was no statistically significant difference among the three groups (ANOVA).

group B: 4.6, and group C: 5.0; Fig. 2). There were no statistically significant differences among the three groups.

Histological findings

Two weeks after the operation, the histological evaluation by hematoxylin and eosin staining revealed that the defect was filled with woven bone and newly formed microvessels around the transplanted tissue. The osseous continuity was not found in the defect area, and fibrous tissue intervened. In group B, fibrous tissue to be found in woven bone was more prevalent in the tissue sample 2 weeks after the surgical procedure as compared to groups A and C. The defective part of the bone began to connect with lamellar bone 4 weeks after the surgical procedure. A continuous bone cortex was seen 6 weeks after the operation, and medullary cavity was noted in the healed defect area after 8 weeks. Immune reaction such as accumulation of lymphocytes was not observed (Fig. 3a–c). All the groups tested negative for Safranin-O staining (data not shown).

FISH analysis

To evaluate the survival period and transplanted cells, FISH analysis was conducted. The specificity of the rat Y-probe in the FISH assay was confirmed in control sections of a male rat. The signal was found as a single red spot in nuclei, and the color reaction was absent in female cells. The detection efficiency was found to be 66.3% ± 3.1% in male positive-control samples (Fig. 4a). In the experimental samples, the signals were detected as staining signals in the cells encapsulated by a mineralized matrix, residing within the bone marrow and around the bone matrix.

Within the bone marrow site, 2 weeks after the surgical procedure, the transplanted cells' survival rate was 41.1% in group A and 41.3% in group B. There was no significant difference between groups A and B. Nevertheless, 4 weeks after the surgical procedure, the rate was 25.7% in group A and 21.6% in group B, and transplanted cells survived much more in group A than in group B at 4 weeks after the surgical procedure ($P < 0.05$). The survival rate was 21.8% in group A and 19.0% in group B at 6 weeks after the surgical procedure, and 0.5% and 0.5%, respectively, 8 weeks after the surgical procedure, but at both time points, there was no significant difference between groups A and B.

Around the bone matrix, 2 weeks after the surgical procedure, the transplanted cells' survival rate was 51.2% in group A and 51.7% in group B. Four weeks after the surgical procedure, it was 43.4% in group A and 40.7% in group B. There was no significant difference between groups A and B at 2 and 4 weeks after the surgical procedure. In contrast, 6 weeks after the surgical procedure, the survival rate was 29.8% in group A and 21.7% in group B, and the transplanted cells survived much better in group A than in group B at 6 weeks after the surgical procedure ($P < 0.05$). Eight weeks after the surgical procedure, the survival rate in groups A and B was 0.8% and 0.5%, respectively, but the difference was not significant. In group C, the survival rate of donor cells was 80.5% in 2 weeks, 68.6% in 4 weeks, 34.1% after 6 weeks, and 18.7% in 8 weeks.

Donor cells showed a lower survival rate in group B than in group A at 4 weeks after the operation in the bone marrow area and 6 weeks after the operation in

