Can the Palatine Tonsil be a Source of Mesenchymal Stem Cells with Immunomodulatory Property?

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－ ABSTRACT －

Background and Objectives：Mesenchymal stem cells (MSCs) are multipotent cells that can differentiate into various cell types and are isolated from various other human adult and fetal tissues. Our study is to isolate palatine tonsil-derived MSCs (T-MSCs) and evaluate their differentiation potential and immunomodulatory effects, compared with bone marrow-derived MSCs (BM-MSCs). **Methods**：T-MSCs were isolated from human palatine tonsil. The expression of surface markers of T-MSCs was assessed by flow cytometric analysis. Differentiation potential of T-MSCs was analyzed histochemically and by the expression of lineage-related marker genes. Immunomodulatory effects were evaluated by mixed lymphocyte reactions (MLR) and mitogen proliferation assays with phytohemagglutinin (PHA). **Results**：T-MSCs were isolated from palatine tonsil and displayed a similar morphology to BM-MSCs. T-MSCs were negative for CD 31, CD45, CD117, HLA-DR and positive for CD44, CD73, CD90, and CD105 in flow cytometric analysis. The expression of surface phenotypes and differentiation potential of T-MSCs exhibited the similar finding as BM-MSCs. T-MSCs showed the significant inhibition of the proliferation of T cells stimulated by allergenic T cells in MLR study and by PHA stimuli in mitogen proliferation assay and also significant decrease of the secretion of TNF-α and IFN-γ ($p < 0.05$). The HLA-G5 secretion by T-MSCs showed the significant increase in MLR and mitogen proliferation assays ($p < 0.05$). T-MSCs do satisfy the phenotypical and functional definition of MSCs. T-MSCs also displayed immunomodulatory effects that were associated with inhibiting T-cell proliferation, decreasing soluble factors, and increasing HLA-G5 secretion in response to various stimuli. **Conclusions**：We propose that T-MSCs provide a good alternative for allogeneic MSCs in therapeutic applications. **(J Clinical Otolaryngol 2012;23:90-100)**

KEY WORDS：Palatine tonsil · Mesenchymal stem cell · Immune modulation.

Introduction

Mesenchymal stem cells (MSCs) were first identified in bone marrow stromal cells and can differentiate along multiple mesenchymal lineages, including chondrocytes, osteoblast, and myoblasts.[1,2] MSCs have great therapeutic potential because of their ability to self-renew and differentiate into multiple tissues. The therapeutic potential of multi-lineage stem cell for application such as tissue engineering and gene therapy is enormous. Although bone marrow is a good source of MSCs, traditional bone marrow procurement procedures in human are painful, frequently requiring general or spinal anesthesia, and may yield low numbers of MSCs upon processing.[3] Therefore, there are

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limitations to the application in the regenerative medicine using bone marrow MSCs (BM-MSCs). An alternative source of MSCs that is obtainable in large quantities under local anesthesia with minimal discomfort would be advantageous. More recent studies have reported the isolation of MSCs from various other human adult and fetal tissues, including fat, bone, synovium, skin, thymus, periodontal ligament, placenta and amniotic fluid.[3-6)]

Tonsillectomy for chronic tonsillar hypertrophy or chronic tonsillitis is one of most common operation in ENT field. Tonsillar epithelium is derived from the second pharyngeal pouch (endodermal origin). During fetal development, tonsillar tissue is invaded by lymphoid tissue (mesodermal origin). So, embryologically, there is the possibility of a source of MSCs. Recent study has reported the palatine tonsil is a new source of MSCs.[7)] The human palatine tonsil is readily accessible to otolaryngologists and easily obtained by tonsillectomy or punch biopsy, particularly in young donors.

The clinical application of MSCs requires large quantities of cells for injection or infusion. Because of the limitation of obtaining sufficient autologous stem cells, MSCs from allogeneic donor could constitute a valuable alternative source of stem cells for therapeutic application. A prerequisite when considering allogeneic MSCs for clinical therapeutic purpose is the characterization of their immunological properties in allogeneic condition. Although palatine tonsil-derived MSCs (T-MSCs) could be a good source of autologous MSCs, it also could be a new candidate of allergenic MSCs because it is easy to obtain tonsillar tissue by tonsillectomy that is one of most common surgery. Consequently, palatine tonsil-derived mesenchymal stem cells (T-MSCs) may provide an alternative source of MSCs for allergenic donor. Our study is to isolate T-MSCs and evaluate their differentiation potential and immunomodulatory effects, compared with BM-MSCs.

