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Adipose tissue-derived stem cells for cell therapy of airway allergic diseases in mouse

Hyeong-Jun Jang¹, Kyu-Sup Cho¹, Hee-Young Park, Hwan-Jung Roh^{*}

Department of Otorhinolaryngology and Medical Research Institute, Pusan National University School of Medicine, Busan, South Korea

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ABSTRACT

The purpose of this study was to compare murine mesenchymal stem cells (MSCs) isolated from bone marrow (BM) and adipose tissue (AT) for the selection of suitable MSCs in cell therapy of an airway allergic animal model. We compared MSCs of BALB/c mice derived from BM and AT with respect to proliferation potential, immunophenotype, and multilineage differentiation capacity. In proliferation potential, MSCs from AT (ASCs) showed higher fibroblastoid colony-forming units frequencies and colony-forming efficiency than MSCs from BM (BMSCs). The flow cytometry analysis showed that both ASCs and BMSCs expressed MSCs-related antigens (CD90 and CD105), whereas they did not express hematopoiesis-related antigens (CD45 and CD11b). There was no significant difference in adipogenic, osteogenic, and chondrogenic differentiation between the murine ASCs and BMSCs. In conclusion, the present study has shown that ASCs had higher CFU-F frequencies and colony-forming efficiency than BMSCs. ASCs and BMSCs presented a similar surface immunophenotype and multilineage differentiation capacity. Therefore, ASCs in BALB/c mice might be a more useful material for cell therapy of the airway allergic experiment due to the abundance, relatively easy harvesting and high proliferation potential.

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Introduction

Mesenchymal stem cells (MSCs) found in many adult tissues are multipotent cells able to self-renew and differentiate into multiple lineages including bone (Delorme and Charbord, 2007; Liu et al., 2007; Titorencu et al., 2007), fat (Delorme and Charbord, 2007; Liu et al., 2007), cartilage (Delorme and Charbord, 2007; Liu et al., 2007), and muscle (Bhagavati and Xu, 2004; Delorme and Charbord, 2007) in the appropriate microenvironment. MSCs may be useful in the repair or regeneration of damaged tissues in *Osteogenesis imperfecta*, myocardial infarction, injured lung, and stroke (Horwitz et al., 2001; Li et al., 2002; Shake et al., 2002; Rojas et al., 2005). In addition, MSCs possess immunomodulatory properties with therapeutic potential to prevent graft-versus-host disease in allogeneic hematopoietic cell transplantation (Le Blanc et al., 2004). Recently we demonstrated that MSCs also can ameliorate experimental airway allergic diseases such as allergic rhinitis and asthma (Cho et al., 2009; Cho and Roh, 2009).

MSCs isolated and cultured from bone marrow (BM) (BMSCs) and adipose tissue (AT) (ASCs) appear to be morphologically similar

and express very similar cell-surface markers (Musina et al., 2005; Kern et al., 2006; Bieback et al., 2008; Peng et al., 2008). However, recent studies have demonstrated that MSCs isolated from several different sources are not a homogenous population and that their differentiation potential may vary depending on the sources and the species (Im et al., 2005; Yoshimura et al., 2007; Hayashi et al., 2008; Koga et al., 2008). In a comparison of human MSCs isolated from BM, umbilical cord blood, and AT, all three sources presented a similar capacity for chondrogenic and osteogenic differentiation however, they differed in their adipogenic potential (Rebelatto et al., 2008). It has been shown that major differences were observable in the frequencies of colony and their expansion potential, although there are no significant differences concerning the morphology and immune phenotype of human MSCs derived from various sources (Bieback et al., 2008). In the study of MSCs derived from BM, cartilage, and AT in the rat, ASCs showed the highest proliferation potential, followed by MSCs derived from BM and cartilage and MSCs from three sources displayed osteogenic, adipogenic, and chondrogenic differentiation potential (Peng et al., 2008).

The mouse is a suitable experimental model to study the immune modulating effect of MSCs in an airway allergic animal model. Although the selection of ideal murine MSCs is apparently crucial for the outcome of *in vivo* airway allergic experiments with MSCs, there are no reports comparing the properties of MSCs according to tissue sources in mice, especially BM and AT.

^{*} Corresponding author at: Department of Otorhinolaryngology, Pusan National University Yangsan Hospital, Beomeo-ri, Mul-geum eup, Yang-san Si, Gyeongsangnam-Do, 626-770, South Korea.

E-mail address: rohjh@pusan.ac.kr (H.-J. Roh).

¹ These authors contributed equally to the content of this article.

The purpose of this study was to compare murine MSCs isolated from BM and AT for the selection of suitable MSCs in cell therapy of an airway allergic animal model.

Materials and methods

Animals

Eight-week-old female BALB/c mice bred in the specific pathogen free animal facility were used. The animal study protocol was approved by the Institutional Animal Care and Use Committee of the Pusan National University School of Medicine.

