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Comparison of immunomodulatory effects of placenta mesenchymal stem cells with bone marrow and adipose mesenchymal stem cells

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ABSTRACT

Mesenchymal stem cells (MSCs) are powerful sources for cell therapy in regenerative medicine because they can be isolated from various tissues, expanded, and induced into multiple-lineages. Of note, their immunomodulatory effects maximize the therapeutic effects of stem cells engrafted on host, making them an especially attractive choice. Recently, several varieties of placenta-derived stem cells (PDSCs) including chorionic plate-derived MSCs (CP-MSCs) have been suggested as alternative sources of stem cells. However, comparative studies of immunomodulatory effects for CP-MSCs among various MSCs are largely lacking. We examined and compared immunomodulatory function of CP-MSCs with that of BM-MSCs and AD-MSCs using co-culture system with activated T-cells derived from human umbilical cord blood (UCB) exposed to anti-CD3 and anti-CD28 which are T-cell activating monoclonal antibodies. All MSCs expressed markers of stem cells and three germ layers by RT-PCR. These cells also exhibited comparable immunomodulatory effects when they were co-cultured with activated T-cells in dose-dependent manner. However, expression of HLA-ABC and HLA-G was highly positive in CP-MSCs compared to other MSCs, and higher levels of cytokines of IL-2, IL-4, IL-13, and GM-CSF were detected in dose-dependent manner in CP-MSCs. Taken together, the results of the present study suggest that while CP-MSCs, BM-MSCs, and AD-MSCs all have immunomodulatory effects, CP-MSCs may have additional advantage over the other MSCs in terms of immunomodulation. In conjunction with other previous studies, CP-MSCs are suggested to be a useful stem cell source in cell therapy.

1. Introduction

Mesenchymal stem cell (MSC) is defined by adherency to tissue culture plastic and capacity to differentiate into multiple lineages with profiles of certain cell surface markers [1]. MSCs were originally isolated from bone marrow (BM) with subsequent isolation from other organs including adipose tissue, placenta, and umbilical cord blood [2–5]. MSCs have gained much attention because they have demonstrated a great potential for clinical use with the capacity to differentiate not only into mesoderm but also endoderm and neuroectoderm [6,7]. Furthermore, the characteristics of differentiation in tissue-specific manner endow a great promise to the use of these multipotent stem cells in the fields of regenerative medicine [8,9].

Besides the regenerative function, another important potential of MSCs is immune-related property [10]. In a number of *in vitro* studies, immunosuppressive effects of MSCs and their mechanism have been well described in which suppression of T-cell proliferation and inhibition of dendritic cell differentiation have been suggested as the key events. Immunosuppressive effects have been further confirmed in *in vivo* studies and are being evaluated in clinical trials in diseases such as refractory graft-versus-host disease and Crohn's disease. In addition, immune privilege and hypoimmunogenicity of MSCs are other aspects of immune-related property. Although controversy exists, there are evidences that MSC might be immune-privileged to freshly isolated NK cells preventing them from lysis and that low expression of human leukocyte antigen (HLA) major histocompatibility complex (MHC) class I with no expression of co-stimulatory molecules may play an important role. These properties are anticipated to give advantage to survival and engraftment of MSCs in transplanted setting [11,12].

Abbreviations: AD-MSCs, adipose-derived MSCs; AFP, a-fetoprotein; BM-MSCs, bone marrow-derived MSCs; CCl_4, carbon tetrachloride; CP-MSCs, chorionic plate-derived mesenchymal stem cells; GM-CSF, granulocyte macrophage colony-stimulating factor; HLA, human leukocyte antigen; IFN-γ, interferon-gamma; MHC, histocompatibility complex; MNCs, mononuclear cells; MSCs, mesenchymal stem cells; PDSCs, placenta-derived stem cells; TNF-α, tumor necrosis factor-alpha; UCB, umbilical cord blood.