Materials and Methods

We used BM-MSC that was previously established in our institute to validate the character of T-MSC.[8)]

T-MSC isolation and culture

This study using human tonsil tissue and peripheral blood samples was approved by the institutional review board. Tonsils were obtained after informed consent from patients (5 to 12 years old) undergoing tonsillectomy as a result of chronic tonsillar hypertrophy. To isolate T-MSCs, tonsils were washed extensively with equal volumes of phosphate buffered saline (PBS), and tissues were digested at 37°C for 30 min with 0.075% collagenase type I (Sigma, St. Louis, MO). Enzyme activity was neutralized with α modified Eagle's medium (α-MEM), containing 10% fetal bovine serum (FBS) and centrifuged (1,200 × g, 10 min) to obtain a pellet. The pellet was filtered through a 100-μm nylon mesh to remove cellular debris and incubated overnight at 37℃/5% CO_2 in control medium (α-MEM, 10% FBS, 100 unit/mL of penicillin, 100 μg/mL of streptomycin). Following incubation, the plates were washed extensively with PBS to remove residual non adherent cells. The resulting cell population was maintained at 37℃/5% CO_2 in control media. One week later, when a monolayer of adherent cells had reached confluencey, cells were trypsinized (0.05% trypsin-EDTA ; Sigma, USA) re-suspended into media and subcultured at a concentration of 2,000 cells/cm^2. To isolate BM-MSCs, we performed methods as previously described.

Flow cytometric analysis

Flow cytometric analysis was used to characterize the phenotypes of the T-MSCs and BM-MSCs. At least 50,000 cells (in 100 μL PBS/0.5% BSA/2 mmol/L EDTA) were incubated with FITC conjugated monoclonal antibodies against human CD105, CD90, CD44, CD73, CD45, CD31, CD117, HLA-DR (BD Biosciences Clontech, Palo Alto) or with the respective isotype control. Labeled cells were analyzed by flow cytometry using a FACS Caliber flow cytometer and the Cell Quest Pro software (BD Biosciences, USA).

Cellular proliferation assays

Tonsil stem cells were plated at a density of 10^3 cells per well in a 96 well plate. Cell viability was assessed by an MTT assay after 24, 48, and 72 h of culture.

Multilineage differentiation

T-MSCs were analyzed for their capacity to differentiate towards the adipogenic, osteogenic, and chondrogenic lineages. Adipogenic differentiation was induced by culturing T-MSCs for 2 weeks in adipogenic media (1 uM dexamethasone, 100 μg/mL 3-isobutyl-1 methylxanthine (IBMX), 5 μg/mL insulin, and 60 μM indomethacine, 10% FBS in α-MEM) and assessed by oil red O (Sigma, St. Louis, MO) staining as an indicator of intracellular lipid accumulation. Prior to staining, the cells were fixed for 15 min at room temperature in 70% ethanol. Cells were then incubated in 2% oil red O reagent for 1 h at room temperature. Excess stain was removed by washing with 70% ethanol and distilled water to visualize lipid droplets.

Osteogenic differentiation was induced by culturing T-MSCs and BM-MSCs for 2 weeks in osteogenic media (0.1 mM dexamethasone, 10 μM β glycerophosphate, and 50 μg/mL ascorbic acid, 10% FBS in α MEM) and examining extracellular matrix calcification by alizarin red S (Sigma, St. Louis, MO) staining. For alizarin red S staining, cells were fixed with 70% ethanol and washed with distilled water, and incubated in 2% alizarin red solution for 15 min at room temperature, followed by numerous washes with distilled water.

Chondrogenic differentiation was induced using the micromass culture technique. Briefly, 10 μL of concentrated MSC suspension (3×10^5 cells/mL) was plated into the center of each well and allowed to attach at 37℃ for 2 h. Chondrogenic media [CM, 1% FBS, 0.1 mM dexamethasone (Sigma, USA), 50 μg/mL ascorbic acid, ITS+1 (insulin-transferrin-selenium ; Sigma), 10 ng/mL TGF β1 (Sigma), 10 ng/mL in α-MEM] was gently overlaid so as not to detach the cell nodules, and the culture was maintained in CM for 4 weeks before analysis. Chondrogenesis was confirmed by immunohistochemistry for collagen type-II staining. 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). Antibody-bound cells were detected with a peroxidase substrate kit (Vectastain ABC kit ; Vector Laboratories, Burlingame, CA). Sections were washed, counterstained with hematoxylin, and examined by light microscopy.

Real-time quantitative reverse transcription-polymerase chain reaction (PCR) for gene profiling

Total RNA was isolated from MSCs or PBMCs from day 21 monolayers and pellet cultures, using the Trizol reagent (Invitrogen Corporation). Isolated RNA was then reverse-transcribed using random hexamers. Real-time PCR were performed using 10 ng of cDNA and SYBR Green mix (Bio-Rad Laboratories, Inc.). Gene-specific primers were designed based on GenBank cDNA sequences, as described previously.[7] Lipoprotein lipase (LPL) and peroxisome proliferator-activated receptor-gamma (PPARγ) were used as genetic markers of adipogenesis, alkaline phosphatase (ALP) and osteocalcin (OC) was used as genetic markers of osteogenesis, and collagen type II α1 (COL2) and aggrecan (AGN) were used as genetic markers of chondrogenesis. Expression levels are presented as a fold increase over GAPDH, using the formula $2^{(\Delta Ct)}$, where ΔCt=Ct of target gene-Ct of GAPDH.