Isolation and culture of MSCs

AT was obtained from abdominal fat of BALB/c mice. Because the amount of stromal cells obtained from AT and BM of each mouse is too small, AT and BM were collected from ten BALB/c mice. To isolate mouse AT stromal cells, adipose tissue was washed extensively with an equal volume of phosphate-buffered saline (PBS), and digested with 0.075% collagenase type I (Sigma–Aldrich, St. Louis, MO) at 37 °C for 30 min. Enzyme activity was neutralized with α -modified Eagle's medium (α -MEM, Sigma–Aldrich, St. Louis, MO) containing 10% fetal bovine serum (FBS) and centrifuged at 1200 $\times$ g for 10 min to obtain a pellet. The pellet was filtered through a 100 μ m nylon mesh to remove cellular debris and incubated overnight at 37 °C/5% CO₂ in control medium (α -MEM, 10% FBS, 100 unit/ml of penicillin, 100 μ g/ml of streptomycin). Following incubation, the plate was washed extensively with PBS to remove residual non-adherent red blood cells. The resulting cell population was maintained at 37 °C/5% CO₂ in control media.

BM was collected from femurs and tibias of BALB/c mice. BM was extruded by inserting a 22-gauge needle into the shaft of the bone and flushed out with 1 ml Hanks' balanced salt solution (Invitrogen, Carlsbad, CA).

Mononuclear cells from BM were separated by centrifugation at 1000 $\times$ g for 20 min in a Ficoll-Hypaque (Sigma–Aldrich, St. Louis, MO) gradient (density = 1.077 g/cm³) suspended in control medium at a concentration of 1×10^6 cells/cm². The mononuclear cells were immunodepleted of granulomonocytic cells using CD11b micromagnetic beads (Miltenyi Biotec, Bergish-Gladbach, Germany). Cultures were maintained at 37 °C in a humidified atmosphere containing 5% CO₂. One-week later, when the monolayer of adherent cells has reached confluence, cells were trypsinized (0.05% trypsin-EDTA; Sigma–Aldrich, St. Louis, MO), resuspended in α -MEM containing 10% FBS, and subcultured at a concentration of 2000 cells/cm². The 3rd or 4th passage of the ASCs was used for experiments.

Colony-forming assay

To evaluate colony number per nucleated cells, mononuclear cells from BM and stromal vascular cells from AT were plated at 10^6 cells/100 cm² dish in three dishes each and cultured for 7 days. After 7 days of primary culture, dishes were washed twice with PBS, fixed with 4% paraformaldehyde, and stained with 0.5% crystal violet for 5 min. The number of cell colonies from each dish was counted at a magnification of $\times 100$ under an optical microscope. Colony numbers per nucleated cell of ASCs and BMSCs were calculated. Colonies less than 2 mm in diameter and faintly stained colonies were ignored.

To evaluate colony number per adherent cells, 100 adherent cells at passages 1 and 2 were re-plated and cultured for 7 days in 100 cm² dishes. The cells were subsequently fixed with 4% paraformaldehyde, stained with 0.5% crystal violet for 5 min, and washed twice with distilled water. Colony number per adherent

cell of ASCs and BMSCs at passages 2 and 3 was then counted. Colonies less than 2 mm in diameter and faintly stained colonies were ignored.

The cell number per colony of ASCs and BMSCs at passages 2 and 3 was also evaluated from the mean number of total cells divided by the mean number of colonies.

Immunophenotypic analysis

Flow cytometric analysis was used to characterize the phenotypes of the ASCs and BMSCs. At least 50,000 cells (in 100 μ l PBS/0.5% BSA/2 mmol/L EDTA) were incubated with FITC-labeled monoclonal antibodies against mouse CD105, CD90, CD45, CD11b (BD Biosciences Clontech, Palo Alto, CA) or with the respective isotype control. After washing the labeled cells were analyzed by flow cytometry using a FACS Calibur flow cytometer and the Cell Quest Pro software (BD Biosciences, San Diego, CA).

Multilineage differentiation of ASCs and BMSCs

ASCs and BMSCs were analyzed for their capacity to differentiate toward the adipogenic, osteogenic, and chondrogenic lineages.

Adipogenic differentiation was induced by culturing ASCs and BMSCs for 2 weeks in adipogenic media (1 μ M dexamethasone, 100 μ g/ml 3-isobutyl-1 methylxanthine (IBMX), 5 μ g/ml insulin, and 60 μ M indomethacin, 10% FBS in α -MEM) and assessed using an Oil Red O stain as indicator of intracellular lipid accumulation. Prior to staining, the cells were fixed 15 min at room temperature in 70% ethanol. The cells were incubated in 2% Oil Red O reagent for 1 h at room temperature. Excess stain was removed by washing with 70% ethanol, followed by several changes of distilled water. In order to quantify the ratio of adipogenic differentiation, optical densities were measured at 510 nm with a spectrophotometer after samples were treated with isopropyl alcohol.

Osteogenic differentiation was induced by culturing ASCs and BMSCs for 2 weeks in osteogenic media (0.1 mM dexamethasone, 10 μ M β -glycerophosphate, and 50 μ g/ml ascorbic acid, 10% FBS in α -MEM) and examined for extracellular matrix calcification by Alizarin red S staining. For Alizarin Red S staining, the cells were fixed with 70% ethanol and washed with distilled water. The cells were incubated in 2% Alizarin red solution for 15 min at room temperature. The cells were washed several times with distilled water. In order to evaluate alkaline phosphatase (ALP) activity, which is recognized as an early osteoblastic marker, samples were treated with 100 μ l 50 mM p-nitrophenyl phosphatase hexahydrate containing 1 mM MgCl₂, and incubated for 20 min at 37 °C. Optical densities were measured at 405 nm with a spectrophotometer.