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MSCs from diverse origins are currently available, and each type has its own strength and shortcomings. Recently, placenta-derived MSCs (PDSCs) have been suggested as an alternative source of stem cells. Similar to other MSCs derived from bone marrow and adipose, PDSCs were shown to differentiate into 3 germ layers and have immunomodulatory properties [13,14]. The merits of using PDSCs lie in that they are free from ethical concern in procurement and that relatively large amount of PDSCs can be readily obtained. Moreover, from a developmental point of view, placenta is an organ that originates during the early period of embryological stages and is fundamental for maintaining fetomaternal tolerance, which possibly indicate that PDSCs might harbor similar traces as well [15,16]. Our group has recently shown that chorionic plate-derived mesenchymal stem cells (CP-MSCs), a subset of PDSCs, can be used in *in vitro* screening system for hepatotoxicity, in which CP-MSCs were better than the bone marrow-derived mesenchymal stem cells (BM-MSCs). We also performed *in vivo* study to document the feasibility of CP-MSCs in ameliorating liver damage in CCl_4-induced cirrhotic rat model [17,18]. However, no study has been conducted yet to directly compare immune-related aspects of CP-MSCs with other MSCs.

Therefore, in this study, we investigated differences in immunomodulatory function between CP-MSC and other MSCs including BM-MSCs and adipose-derived MSCs (AD-MSCs).

2. Materials and methods

2.1. Culturing of CP-MSCs, BM-MSCs, and AD-MSCs

Full term normal human placentas (gestation ≥ 37 weeks) without medical, obstetrical, or surgical complications were collected after informed consent. Sample collection and utilization for this research were approved by the Institutional Review Board of CHA General Hospital, Seoul, Korea. To isolate CP-MSCs, the chorioamniotic membrane was peeled off and separated from the chorionic plate of placenta. They were chopped into small pieces, then washed with phosphate-buffered saline (PBS), and initially digested with 0.5% collagenase IV in PBS at 37.8 °C for 40 min, followed by vigorous shaking for 15 min at 15-min intervals over 90 min in 0.25% collagenase IV at 37.8 °C. An equal volume of Dulbecco's modified Eagle's medium (DMEM; Gibco-Invitrogen, Grand Island, NY) containing 10% fetal bovine serum (FBS; Gibco-Invitrogen) was added and centrifuged at 1000 *g* for 5 min to get the cell pellet. The harvested cells were resuspended in the culture medium contained DMEM/F12 (Gibco-BRL) supplemented with penicillin (100 U/ml), streptomycin (100 mg/ml), 25 ng/ml FGF4 (Peprotech, Inc., NJ), 1 mg/ml heparin (Sigma), 50 mg/ml gentamicin (Gibco-BRL), and 10% FBS (Gibco-BRL) and then incubated at 37.8 °C in an incubator with 5% CO_2. BM-MSCs (Cambrex Bioscience Walkersville, East Rutherford, NJ), AD-MSCs (provided by Dr. JH Sung, CHA University, Korea), and normal fibroblast cell line WI-38 (ATCC; Manassas, VA) were cultured using a culture medium containing alpha-MEM (Gibco-BRL-Invitrogen), supplemented with penicillin (100 U/ml), streptomycin (100 mg/ml), 1 mM sodium pyruvate (Gibco-BRL), and 10% FBS (Gibco-BRL).

2.2. RT-PCR

For RT-PCR analysis, CP-MSCs, BM-MSCs, AD-MSC, and WI38 were homogenized and lysed in 1 ml of TRIzol (Invitrogen, Carlsbad, CA). Total RNA was extracted with 200 ml of chloroform and precipitated with 500 ml of 80% (v/v) isopropanol. After the supernatant was removed, the RNA pellet was washed with 75% (v/v) ethanol, air-dried, and dissolved in 0.1% (v/v) diethyl pyrocarbonate-treated water. The RNA concentration was determined by measuring absorbance at 260 nm using a spectrophotometer. A reverse transcription reaction was performed with 1 mg of total RNA and SuperScriptTM III reverse transcriptase (Invitrogen). The cDNA was amplified using h-Taq DNA polymerase (Solgent, Seoul, Korea), according to the manufacturer's instructions. First-strand cDNAs were amplified in a final volume of 20 ml containing 0.5 U Taq DNA polymerase (Solgent) and 20 pmol of each human-specific target primers. The PCR primers and the size of the amplified products are shown in Table 1. Amplification reactions were performed on the following conditions: denaturation at 95.8 °C for 15 min followed by 35–40 cycles of denaturation at 95.8 °C for 30 s, annealing at 50–60.8 °C for 40 s, and elongation at 72.8 °C for 5 min. The PCR products were visualized and photographed following electrophoresis on a 1% (w/v) agarose gel containing 0.5 mg/ml ethidium bromide. cDNA samples were adjusted to yield equal actin amplifications.