Mixed lymphocyte reaction (MLR) and mitogen proliferative assays

Human peripheral blood mononuclear cells (PBMCs) were obtained from heparinized whole blood samples or buffy coats donated by healthy subjects, after informed consent was obtained, using density gradient centrifugation (Lymphoprep ; Axis-Shield, Oslo, Norway, http://www.axis-shield.com). MLR cultures were set up with 2×10^5 purified PBMCs as responder cells and equal number of irradiated (3,000 cGy) HLA mismatch PBMCs as stimulators. In T-MSC/MLR cocul-

ture experiments, MLR were performed using coculture with various T-MSCs concentration at 0, 1×10^4, 2×10^4, 4×10^4.

All cultures were performed in triplicate, using round-bottomed 96-well tissue culture plates (Corning), in a final volume of 200 μL complete medium. After 4 days d of incubation, ^{3}H-thymidine was added overnight and thymidine incorporation was measured using a β-scintillation counter and is expressed in counts per minute (cpm).

In mitogen proliferation assays, PBMCs were seeded in triplicate at a concentration of 2×10^5 cells/well in 96-well flat-bottom plates in the presence of 10 μg/mL phytohemagglutinin (PHA, Sigma, St. Louis, MO). Medium or T-MSCs were added to the various MLR (T-MSCs : PBMCs) at a 1 : 20, 1 : 10, 1 : 5, or 1 : 0 ratio. After 4 days of incubation, ^{3}H thymidine was added overnight and thymidine incorporation measured using a β scintillation counter and is expressed in counts per minute (cpm).

Production of soluble factors from T-MSCs and BM-MSCs

The levels of interleukin (IL)-10, tumor necrosis factor (TNF)-α, interferon (IFN)-γ and HLA-G5 were quantified using commercially available enzyme-linked immunosorbent assays (ELISA ; R&D system, USA) from supernatants obtained from MLR and mitogen proliferation assays. HLA-G5 was detected by flow cytometric analysis. To analyze the surface HLA-G5, cells were stained with PE-conjugated anti-human HLA-G5a (clone 87G ; Exbio) and measured using a FACScalibur (BD bioscience), and data analysis was performed using Cellquest pro software (BD bioscience).

Statistical analyses

Statistical analyses were performed using the SPSS software (ver. 15.0 ; Chicago, IL, USA. Differences between levels of cpm and absorbance were evaluated using the t-test and the chi squared test. A p-value $<$ 0.05 was deemed to indicate statistical significance.

Results

T-MSC Isolation

The cell yield from each tonsil ranged from 1 to 5×10^9 cells, with the majority being non adherent and, likely, of hematopoietic origin. Following repeated washes, adherent cells that had fibroblast-like spindle-shaped appearances were identified ; however, large round cells were also still present. After multiple trypsinization steps, an increased number of homogeneous fibroblast-like cells with extended cytoplasmic processes was observed. The cells obtained from BM-MSCs were similar to those from T-MSCs (Fig. 1). The doubling time was higher in T-MSCs (73.5 ± 4.2 h), compared with BM-MSCs (96.0 ± 2.9 h). The proliferation profiles of BM-MSCs and T-MSCs were not significantly different. The cells per well did not limit

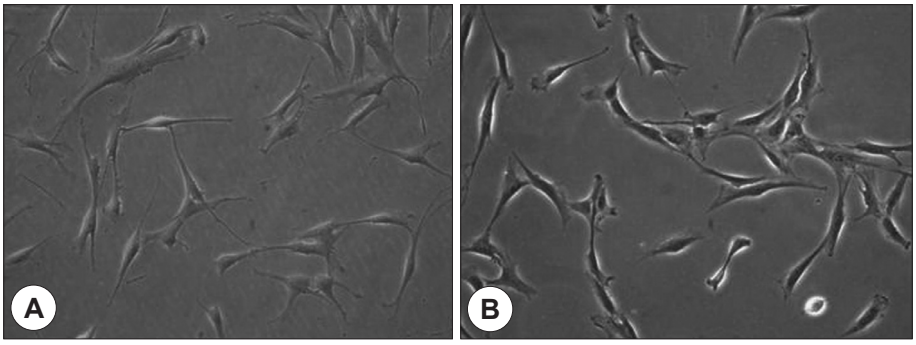

Fig. 1. Morphology of T-MSCs and BM-MSCs. The fibroblast-like spindle-shaped appearances of T-MSCs (A) is similar to BM-MSCs (B)(×200).

Table 1. Comparison of the expression of surface markers of mesenchymal stem cells derived from bone marrow and palatine tonsil as analyzed by flow cytometry

Surface phenotypes	BM-derived MSCs	Tonsil-derived MSCs
CD31	1.12±0.5	2.20±1.1
CD45	0.42±0.8	3.99±0.8
CD117	1.69±1.1	4.30±1.2
HLA-DR	0.26±0.2	1.51±0.8
CD 44	97.77±1.4	96.11±3.2
CD 73	97.11±2.2	97.88±1.8
CD 90	96.70±2.1	98.55±1.2
CD 105*	43.98±3.3	98.51±1.2

* : $p<0.05$

the proliferation of MSCs during culture.