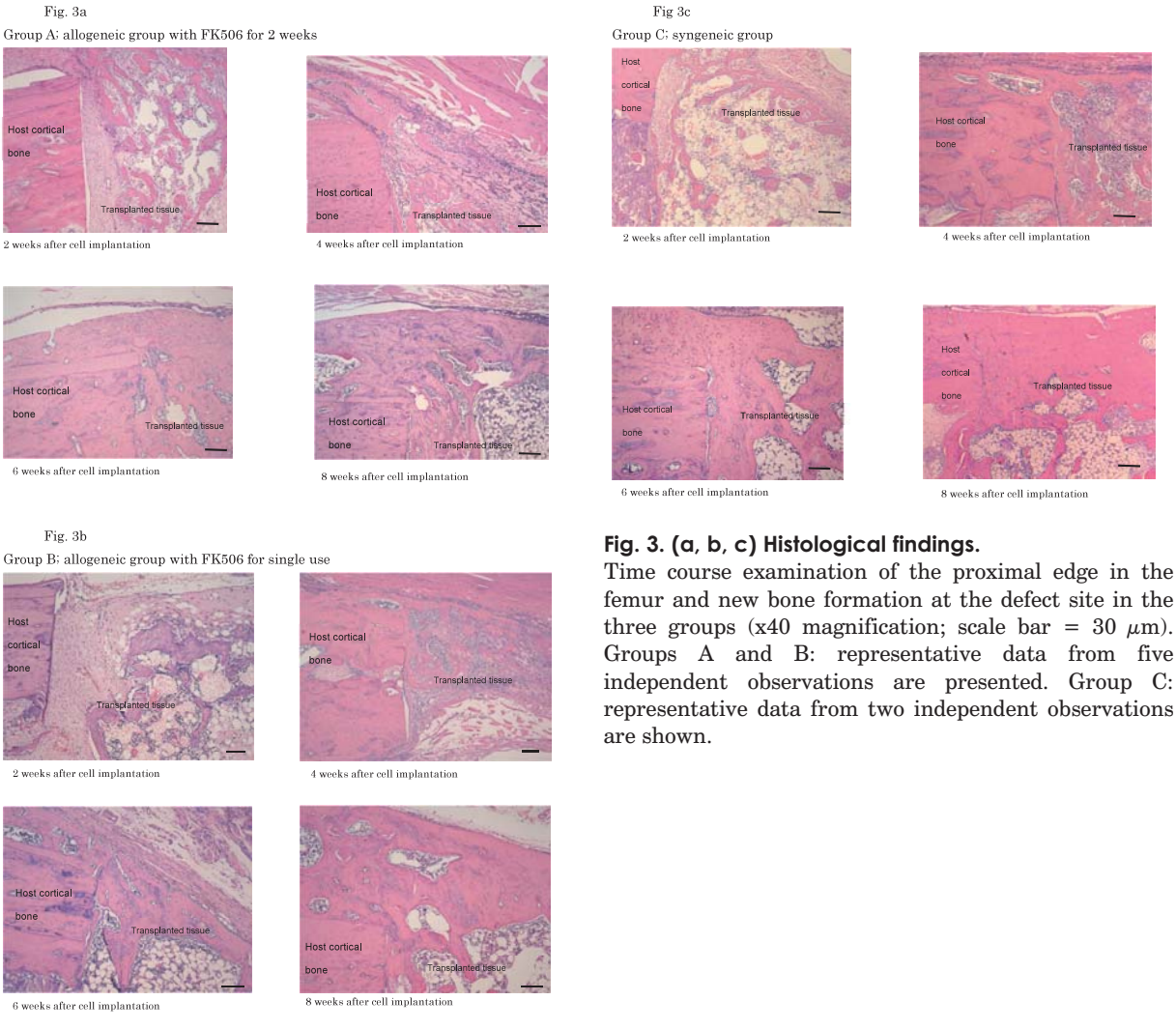

Fig. 3. (a, b, c) Histological findings.
Time course examination of the proximal edge in the femur and new bone formation at the defect site in the three groups (x40 magnification; scale bar = 30 μm). Groups A and B: representative data from five independent observations are presented. Group C: representative data from two independent observations are shown.

the bone matrix. Eight weeks after the transplant, no donor cells were detected in any group on the border of host bone marrow and new bone, except for group C, and ~20% of cells in the bone matrix were donor cells (Fig. 4b).

Discussion

The elderly population increases year by year, and pseudarthrosis and/or nonunion after a fracture, with a bone defect at the surgical site, increase in prevalence[1), 6), 16)]. Treatment of the defective part of bone often requires an autologous bone transplant; however, in the elderly with osteoporosis, autologous bone tissue of good quality is often insufficient. As for the cases of low potency of bone regeneration and a large bone defect, there have been many reports on the use of rhBMP-2, and MSCs are used for bone regeneration[4), 8)–10), 28), 32)].

In various studies, there are reports on bone formation under the influence of implanted rhBMP-2 at a defect site in a bone, subcutis, or muscle. Yasko *et al*. showed that when they infiltrated rhBMP-2 into decalcified bones in a rat model of a thigh bone defect, 11.0 μg of rhBMP-2 induced enough bone formation[25)]. Fujimura *et al*. reported that 2.0 μg of rhBMP-2 used with FGF in a rat model of subcutaneous implantation induced bone formation successfully[27)]. Barnes *et al*. implanted 2.0 mg of rhBMP-2 into a monkey model of spinal fusion and achieved spinal bone union[35)]. A large quantity of rhBMP-2 is required for clinical use[36), 37)]. Because of the use of a small quantity of rhBMP-2 to obtain effective bony formation, a combination of a cell