Chondrogenic differentiation was induced using the micro-mass culture technique. Briefly, 10 μ l of a concentrated MSC suspension (3×10^5 cells/ml) was plated into the center of each well and allowed to attach at 37 °C for 2 h. Chondrogenic media (CM, 1% FBS, 0.1 mM dexamethasone (Sigma–Aldrich, St. Louis, MO), 50 μ g/ml ascorbic acid, ITS + 1 (insulin–transferrin–selenium; Sigma–Aldrich, St. Louis, MO), 10 ng/mL TGF- β 1 (Sigma–Aldrich, St. Louis, MO), 10 ng/mL in α -MEM) was gently overlaid so as not to detach the cell nodules, and cultures were maintained in CM for 4 weeks prior to analysis. Chondrogenesis was confirmed by immunohistochemistry.

Micromass were harvested after 4 weeks culture and immediately frozen in optimum cutting temperature medium. 10 μ m thick sections were cut, fixed in 4% paraformaldehyde. For collagen type II staining, sections were blocked with 10% horse serum, incubated with purified anti-mouse collagen type II antibody (BD Bioscience, San Jose, CA) for 1 h, and washed with PBS (pH 7.4). Cells with bound antibodies were detected with a peroxidase substrate kit (Vectastain ABC kit; Vector Laboratories, Burlingame, CA). Sections were

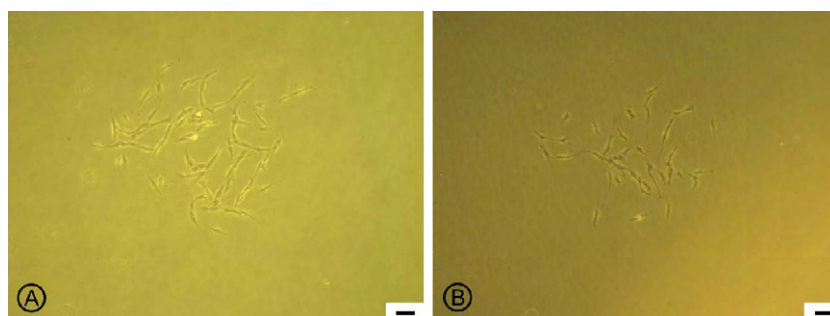


Fig. 1. Morphology of adherent cells after initial plating. Fibroblastoid adherent cells derived from adipose tissue (A) and bone marrow (B) were similar in morphology. Scale bar = 30 μ m.

washed, counterstained with hematoxylin, and examined by light microscopy.

Statistical analysis

Data were presented as mean $\pm$ SEM. The statistical significance was assessed by unpaired *t* test using the SPSS software package (version 12.0, SPSS Inc., IBM, Chicago, IL). A value of $p < 0.05$ was considered to be significant.

Results

Isolation characteristics of ASCs and BMSCs

At an initial plating density of 1×10^6 cells/cm², both BM- and AT-derived fibroblastoid cells formed a monolayer 4–5 days after initial plating (Fig. 1). The number of fibroblastoid colony-forming

units (CFU-F) per 1×10^6 mononuclear cells in the BM and 1×10^6 stromal vascular cells in the AT was named as CFU-F frequencies. CFU-F frequencies were 35 ± 5 in BM and 350 ± 25 in the AT. The colony number per nucleated cell, namely colony-forming efficiency at passage 1, was much higher in AT than BM ($p < 0.001$). Although colony number per adherent cell and cell number per colony at passages 2 and 3 were higher in AT than BM, there were no significant differences between AT and BM (Fig. 2).

Immunophenotypic characterization

Cultures of ASCs and BMSCs were analyzed for expression of cell-surface markers. ASCs and BMSCs were negative for the hematopoietic lineage marker, CD45 and CD11b. The percentage of CD105 and CD90 positive cells was 20.5%, 57.8% in BMSCs and 21.6%, 55.6% in ASCs, respectively (Fig. 3).