2.3. T-cell proliferation analysis using ELISA

To assess the ability of MSCs to suppress T-cell proliferation, MSCs were first treated with mitomycin C of 50 μg/ml for 50 min to inactivate the proliferation of MSCs themselves. Next, human umbilical cord blood (UCB) mononuclear cells (MNCs) were prepared by centrifugation on a Ficoll Hypaque density gradient, and 2×10^5 cells of UCB MNCs were co-cultured with 2×10^3, 4×10^3, 1×10^4, and 2×10^4 cells of each MSC per well in 96-well culture plate with or without 1 μg/ml anti-CD3 and anti-CD28 T-cell activating mAbs (eBioscience, Inc., San Diego, CA, USA) for 72 h. To analyze the suppression of proliferative response of T-cell clonal expansion, clustering of T-cells was examined in bright field and BrdU ELISA (Roche) was performed according to the manufacturer's protocol at 72 h cultivation.

2.4. Flow cytometry analysis

For flow cytometry analysis, CP-MSCs, BM-MSCs, AD-MSC, and WI-38 (5×10^5 cells) were dissociated with cell dissociation buffer (Gibco-Invitrogen) and washed with PBS containing 2% (v/v) FBS. They were incubated with isotype control IgG or antigen-specific antibodies with various fluorescence-conjugated anti-human IgG antibodies (diluted 1:200; BD Biosciences, San Diego) for 30 min and propidium iodide (PI, 5 ng/ml; Sigma-Aldrich) was used to identify nonviable cells. Flow cytometry analysis was performed using a vantage Flow Cytometer (BD Biosciences, San Jose, CA).

2.5. Multiplex supernatant cytokine assay (Luminex)

The supernatant from co-culture of MSCs and T-cells was harvested with or without 1 μg/ml anti-CD3 and anti-CD28 activating mAbs after 72 h cultivation, and 50 μl of supernatant was combined

Table 1
Sequence of primers and length of fragments used for RT-PCR.

Genes	Sequence	Tm (°C)	Size (bp)
Oct4	F: 5′-ACA CTC GGA CCA CGT CTT TC-3′ R: 5′-CGT TCT CTT TGG AAA GGT GTT C-3′	54	300
Nanog	F: 5′-TTC TTG ACT GGG ACC TTG TC-3′ R: 5′-GCT TGC CTT GCT TTG AAG CA-3′	54	300
Sox2	F: 5′-GGG CAG CGT GTA CTT ATC CT-3′ R: 5′-AGA ACC CCA AGA TGC ACA AC-3′	52	200
NF-68	F: 5′-GAG TGA AAT GGC ACG ATA CCT A-3′ R: 5′-TTT CCT CTC CTT CTT CTT CAC CTT C-3′	58	500
Cardiac	F: 5′-GGA GTT ATG GTG GGT ATG GGT C-3′ R: 5-AGT GGT GAC AAA GGA GTA GCC A-3′	58	500
AFP	F: 5′-AGC TTG GTG GAT GAA AC-3′ R: 5′-TCC AAC AGG CCT GAG AAA TC-3′	50	200
HLA-G	F: 5′-GCG GCT ACT ACA ACC AGA GC-3′ R: 5′-GCA CAT GGC ACG TGT ATC TC-3′	58	900
TERT	F: 5′-GAG CTG ACG TGG AAG ATG AG-3′ R: 5′-CTT CAA GTG CTG TCT GAT TCC AAT G-3′	55	300
β-actin	F: 5′-TCC TTC TGC ATC CTG TCA GCA-3′ R: 5′-CAG GAG ATG GCC ACT GCC GCA-3′	58	300