Phenotypes analysis of the various cell preparations

The antigenic phenotypes of all cell preparations were investigated by flow cytometry. T MSCs and BM-MSCs were uniformly positive for CD44, CD73, and CD90. Higher levels of CD105 surface expression were observed in T-MSCs compared with BM MSCs (Table 1). CD31, CD45, CD117, and HLA-DR were negative in both MSCs. CD31 and CD45 negativity confirmed that the T-MSCs were of non-hematopoietic lineage. The expression of surface phenotypes of T-MSCs exhibited the similar cell surface phenotype as BM-MSCs. However, we observed significant differences concerning the expression of CD105.

Multi-lineage differentiation potential

As previously described, when cultured in the appropriate medium, T-MSCs and BM-MSCs were capable of *in vitro* differentiation into adipocytes, osteoblast, and chondrocytes.

Adipogenesis

Following treatment of BM-MSCs and T-MSCs with adipogenic supplements, both MSCs were maintained in expansion medium. Multiple lipid droplets were observed in the cytoplasm of both MSCs, suggesting similar morphological changes (Fig. 2A). Quantitative RT-PCR analysis revealed a significant increase of LPL and PPARγ expression in both T-MSCs and BM-MSCs (Fig. 3A, B)($p<0.05$). The expression of adipogenic genes of T-MSCs and BM-MSCs were similar.

Osteogenesis

Osteogenic induction enabled both MSCs to be transformed from spindle-shaped to flattened and spread cells. Additionally, both osteoblast-like cells and matrix mineralization were observed (Fig. 2B). Quantitative RT-PCR analysis revealed a significant increase of OC and ALP expression in both T-MSCs and BM-MSCs (Fig. 3C, D)($p<0.05$). The expression of osteogenic genes of T-MSCs exhibited the similar to the findings of BM-MSCs.

Chondrogenesis

An accumulation of matrix sulfated proteoglycan was detected in both MSCs and the intensity of staining did not differ between the MSCs (Fig. 2C). Quantitative RT-PCR analysis revealed a significant increase of AGN and COL2 expression in both T-MSCs and BM-MSCs (Fig. 3E, F)($p<0.05$). The expression of adipogenic genes of T-MSCs and BM-MSCs were similar.

Effects of T-MSCs on MLR and mitogen proliferation assay

MLR and mitogen proliferation assays were performed to evaluate the immunomodulatory properties of both allogeneic MSCs. Two-way MLR between two mismatched HLA-DR PBMCs was suppressed using T-MSCs at varying cell densities. Changes in DNA synthesis profiles (cpm) in the absence or presence of various concentrations of MSCs were also observed. Fig. 4A demonstrates that the lowest concentration of T-MSCs (T-MSCs to responder cell ratio of 1 : 5) produced a marked decrease in lymphocyte proliferation ($p<0.001$). Similar effects were observed at higher concentrations of MSCs and the degree of inhibition did not differ between T-MSCs and BM-MSCs. In addition to allogeneic stimuli, we evaluated the immunosuppressive effect of both MSCs in mitogen (PHA)-induced proliferation assays. T-MSCs and BM-MSCs strongly inhibited the PHA-induced proliferation of