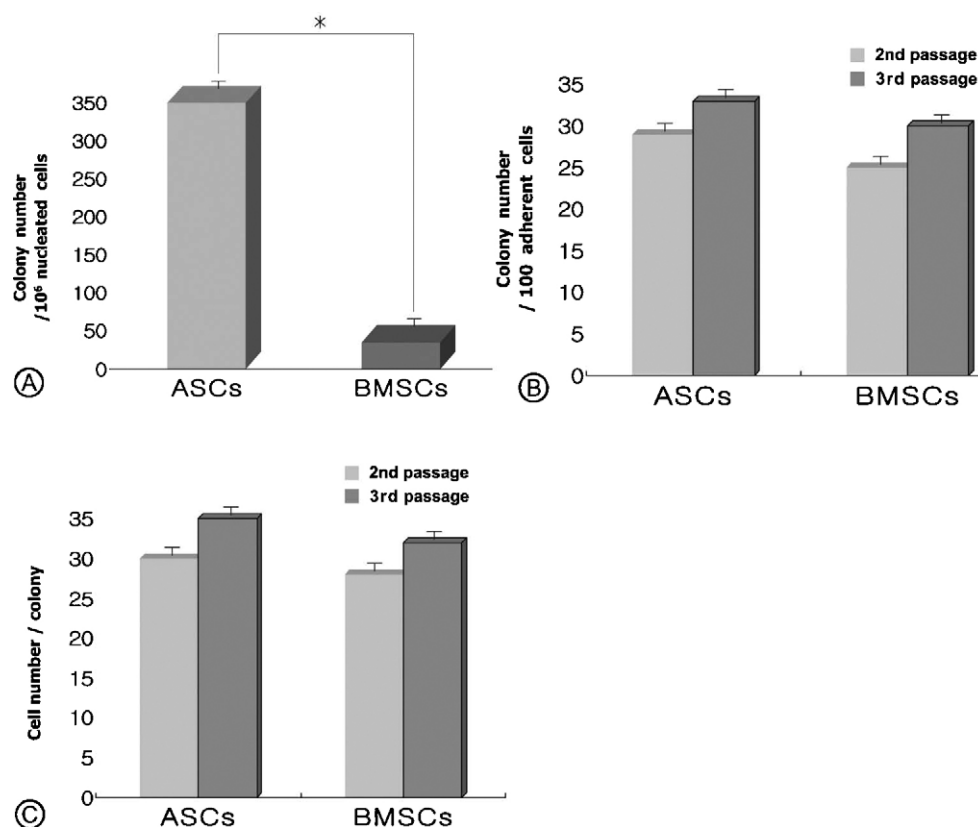


Fig. 2. Colony-forming assays of MSCs from adipose tissue (ASCs) and bone marrow (BMSCs). The colony number per nucleated cell, namely colony-forming efficiency at passage 1 was significantly higher in ASCs than BMSCs. However colony number per adherent cell and cell number per colony at passages 2 and 3 did not differ between the ASCs and BMSCs. Data expressed mean $\pm$ SEM, $*p < 0.001$.

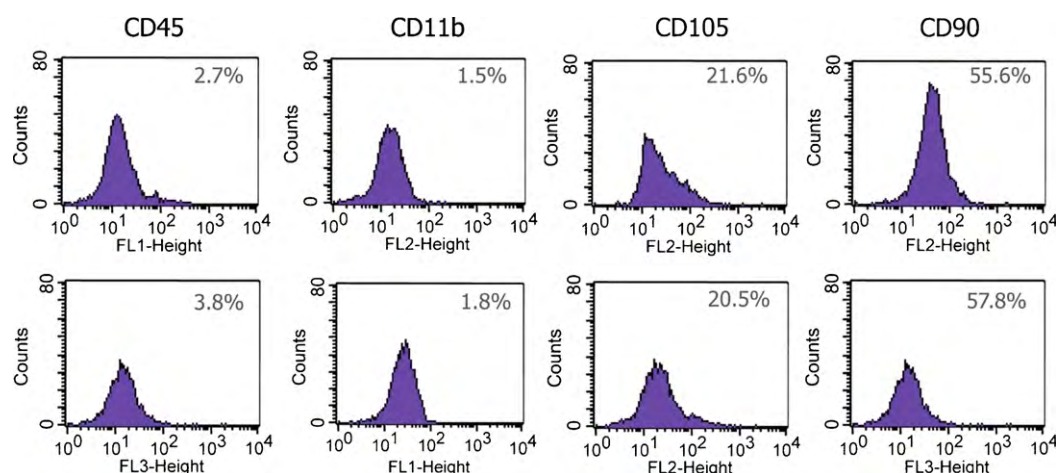


Fig. 3. Immunophenotypic analysis of MSCs from adipose tissue (ASCs) (A) and bone marrow (BMSCs) (B). Flow cytometry showed negative expression for CD45 and CD11b and positive expression for CD105 and CD90.

Adipogenic differentiation capacity

Adipogenic differentiation was demonstrated by the accumulation of neutral lipid vacuoles indicated by Oil Red O staining. After adipogenic induction, a significant fraction of the cells contained multiple, intracellular lipid-filled droplets that stained by Oil Red O in the ASCs and BMSCs (Fig. 4A and B), although there was no red staining in the control ASCs and BMSCs group (Fig. 4C and D). Optical densities were significantly higher in both ASCs and BMSCs group than in the control group ($p < 0.001$). However, there was no significant difference between the ASCs and BMSCs group (Fig. 5).

Osteogenic differentiation capacity

Osteogenic differentiation was confirmed by the detection of an osteogenic phenotype consisting of an increased expression of ALP and the deposition of Alizarin Red S stained mineralized matrix. Calcification appeared as red regions within the cells monolayer.

Consistent with osteogenesis, several red regions in Alizarin red S staining, indicative of calcified extracellular matrix, were observed in ASCs and BMSCs treated for 2 weeks in osteogenic media (Fig. 6A and B). No red regions were detected in control ASCs and BMSCs group (Fig. 6C and D). ALP activities were significantly higher in both ASCs and BMSCs group than control group ($p < 0.001$). However, there was no significant difference between the ASCs and BMSCs group (Fig. 7).

Chondrogenic differentiation capacity

Compared with control group, chondrogenic differentiation of ASCs and BMSCs was confirmed by the formation of a sphere in micromass culture. The size of a sphere was not different between ASCs and BMSCs group. Chondrogenesis was further studied by analyzing the expression of a cartilage-specific type II collagen. There was no significant difference in the expression of collagen type II between the ASCs and BMSCs group (Fig. 8).

