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B7-H1 Inhibits T Cell Proliferation Through MHC Class II in Human Mesenchymal Stem Cells

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ABSTRACT

B7-H1 on mesenchymal stem cells (MSCs) is known to modulate immune response. However, its expression pattern and exact immunomodulatory mechanism are unclear. In this study, we examined the immunomodulatory mechanism through the expression pattern of B7-H1 and major histocompatibility complex class II in various MSCs. Human bone marrow, adipose tissue, and cord blood MSCs were isolated and cultured. B7-H1, HLA-ABC, and HLA-DR expression on MSCs by interferon-γ (IFN-γ) was detected time-dependently by flow cytometry. The inhibitory effect of MSCs on T lymphocytes was observed in phytohemagglutinin antigen–induced T cell proliferation assay. The expression of B7-H1 was rapidly induced, but the expression of HLA-DR was induced at 48 hours after IFN-γ treatment. The inhibitory effect of MSCs on T cell proliferation could be restored when the anti-B7-H1 monoclonal antibody was used to block the B7-H1, or when the HLA-DRα small interfering RNA was used to interfere with its expression. These results show that MSCs could inhibit the T cell proliferation and activation by B7-H1 depending on the presence of HLA-DR. Therefore, MSCs would have a strong effect on immune diseases such as graft-versus-host disease and autoimmune diseases when MSCs are primed with IFN-γ 48 hours before transplantation.

MESENCHYMAL STEM CELLS (MSCS) were first characterized from bone marrow (BM) by Friedenstein et al, and have been identified from many different tissue sources, such as adipose tissue (AT), cord blood (CB), and placenta [1–3]. MSCs are multipotent progenitor cells which can differentiate into a number of cell lineages [4]. Furthermore, they exert immunomodualatory and immunosuppressive properties. Many researchers have focused on paracrine mechanism via soluble factors including indoleamine 2,3-dioxygenase (IDO), hepatocyte growth factor, transforming growth factor-β, interleukin-10 (IL-10), prostaglandin E2, and unidentified factors [2]. Recently cell-to-cell contact mechanism has been reported to be also implicated via B7-H1 and FasL [3]. MSCs undergo functional polarization toward the inhibitory phenotype on exposure to proinflammatory cytokines such as interferon- γ (IFN-γ), tumor necrosis factor −α, and IL-1α. Especially IFN-γ strongly induces immunosuppressive molecules including IDO and B7-H1 [5].

B7-H1 (programmed death ligand-1) is found in various tissues and acts as an inhibitory costimulatory molecule during immune response [5,6]. MSCs also express B7-H1 which is known to modulate immune response [3]. However, its expression pattern and exact immunomodulatory mechanism are unclear [5]. In this study, we examined the immunomodulatory mechanism through the expression pattern of B7-H1 and major histocompatibility complex (MHC) class II in various MSCs.

MATERIALS AND METHODS

Isolation and Culture of Human BM, AT, and CB-MSCs

The Institutional Review Board of Ajou University Hospital approved this study and all samples were obtained with informed consent. BM- and CB-MSCs were isolated and cultured as previously

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described [2]. Briefly, mononuclear cells were isolated from normal BM aspirates or term CB of newborns using Ficoll-Hypaque density gradient centrifugation (Histopaque-1077, Sigma-Aldrich, St Louis, MO, United States). Cells were seeded on uncoated T25 culture flasks (Nalge Nunc, Naperville, IL, United States) at a density of 3×10^5 cells/cm^2 in low glucose Dulbecco's modified Eagle's medium (LG-DMEM; Invitrogen-Gibco, Rockville, MD, United States) containing 10% fetal bovine serum (FBS; Invitrogen-Gibco) and 100 U/mL penicillin/streptomycin (Invitrogen-Gibco). The cells were incubated in a humidified atmosphere at 37°C with 5% CO_2, and the medium was changed every 7 days until the adherent fibroblast-like cells reached confluence. Adherent cells were then resuspended with 0.05% trypsin–ethylenediaminetetraacitic acid (EDTA; Invitrogen-Gibco) and reseeded at 2×10^3 cells/cm^2. AT-MSCs were isolated and cultured according to a previous protocol [2]. Briefly, lipoaspirates were washed extensively with equal volumes of Dulbecco's phosphate-buffered saline (DPBS; HyClone, Logan, UT), and the extracellular matrix was digested with 0.075% collagenase A (Roche Applied Science, Penzberg, Germany) at 37°C for 30 minutes. Enzyme activity was neutralized with LG-DMEM containing 10% FBS, 100 U/mL penicillin/streptomycin; samples were centrifuged at 1200 g for 10 minutes. The cell pellet was washed with DPBS and filtered through a 100 μm Nylon mesh (Cell strainer; Becton Dickinson, Franklin Lakes, NJ). After 24 hours in culture, cells were washed with DPBS and incubated in a humidified atmosphere at 37°C with 5% CO_2 in medium. Adherent cells were resuspended with 0.05% trypsin–EDTA and reseeded at 2×10^3 cells/cm^2. In some experiments, 100 U/mL IFN-γ to MSC treatment was performed by 36 hours.