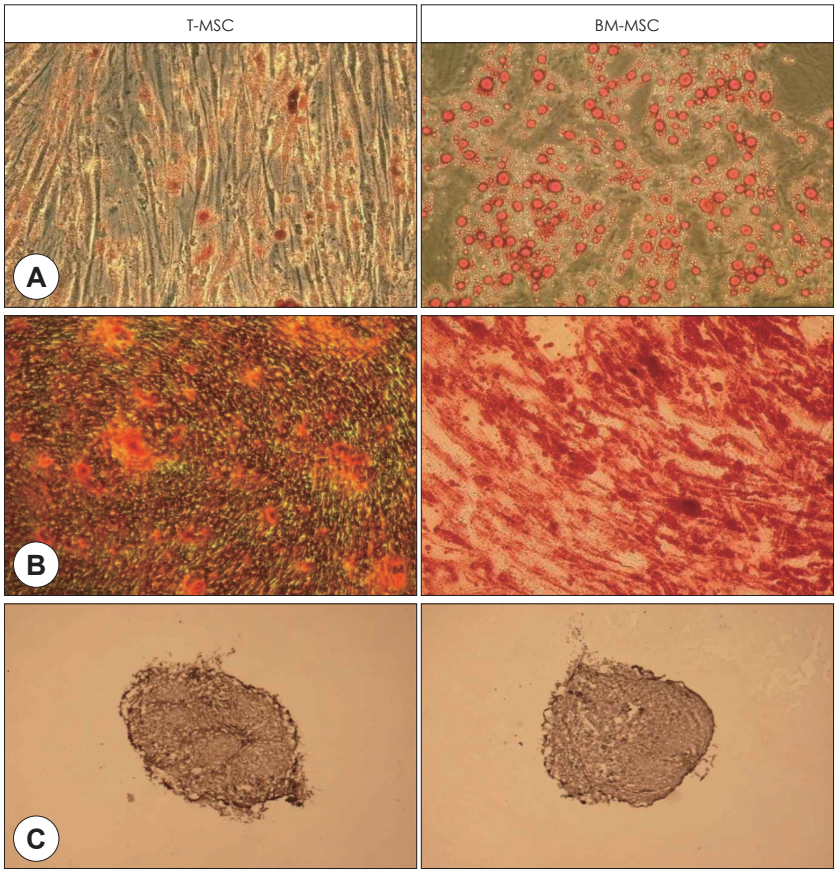

Fig. 2. Histological analysis of differentiation potential of T-MSCs and BM-MSCs. Representative micrscopic images views of the differentiation of T-MSCs compared to BM-MSCs into adipogenic (A), osteogenic (B) and chondrogenic (C) lineages (×200). Oil red O staining shows lipid vacuoles stained red, alizarlin red S staining shows deposition of calcium crystals stained orange to brown, and collagen type II staining shows cartilage-specific proteoglycan.

lymphocytes, to a similar extent (Fig. 4B) ($p < 0.05$). In mitogen proliferation assays, the inhibition at the lowest concentration was less pronounced than in MLR, but was sustained at increasing concentrations. This was also evident when BM-MSCs were added to the reaction. These data demonstrate that both MSCs suppress the proliferation of lymphocytes, to a similar degree, in a cell density-dependent manner.

Secretion of TNF-α, IFN-γ, and IL-10 on MLR and mitogen proliferation assays

Previous studies indicated that elicited soluble factors participate in MSC-mediated immunosuppression. To evaluate these effects, supernatants obtained from MLR and mitogen proliferation assays were assessed for changes in the levels of soluble factors by ELISA. In MLR, both TNF-α and IFN-γ (which reflect T-lymphocyte proliferation and inflammatory condition) decreased significantly at the initial concentration of T-MSCs and BM-MSCS. At increasing concentrations of MSCs, significant changes were not evident. The levels of IL-10 showed no variation at any concentration of MSCs (Fig. 5A). In the mitogen proliferation assays, similar results were observed, with graded responses along the concentrations of MSCs evident. IL-10 levels did not change significantly (Fig. 5B).