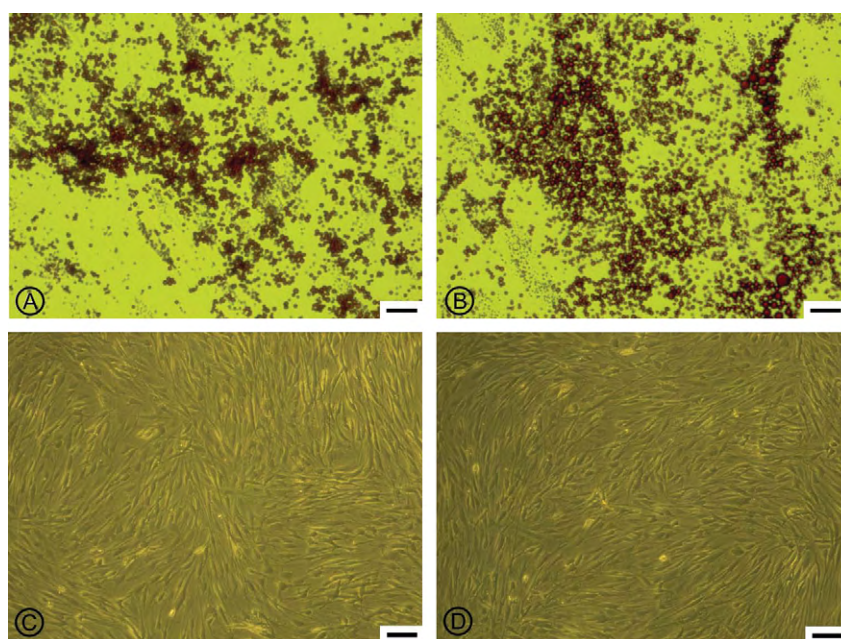


Fig. 4. Adipogenic differentiation of MSCs from adipose tissue (ASCs) and bone marrow (BMSCs). Adipogenesis was detected by the formation of multiple, intracellular lipid-filled droplets stained with Oil Red O in the ASCs (A) and BMSCs (B) under adipogenic differentiation condition. The control groups of ASCs (C) and BMSCs (D) were cultured with non-inductive medium. Scale bar = 50 μ m. Results are a representative of four independent experiments.

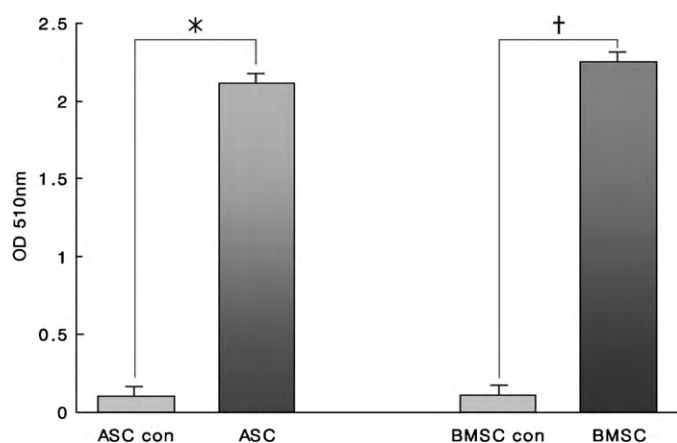


Fig. 5. Spectrophotometric analysis of Oil Red O staining of ASCs and BMSCs. Optical densities were significantly higher in both ASCs and BMSCs than control group. However, there was no significant difference between the ASCs and BMSCs group (C). Data expressed mean $\pm$ SEM ($n=4$, each group). (*), (†) $p < 0.001$.

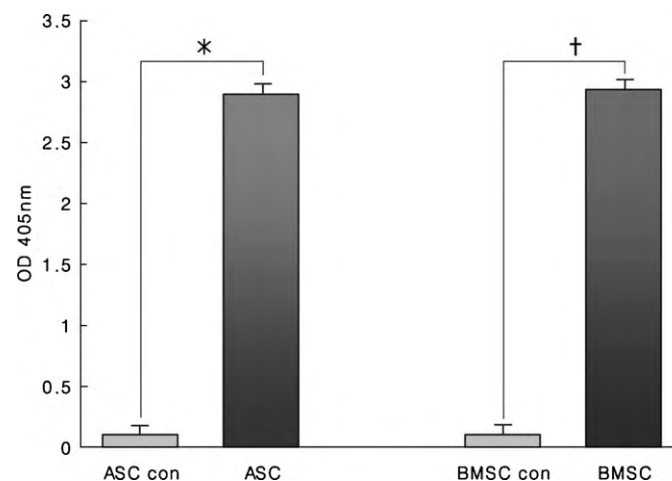


Fig. 7. Analysis of alkaline phosphatase activity. Optical densities were significantly higher in both ASCs and BMSCs than control group. However, there was no significant difference between the ASCs and BMSCs group. Data expressed mean $\pm$ SEM ($n=4$, each group). (*), (†) $p < 0.001$.