T-cell Proliferation: 5-bromo-2′-deoxyuridine Incorporation

BM-, AT-, and CB-MSCs were seeded at 3.75×10^4 cells/cm^2 in high glucose DMEM, 10% FBS, 100 U/mL penicillin/streptomycin. After 24 hours, 10 μg/mL mitomycin C (Sigma-Aldrich) was added to inhibit MSC proliferation, and cells were incubated for 2 hours at 37°C followed by five extensive washes with medium. Peripheral blood mononuclear cells (PBMCs) were isolated by density gradient centrifugation. A volume of 3×10^5 PBMCs/cm^2 were added and stimulated with 1 μg/mL phytohemagglutinin (PHA; Sigma-Aldrich). In case of transwell assay, PBMCs were added at the transwell upper-side. PHA-activated PBMCs were cultured in the presence or absence of MSCs. Cultures were plated in triplicate and incubated for 2 to 4 days before addition of 5-bromo-2′-deoxyuridine (BrdU). After 18 hours, proliferation was assessed using the BrdU-Assay kit (Roche Applied Science) according to the manufacturer's protocol.

Flow Cytometry

Antibodies against the human antigens HLA-ABC and HLA-DR were purchased from Becton Dickinson. Antibodies against B7-H1 were purchased from eBioscience (San Diego, Calif, United States). A total of 5×10^5 cells were resuspended in 0.2 mL PBS and incubated with fluorescein isothiocyanate- or phycoerythrin-conjugated antibodies for 20 minutes at room temperature. The fluorescence intensity of the cells was evaluated by flow cytometry using a flow cytometer (FC 500; Beckman Coulter) and the data were analyzed with the CXP software (Beckman Coulter).

Blocking Experiment

Neutralizing monoclonal antibody to 100 ng/mL human B7-H1 (eBioscience) was added 4 hours before PHA-induced T cell proliferation assay. The same concentration of mouse immunoglobulin G (vehicle) was used as an isotype control.

RNA Interference

MSCs were plated 24 hours before small interfering RNA (siRNA) transfection. Cells were incubated with siRNA-lipofectamine 2000 (Invitrogen) complex for 18 hours at 37 °C. The medium was then changed, and the transfected cells were incubated for an additional 12 hours until the target gene was effectively downregulated. siRNAs targeting HLA-DRα (sc-37113) and scrambled siRNA (sc-37007) were all purchased from Santa Cruz Biotechnology.

Statistical Analysis

Data were expressed as the mean ± standard deviation (SD). The results were considered significant when $P < .05$.

RESULTS

It has been shown that MSCs suppress the proliferation and activation of T cells stimulated with PHA. To determine the

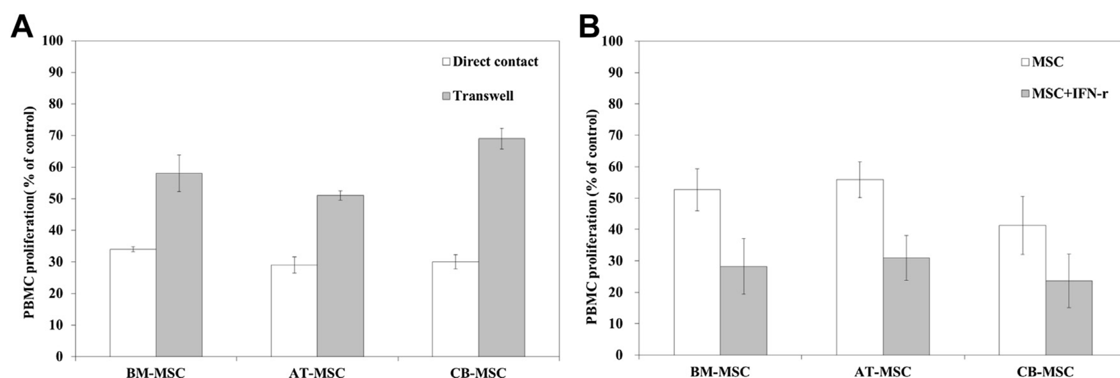

Fig 1. Immunosuppressive potential of BM-, AT-, and CB-MSCs. **(A)** PHA-induced proliferation of T cells in PBMCs in the absence or presence of different numbers of MSCs was evaluated on day 3 as the percentage of BrdU+ cells in direct contact (*white bar*) or transwell (*gray bar*) system. **(B)** The suppressive effect of IFN-γ−primed (*gray bar*) and naïve (*white bar*) MSCs was compared. T cell proliferation assay was performed after MSCs were primed with IFN-γ (100 U/mL) for 36 hours. The inhibition of T cell proliferation was more significant in IFN-γ−primed MSCs group than naïve MSCs group.

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