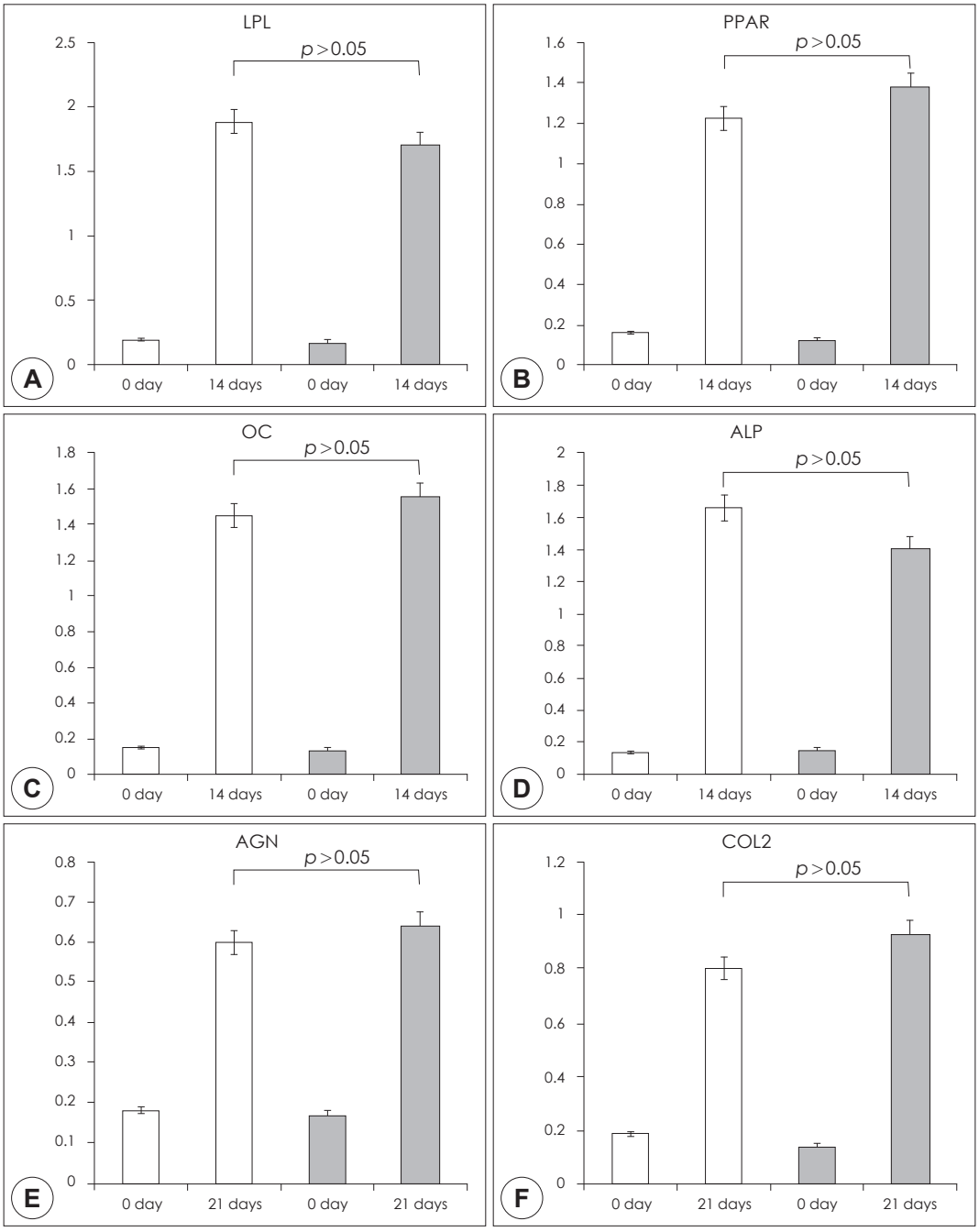

Fig. 3. Gene expression analysis of differentiation potential of T-MSCs and BM-MSCs. Adipogenic genes are (A) lipoprotein lipase (LPL) and (B) proliferator-activated receptor-gamma (PPARγ), osteogenic genes are (C) osteocalcin (OC) and (D) alkaline phosphatase (ALP), and chondrogenic genes are (E) aggrecan (AGH) and (F) collagen type II a1 (COL2). Quantitative RT-PCR analysis revealed a significant increase of LPL, PPARγ, OC, ALP, AGH, and COL2 expression in both T-MSCs and BM-MSCs ($p<0.05$). The patterns of differentiation markers of T-MSCs and BM-MSCs were similar. Vertical axis indicates the relative gene expression level.

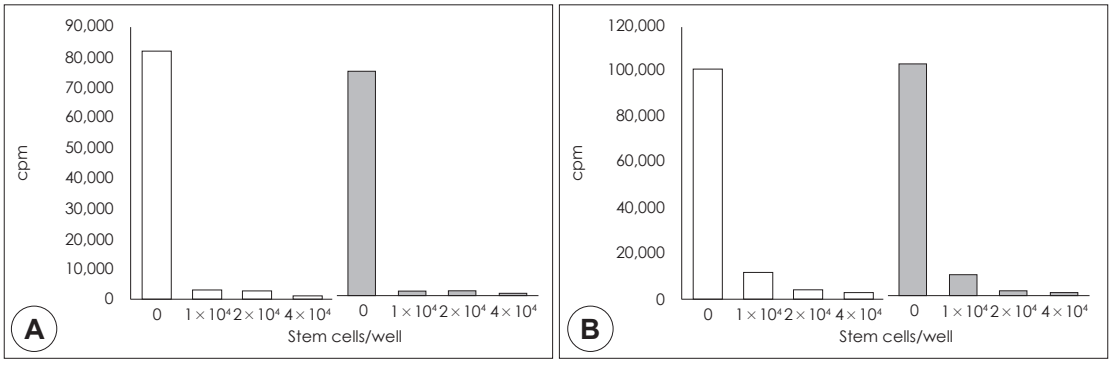

Fig. 4. Inhibition of lymphocyte proliferation in presence of T-MSCS and BM-MSCs. (A) MLR and (B) mitgen proliferation assay showed immunomodulatory effects of both MSCs. T-MSCs and BM-MSCs inhibited proliferation of lymphocytes in both the reactions.

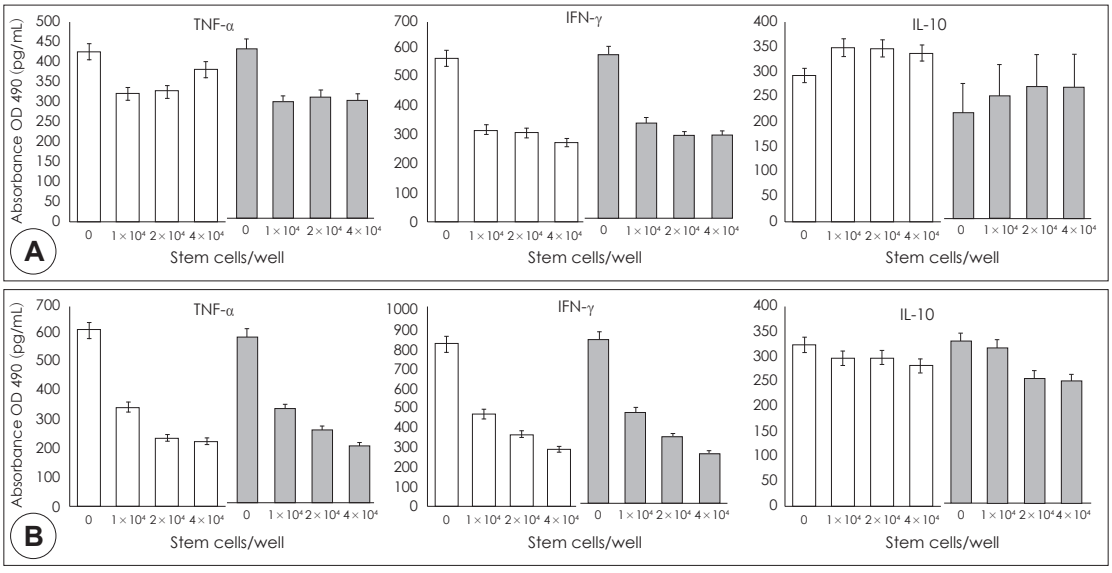

Fig. 5. Change of molecules participating immunomodulatory reaction of T-MSCs. (A) MLR and (B) mitogen proliferation assay. In both reactions, TNF-a and IFN-γ decreased.