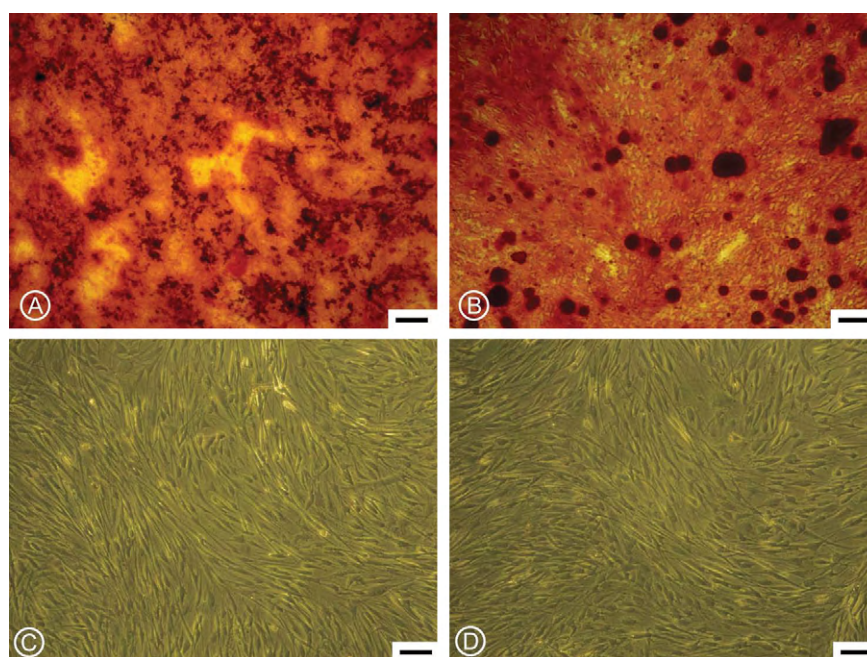


Fig. 6. Osteogenic differentiation of MSCs from adipose tissue (ASCs) and bone marrow (BMSCs). Osteogenesis was demonstrated by several dark red regions stained with Alizarin Red S, indicative of calcified extracellular matrix in the ASCs (A) and BMSCs (B) under osteogenic differentiation condition. The control groups of ASCs (C) and BMSCs (D) were cultured with non-inductive medium. Scale bar = 50 μ m. Results are a representative of four independent experiments. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.).

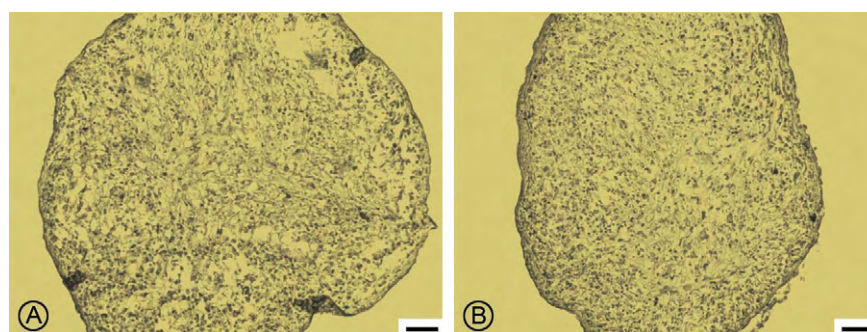


Fig. 8. Chondrogenic differentiation of MSCs from adipose tissue (ASCs) and bone marrow (BMSCs). Chondrogenesis was demonstrated by the formation of a sphere and type II collagen expression in the ASCs (A) and BMSCs (B). There was no difference in the size of a sphere and the immunohistochemical staining for type II collagen between the ASCs and BMSCs group. Scale bar = 50 μ m. Results are a representative of four independent experiments.

Discussion

MSCs consist of multipotent progenitors capable of differentiating into various different tissues (Delorme and Charbord, 2007). In contrast with stem cells derived from the embryo or fetus, adult MSCs are not ethically restricted and easy to obtain. MSCs were first identified in the BM by their adherence to plastic dishes and forming the colonies with a fibroblast-like morphology (Friedenstein et al., 1996). Several recent studies have shown that human MSCs are also present in other tissues, such as muscle (Asakura et al., 2001), periosteum (De Bari et al., 2001a,b), synovium (De Bari et al., 2001a,b), and subcutaneous adipose tissue (Zuk et al., 2002). MSCs isolated and cultured from various tissues appear to be morphologically quite similar in the expression of commonly used cell-surface markers (Musina et al., 2005). BMSCs have been isolated from various species, including rabbit (Johnstone et al., 1998), rat (Javazon et al., 2001), and mouse (Peister et al., 2004). Although properties of MSCs from different species and tissues have partially similar characteristics, significant differences have been observed according to tissue source and species (Im et al., 2005; Yoshimura et al., 2007; Bieback et al., 2008; Hayashi et al., 2008; Koga et al., 2008; Rebelatto et al., 2008). For example, it has been shown that synovium-derived MSCs were superior to MSCs from other sources for clinical application (Sakaguchi et al., 2005). Recently, strain-dependent variations in the amount of MSCs derived from BM in mice have been reported (Peister et al., 2004; Sung et al., 2008). The MSCs of C57BL/6 strain showed the highest number of CFU-F among all strains of mice, and CFU-F of the ASCs were significantly greater than that of the BM and compact bone MSCs in all strains of mice, suggesting that C57BL/6 ASCs might be the best source of MSCs in mice (Sung et al., 2008).

In view of clinical applications of MSC-based cell therapy for airway allergic disease, such as allergic rhinitis and asthma, suitable MSCs for *in vivo* immunomodulatory effect of MSCs in airway allergic animal model have to be determined. Because BALB/c mice are established airway allergic mouse models in view of the fact that BALB/c mice, with Th2 tendency, respond well to allergen stimulation, whereas C57BL/6 mice, with Th1 tendency, respond less well (Okano et al., 1999), female BALB/c mice were used to isolate MSCs from BM and AT in this study. We have analyzed MSCs from mouse BM and AT from the standpoint of the frequencies of colonies, colony-forming efficiency, colony number per adherent cells, cell number per colonies, immunophenotype, and multilineage differentiation capacity.