Fig.4a

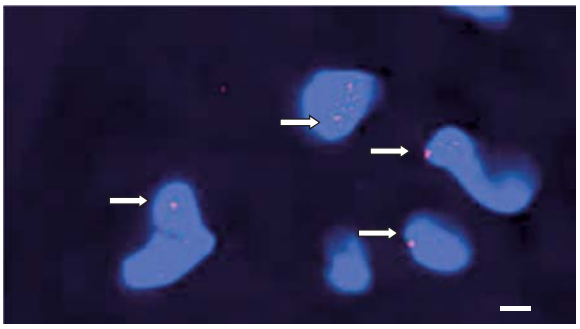

Fig.4b

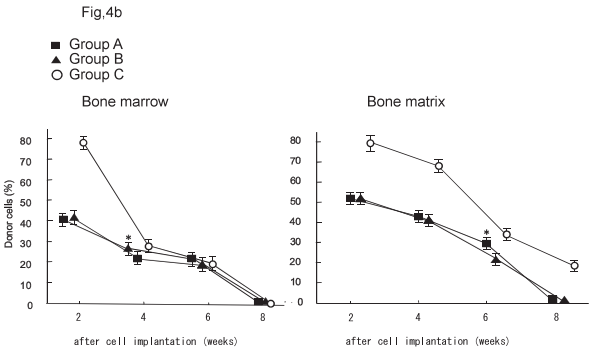

Fig. 4. Survival rates of transplanted cells.
The rat model of a femoral segmental defect was set up, and then each rat received an implant of bone marrow-derived MSCs as described in Table 1. The rats were euthanized 2, 4, 6, and 8 weeks after the implantation, and the donor cells (from male rats) were detected by FISH with a Y chromosome probe. (a) Representative FISH-positive cells. The Y-probe-positive cells (white arrows) were detected in newly formed bone in the defect area (x400 magnification; scale bar = 5 μm). (b) The percentages of donor cells. The surviving donor cells in the area of bone marrow (left panel) or bone matrix (right-hand panel) were quantified by microscopic analysis. *$P < 0.05$, comparison of groups A and B by unpaired Student's t test (n = 5).

transplant and development of a carrier was reported[35)−37)]. In those studies, usefulness of MSCs is described as one of the transplanted cell types. MSCs are present in many tissues including bone marrow, muscle, fat, and blood, and rhBMP-2 stimulates MSCs to multiply and differentiate, then induces bone formation. In this study, we tested whether bone formation occurs after administration of a small dose of rhBMP-2 during treatment with MSCs, and we observed enough bone formation with 6 μg of rhBMP-2; this is approximately half of the dose reported by Yasko *et al*.[25)].

Bone marrow-derived MSCs have often been used for the treatment of a bone defect site[16), 17), 32), 33), 38)]. In contrast, differentiation potency and cell activity of MSCs are more likely to be insufficient for a treatment designed to promote bone formation. When sampling of enough MSCs from the patients is difficult, an allogeneic cell transplant seems to be effective. In various articles about bone regeneration using MSCs, most studies involve a syngeneic cell transplant, but the research on allogeneic stem cell transplants is limited. The use of an immunosuppressive drug is required during bone regeneration after an allogeneic cell transplant. Tsuchida *et al*. demonstrated repair in a rat model of a femoral segmental defect using allogeneic MSCs that carried the BMP-2 gene introduced by means of an adenovirus[21)]. As an immunosuppressive drug, FK506 was injected intramuscularly for 3 weeks after the surgical procedure, and sufficient bone formation was achieved by grafting allogeneic MSCs, but the problem with safety of adenoviruses was not solved. Therefore, we tried to accomplish bone formation without the use of a virus: by means of rhBMP-2. We inhibited antigenicity of the allogeneic MSCs without the use of a virus and verified whether bone formation was achieved with a single dose of the immunosuppressive drug. In group B, FK506 was given in a single dose, and bone formation quantity tended to be scarce 2−4 weeks after the operation in comparison with group A, which received FK506 for 2 weeks after the surgical procedure. Nevertheless, sufficient bone formation was achieved in group B after 6−8 weeks as effectively as in group A. Furthermore, continuity of cortical bone, and trabecular formation were detected by the histological analysis 6 weeks after the surgical procedure in group B. Thus, we were able to achieve sufficient bone formation in the MSC model of an allogeneic transplant when we used a single dose of FK506 at 10 mg per kilogram of body weight. Furthermore, it is reported that MSCs have immunosuppressive effects. It has been shown that MSCs reduce the incidence and severity of graft versus host disease (GVHD) after an allogeneic transplant[39), 40)]. In the present study, the

use of a single dose of FK506 had the immunosuppressive effects.