Expression of HLA-G5 in MLR and mitogen proliferation assays

Both flow cytometry and ELISA were performed to evaluate the levels of HLA-G5 expression. Increases in HLA-G5 expression were observed following allogeneic or mitogenic stimulation. HLA-G5 levels increased significantly in the MLR and mitogen proliferation assays as the concentration of T-MSCs increased ($p < 0.05$; Fig. 6A). To validate that T-MSCs do not interact with HLA-G5, T-MSCs were mixed with HLA-G5 without allogeneic stimulus as the control group, where no change in HLA G5 expression was evident. The increase in HLA-G5 expression was significantly higher in the mitogen proliferation assay than control group (Fig. 6B).

Discussion

The isolation of MSCs from various other human adult and fetal tissues, including fat, bone, synovium, skin, thymus, periodontal ligament, placenta and amniotic fluid, were reported.[3-6] Recent study has report-

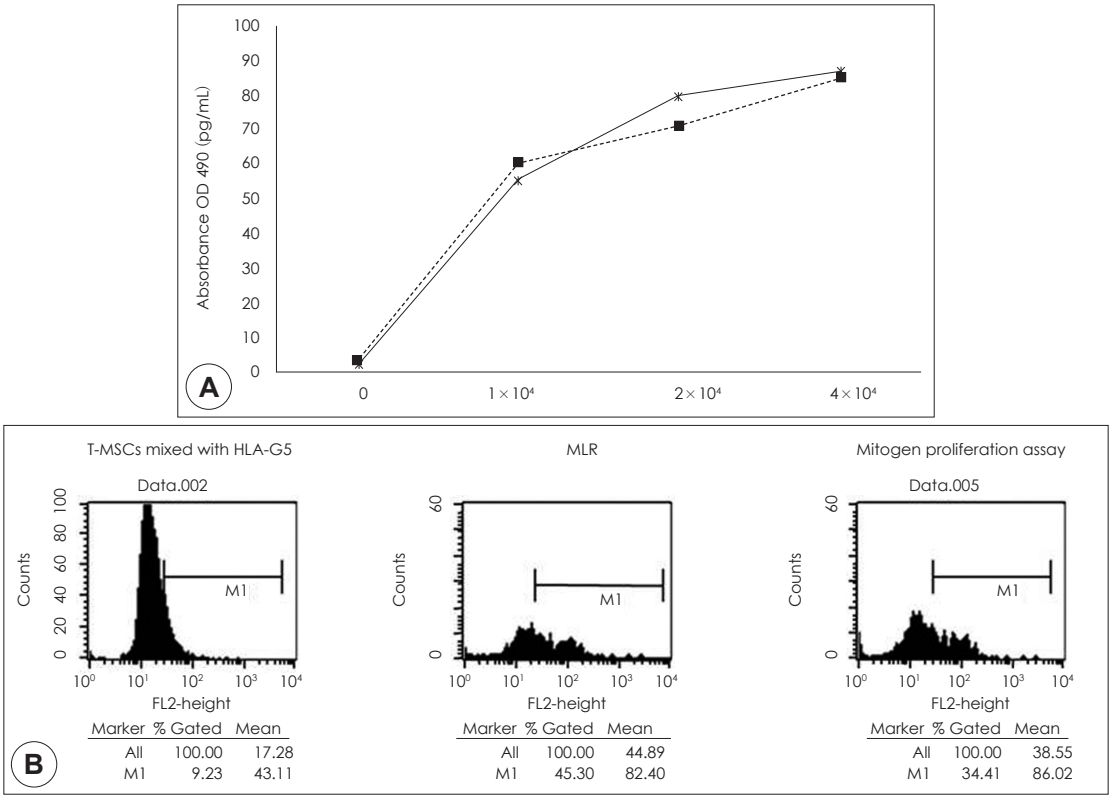

Fig. 6. Role of HLA-G5 was evaluated. HLA- G5 level changed as the concentration of T-MSCs was increased in both the MLR (solid line) and mitogen proliferation assay (dotted line) (A) and it was analyzed by ELISA. Significant change in the presence of T-MSCs was observed versus in the absence of T-MSCs ($p<0.05$). (B) Flow cytometry presenting increased HLA-G5 in MLR and mitogen proliferation assay compared with control group (T-MSCs mixed with HLA-G5).

ed the palatine tonsil is a new source of MSCs.[7] In our study, fibroblast-like cells (T-MSCs) were isolated from palatine tonsil and displayed a similar morphology to BM-MSCs. The proliferation profile of T-MSCs was not significantly different when compared to BM-MSCs. In flow cytometric analyses, T-MSCs were negative for CD 31, CD45, CD117, HLA-DR and positive for CD44, CD73, CD90, and CD105. The expression of surface phenotypes of T-MSCs exhibited the similar cell surface phenotype as BM-MSCs, specifically CD44, CD73, and CD90. However, we observed significant differences concerning the expression of CD105. It needs to be further investigated whether this molecule is important for the function and differentiation of stem cells.