CFU-F frequencies, namely the colony number per nucleated cells, derived from synovium, periosteum, AT, and muscle is much higher than that derived from BM in both rat and human (Sakaguchi et al., 2005; Yoshimura et al., 2007). In human, cell number per colony is much greater in BM than in other mesenchymal tissues, and consequently, the expansion potential of BM cells is similar with that of other tissue-derived cells (Sakaguchi et al., 2005). On the other hand, in rat, cell number per colony is lower in BM than in other mesenchymal tissues, resulting in a lower expansion potential of rat BMSCs (Yoshimura et al., 2007). In this study, CFU-F frequencies and colony-forming efficiency in mice was significantly higher in AT than BM, although there is no significant difference in the colony number per adherent cell and cell number per colony between AT and BM, indicating that the proliferation potential of ASCs in mice is much higher than BMSCs and ASCs could be obtained in larger quantities than BMSCs.

Several previous reports have documented the expression of phenotypic markers in MSCs (Sun et al., 2003; Bhagavati and Xu, 2004; Yamamoto et al., 2007; Sung et al., 2008). Immunophenotypic analysis of this study showed that the surface marker profiles for ASCs and BMSCs in the BALB/c mice were compatible with those previously reported for MSCs. Both ASCs and BMSCs were positive

for CD90 and CD105 expression, whereas they were negative for CD11b and CD45 expression.

Multiple lineage differentiation potential has been considered an important quality of stem cells. In the present study, MSCs from AT and BM in the BALB/c mice were verified to possess osteogenic, adipogenic, and chondrogenic potential. Although several studies showed that BMSCs have exhibited superior capacity towards osteogenic and chondrogenic differentiation and inferior capacity to adipogenic differentiation than ASCs in the human and rat (Liu et al., 2007; Yoshimura et al., 2007; Peng et al., 2008), there was no significant difference in multiple lineage differentiation capacity between the murine ASCs and BMSCs in this study.

This study revealed similarities and differences between murine BMSCs and ASCs. Both ASCs and BMSCs can be regarded as potential materials for stem cell therapy experiments in the airway allergic disease mouse model. It is thought that the differentiation potential is not the first choice, but abundance, frequency, and proliferation potential are more important in therapeutic experiments of MSCs for airway allergic animal model. However, it is difficult to obtain sufficient quantities of MSCs from BM. Subcutaneous adipose tissues are easy accessible, more abundant and a less invasive alternative source of MSCs present in every individual.

In conclusion, ASCs had higher CFU-F frequencies and colony-forming efficiency than BMSCs. Therefore, ASCs in BALB/c mice might be a more useful material for cell therapy of the airway allergic experimental studies owing to the abundance, relatively easy harvesting, and high proliferation potential.

References

- Asakura A, Komaki M, Rudnicki M. Muscle satellite cells are multipotent stem cells that exhibit myogenic, osteogenic, and adipogenic differentiation. *Differentiation* 2001;68:245–53.
- Bhagavati S, Xu W. Isolation and enrichment of skeletal muscle progenitor cells from mouse bone marrow. *Biochem Biophys Res Commun* 2004;318:119–24.
- Bieback K, Kern S, Kocaomer A, Ferlik K, Bugert P. Comparing mesenchymal stromal cells from different human tissues: bone marrow, adipose tissue and umbilical cord blood. *Biomed Mater Eng* 2008;18:S71–76.
- Cho KS, Park HK, Park HY, Jung JS, Jeon SG, Kim YK, et al. IFATS collection: Immunomodulatory effects of adipose tissue-derived stem cells in an allergic rhinitis mouse model. *Stem Cells* 2009;27:259–65.
- Cho KS, Roh HJ. Immunomodulatory effects of adipose-derived stem cells in airway allergic diseases. *Curr Stem Cell Res Ther* 2009(November) [E-Pub ahead of print].
- De Bari C, Dell'Accio F, Luyten FP. Human periosteum-derived cells maintain phenotypic stability and chondrogenic potential throughout expansion regardless of donor age. *Arthritis Rheum* 2001a;44:85–95.
- De Bari C, Dell'Accio F, Tylzanowski P, Luyten FP. Multipotent mesenchymal stem cells from adult human synovial membrane. *Arthritis Rheum* 2001b;44:1928–42.
- Delorme B, Charbord P. Culture and characterization of human bone marrow mesenchymal stem cells. *Methods Mol Med* 2007;140:67–81.
- Friedenstein AJ, Piatetzky-Shapiro II, Petrakova KV. Osteogenesis in transplants of bone marrow cells. *J Embryol Exp Morphol* 1996;16:381–90.
- Hayashi O, Katsube Y, Hirose M, Ohgushi H, Ito H. Comparison of osteogenic ability of rat mesenchymal stem cells from bone marrow, periosteum, and adipose tissue. *Calcif Tissue Int* 2008;82:238–47.