When MSCs are transplanted, it is not yet obvious what kind of roles donor cells and recipient cells play in bone regeneration. Goshima *et al*. published an experiment where they transplanted bone marrow cells from quail into a nude mouse; bone formation due to donor cells occurred 3–4 weeks after the transplant, and the bone remodeling due to recipient cells progressed 8–12 weeks after the transplant[38]. In the present study, there was less pronounced bone formation in group B than in groups A and C at 2 and 4 weeks after the surgical procedure, but bone formation was almost equal 6 and 8 weeks after the operation. Therefore, donor cells were greatly involved in bone regeneration after the early phase of the transplant, but it appears that recipient cells activated the bone metabolic cycle several weeks later. Moreover, based on the examination of transplanted cells by FISH in group B, there was no significant difference from group A at 2 weeks after the surgical procedure in terms of the transplanted cells' survival rate in the marrow, but this rate was lower than that in group A at 4 weeks after the operation. If donor cells could sustain some number of MSCs and cellular activity until 2 weeks after the surgical procedure, then bone regeneration would be possible.

As for immunosuppressive effects of FK506, the half-life of the drug is 7.5–16.9 hours in a mouse body. It seemed difficult to assess the effectiveness of the single dose of this immunosuppressive drug even 2 weeks after the surgical procedure. Because there was a higher concentration of FK506 just after the transplant in group B (10 mg/[kg body weight]) than in group A (1 mg/[kg body weight]), many transplanted cells appear to have survived. There was poor survival of transplanted cells immediately after the transplant in group A, but the 2-week dosing period of FK506 may decrease the number of surviving transplanted cells slowly as compared with group B. Finally, the transplanted cells' survival rate became equivalent in groups A and B 4–6 weeks after the grafting. Furthermore, the survival rate of transplanted cells was higher in group A than in group B in the cortical bone for 6 weeks after the transplant and in the marrow for 4 weeks. This effect seems to be caused by the following: rebuilding of the blood circulation in bone marrow took place earlier than that in cortical bone, and the survival rate of transplanted cells was high.

The functions of transplanted cells in bone regeneration include "autocrine" differentiation directly into bone cells, and "paracrine" roles: the release of cytokines and growth factors and repair of the environment. In this study, no group showed Safranin-O staining of the cartilage matrix. Therefore, the adequate bone formation was not caused by cartilage ossification, and MSCs differentiated into bone cells directly, otherwise, membranous ossification of the recipients may have occurred. In addition, the examination by FISH revealed that the transplanted cells' survival rate in cortical bone 6 weeks after the surgical procedure was 29.8% in group A and 21.7% in group B; hardly any cells survived (0.5% rate) in both groups at 8 postoperative weeks. The bone regeneration 8 weeks after the surgical procedure was hard to evaluate with transplanted cells differentiating directly into osteocytes, but MSCs derived from the recipient seemed to differentiate into osteocytes. In contrast, the transplanted cells' survival rate in the cortical bone was 52.2% in group A and 51.7% in group B at 2 weeks after the surgical procedure. Therefore, at the early transplant stage, the transplanted cells were strongly associated with bone regeneration.

In this study, it was confirmed that bone formation under the influence of rhBMP-2 increased after coadministration with MSCs. It is expected that the effect will decrease if there is an insufficient number of MSCs for the transplant, but determination of the suitable cell count for clinical application is a task for a future study. In addition, bone regeneration was achieved with a single dose of the immunosuppressive drug in this allogeneic model, but it is necessary to explore safer treatment regimens. Besides, after examining the peripheral blood of patients with dysraphism of the long bone, Zimmermann *et al*. demonstrated that BMP-2.4 is not detectable[41]. Regarding the treatment of a large bone defect, the development of a treatment with a tested systemic growth factor and biological therapeutics in combination with a cell transplant is expected. If bone

rebuilding in elderly people runs into the difficulty with autologous cell culture, or in the case of a huge bone defect, e.g., long-range reconstruction of spinal columns, allogeneic MSC grafting seems to show an effect clinically.

On the other hand, transplanted MSCs can induce immune tolerance[39), 40)]. In the present study, when rats were treated with a single dose of FK506, there was a ～6-week period when the immune tolerance responses against alloantigens were possible. Furthermore, the cells eventually disappeared after 8 weeks. Thus, implantation of MSCs may be an ideal system of induction of immune tolerance to alloantigens.

It is thought that regenerative-medicine methods involving iPS cells will change future medical care dramatically. Nevertheless, the treatment with iPS cells derived only from self is difficult because of a medical economic problem; researchers will use an organization created by an iPS bank. In that case, the method of choice will be an allogeneic transplant. According to this study and another study, MSCs have an immunosuppressive effect. In the near future, the success rate of various methods of regenerative medicine may increase because of adaptation of MSCs derived from iPS cells along with iPS cells from reproductive health organizations.

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Disclosure

The authors have no disclosures.

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