The multilineage potential of T-MSCs was shown based on their ability to differentiate into fat, bone, and cartilage. Adiopocytes displayed characteristic lipid droplets and expressed LPL and PPARγ. Analysis of the osteogenic condition showed areas of mineralization and osteoblasts, while OC and ALP were expressed as mRNA transcript markers. Chondrocytes displayed matrix accumulation of sulfated glycosaminoglycans in cell pellets and the expression of AGN and COL2 as mRNA transcript markers. These findings are consistent with Janjanin et al.[7] However, they reported that the expression of OC, ALP, and COL2 in T-MSCs pellets were significantly lower than BM-MSCs. The lower level expression of particular markers of differentiation could be the result of prior in vivo exposure of T-MSCs to high concentrations of inflammatory cytokines, which are characteristically present in donor

tissue of chronic tonsillitis.[7] However, in this study, the levels of mRNA expressed from each differentiated pellets did not differ significantly between T-MSCs and BM-MSCs. The main cause of tonsillectomy in our cases is a chronic tonsillar hypertrophy.

MSCs from allogenic donor could constitute a valuable alternative source of stem cells for therapeutic application because there are some limitations of obtaining sufficient autologous MSCs. MSCs-mediated immune modulation effect is a complex mechanism that involves changes in the maturation of antigen-presenting cells, the suppression of monocyte derived dendritic cells, the cytokine secretion profile of dentritic cells, T cells, and natural killer cells.[9,10] The molecular mechanism that medicates the immunosuppression effect of MSCs is not completely understood.

MSCs exert profound immunosuppression by inhibiting T-cell proliferation and decreasing soluble factors in response to various stimuli in vitro.[9,11] This suppressive effect of MSCs is mediated through several inducible soluble factors, such as transforming growth factor-β, hepatocyte growth factors, interleukin-10, prostaglandin E2, and indoleamine 2,3-dioxygenase.[12-14] Janjanin et al. reported that T-MSCs inhibited the proliferation of T cells stimulated by allergenic T cells or by non-specific mitogenic stimuli (PHA) and decrease the secretion of IFN-γ via an indoleamine 2,3-dioxygenase-dependent mechanism. These results are consistent with our findings that T-MSCs inhibits the proliferation of T cells stimulated by allergenic T cells in MLR study and by PHA stimuli in mitogen proliferation assay and also decreases the secretion of TNF-α and IFN-γ. Janjanin et al.[7] reported that the immunomodulatory activity of T-MSCs was significantly less pronounced than that of BM-MSCs. However, in our study, the immune suppressive effect of T-MSCs was not significantly different when compared to BM-MSCs.

MSCs modify the proinflammatory Th1 profile toward Th2 anti-inflammatory profile.[12,13,15] In our study, T-MSCs inhibit the proinflammatory Th1 cytokines, such as TNF-α and IFN-γ, but do not show significant decreasing secretion of anti-inflammatory Th2 cytokine, such as IL-10. These results are supported that T-MSCs can also modify the conversion from Th1 to Th2 lymphocytes.

One of immune suppression mechanism of MSCs is a histocompatibility locus antigen (HLA)-G molecules.[16] Natural process allows fetal allografts to evade from rejection by the mother. This phenomenon is related with on HLA-G molecules.[17] HLA-G is a non-classical major MHC class I, which is expressed in three types of membrane-bound and four types of soluble isoforms.[18] HLA-G5 is a soluble form that mediates an immunosuppressive effect by inducing apoptosis of $CD8^+$ T cells and own regulating $CD4^+$ T-cell proliferation.[18] HLA-G5 expressed and secreted by MSCs contributes to the suppression of the NK lytic activity and IFN-γ secretion, the direct inhibition of allergenic T-cell responses, and the increase of IL-10 concentration in the alloreaction microenvironment.[19] In the ELISA and flow cytometry assay, T- MSCs that were stimulated by allergenic T cells or by non-specific mitogenic stimuli increased the secretion of HLA-G5. These results are supported that the immune suppression mechanism by T-MSCs are related with the increase of HLA-G5 level.

The current study demonstrated that T-MSCs do satisfy the phenotypical and functional definition of mesechymal stem cells, as has previously been reported in BM-MSCs. T-MSCs also displayed immunomodulatory effects that were associated with inhibiting T-cell proliferation, decreasing soluble factors, and increasing HLA-G5 secretion in response to various stimuli. These findings should contribute to improving clinical therapeutic trials using T-MSCs injection or infusion for immunomodulation purposes. Tonsil tissues that are harvested by otolaryngologist easily in the ENT field provide a new alternative source of MSCs. In future, T-MSCs will be useful and helpful for study using autologous MSCs in ENT field and may provide a good alternative source as allogeneic MSCs in therapeutic application.

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