- Horwitz EM, Prockop DJ, Gordon PL, Koo WW, Fitzpatrick LA, Neel MD, et al. Clinical responses to bone marrow transplantation in children with severe *Osteogenesis imperfecta*. *Blood* 2001;97:1227–31.
- Im GI, Shin YW, Lee KB. Do adipose tissue-derived mesenchymal stem cells have the same osteogenic and chondrogenic potential as bone marrow-derived cells? *Osteoarthritis Cartilage* 2005;13:845–53.
- Javazon EH, Colter DC, Schwarz EJ, Prockop DJ. Rat marrow stromal cells are more sensitive to plating density and expand more rapidly from single-cell-derived colonies than human marrow stromal cells. *Stem Cells* 2001;19:219–25.
- Johnstone B, Hering TM, Caplan AI, Goldberg VM, Yoo JU. In vitro chondrogenesis of bone marrow-derived mesenchymal progenitor cells. *Exp Cell Res* 1998;238:265–72.
- Kern S, Eichler H, Stoeve J, Kluter H, Bieback K. Comparative analysis of mesenchymal stem cells from bone marrow, umbilical cord blood, or adipose tissue. *Stem Cells* 2006;24:1294–301.
- Koga H, Muneta T, Nagase T, Nimura A, Ju YJ, Mochizuki T, et al. Comparison of mesenchymal tissues-derived stem cells for in vivo chondrogenesis: suitable conditions for cell therapy of cartilage defects in rabbit. *Cell Tissue Res* 2008;333:207–15.
- Le Blanc K, Rasmuson I, Sundberg B, Götherström C, Hassan M, Uzunel M, et al. Treatment of severe acute graft-versus-host disease with third party haploidentical mesenchymal stem cells. *Lancet* 2004;363:1439–41.
- Li Y, Chen J, Chen XG, Wang L, Gautam SC, Xu YX, et al. Human marrow stromal cell therapy for stroke in rat: neurotrophins and functional recovery. *Neurology* 2002;59:514–23.
- Liu TM, Martina M, Hutmacher DW, Hui JH, Lee EH, Lim B. Identification of common pathways mediating differentiation of bone marrow- and adipose tissue-derived human mesenchymal stem cells into three mesenchymal lineages. *Stem Cells* 2007;25:750–60.
- Musina RA, Bekchanova ES, Sukhikh GT. Comparison of mesenchymal stem cells obtained from different human tissues. *Bull Exp Biol Med* 2005;139:504–9.
- Okano M, Nishizaki K, Abe M, Wang MM, Yoshino T, Satoskar AR, et al. Strain-dependent induction of allergic rhinitis without adjuvant in mice. *Allergy* 1999;54:593–601.
- Peister A, Mellad JA, Larson BL, Hall BM, Gibson LF, Prockop DJ. Adult stem cells from bone marrow (MSCs) isolated from different strains of inbred mice vary in surface epitopes, rates of proliferation, and differentiation potential. *Blood* 2004;103:1662–8.
- Peng L, Jia Z, Yin X, Zhang X, Liu Y, Chen P, et al. Comparative analysis of mesenchymal stem cells from bone marrow, cartilage, and adipose tissue. *Stem Cells Dev* 2008;17:761–73.
- Rebelatto CK, Aguiar AM, Moretão MP, Senegaglia AC, Hansen P, Barchiki F, et al. Dissimilar differentiation of mesenchymal stem cells from bone marrow, umbilical cord blood, and adipose tissue. *Exp Biol Med* 2008;233:901–13.
- Rojas M, Xu J, Woods CR, Mora AL, Spears W, Roman J, et al. Bone marrow-derived mesenchymal stem cells in repair of the injured lung. *Am J Respir Cell Mol Biol* 2005;33:145–52.
- Sakaguchi Y, Sekiya I, Yagishita K, Muneta T. Comparison of human stem cells derived from various mesenchymal tissues: superiority of synovium as a cell source. *Arthritis Rheum* 2005;52:2521–9.
- Shake JG, Gruber PJ, Baumgartner WA, Senechal G, Meyers J, Redmond JM, et al. Mesenchymal stem cell implantation in a swine myocardial infarct model: engraftment and functional effects. *Ann Thorac Surg* 2002;73:1919–26.
- Sung JH, Yang HM, Park JB, Choi GS, Joh JW, Kwon CH, et al. Isolation and characterization of mouse mesenchymal stem cells. *Transplant Proc* 2008;40:2649–54.
- Sun S, Guo Z, Xiao X, Liu B, Liu X, Tang PH, et al. Isolation of mouse marrow mesenchymal progenitors by a novel and reliable method. *Stem Cells* 2003;21:527–35.
- Titorencu I, Jinga VV, Constantinescu E, Gafencu AV, Ciohodaru C, Manolescu I, et al. Proliferation, differentiation and characterization of osteoblasts from human BM mesenchymal cells. *Cytotherapy* 2007;9:682–96.
- Yamamoto N, Akamatsu H, Hasegawa S, Yamada T, Nakata S, Ohkuma M, et al. Isolation of multipotent stem cells from mouse adipose tissue. *J Dermatol Sci* 2007;48:43–52.
- Yoshimura H, Muneta T, Nimura A, Yokoyama A, Koya H, Sekiya I. Comparison of rat mesenchymal stem cells derived from bone marrow, synovium, periosteum, adipose tissue, and muscle. *Cell Tissue Res* 2007;327:449–62.
- Zuk PA, Zhu M, Ashjian P, De Ugarte DA, Huang JL, Mizuno H, et al. Human adipose tissue is a source of multipotent stem cells. *Mol Biol Cell* 2002;13:4279–95.