Cellular Immunology 264 (2010) 171–179

Contents lists available at ScienceDirect

Cellular Immunology

journal homepage: www.elsevier.com/locate/ycimm

Mesenchymal stromal cells use PGE2 to modulate activation and proliferation of lymphocyte subsets: Combined comparison of adipose tissue, Wharton's Jelly and bone marrow sources

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A R T I C L E I N F O

Article history:
Received 1 April 2010
Accepted 15 June 2010
Available online 18 June 2010

Keywords:
Mesenchymal stromal cells
Bone marrow
Adipose tissue
Wharton's Jelly
Immunomodulation
T-cell subsets
Lymphocyte activation/proliferation
Prostaglandin E2 (PGE2)
Hepatic growth factor (HGF)

A B S T R A C T

Due to their immunomodulatory properties, adipose tissue (AT) and Wharton's Jelly (WJ) constitute valuable alternatives to BM as sources of MSCs for managing graft-versus-host disease. To ensure the efficiency of AT- and WJ-MSCs implies the characterization of their immunomodulatory functions in comparison to those of BM. In this study, we investigated the capacity of AT- and WJ-MSCs to modulate lymphocyte reactions in response to different stimuli as well as the specificity of this immunomodulation. AT- and WJ-MSC displayed potent immunosuppressive effects on lymphocyte responses in a dose-dependent manner. These effects included the prevention of lymphocyte activation as well as the suppression of T-cell proliferation regardless of the stimuli used to activate lymphocytes. These effects were mediated through the expression of COX1/COX2 enzymes and by the production of PGE2. $CD4^+$ and $CD8^+$ T-lymphocytes were equally targeted by MSCs demonstrating that the immunomodulation was not restricted to a specific T-cell subpopulation.

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1. Introduction

Mesenchymal stromal cells (MSCs) are multipotent progenitors derived from different sources including bone marrow (BM) [1] and other adult and fetal tissues [2–4]. Among these tissues, adipose tissue (AT) [5] and Wharton's jelly (WJ) of the umbilical cord [6] have been considered as potential alternatives to BM. MSCs are characterized by their ability to differentiate into multiple lineages [7–8] and represent an attractive candidate for tissue regeneration and repair [9]. Furthermore, BM-MSCs display immunoregulatory activities and have emerged as promising tool for managing graft-versus-host disease (GVHD) and modulating autoimmune disorders [10–11]. MSCs exert immunosuppressive effects in vitro through the regulation of different immune cells. MSCs can suppress T-lymphocyte activation and proliferation induced by cellular or mitogenic stimuli [12]. Nevertheless, conflicting data still exist in the literature regarding the mechanisms by which MSCs modulate immune cells. The potential mechanisms include both direct cell–cell contact and the production of soluble mediators. These immunoregulatory factors include hepatic growth factor (HGF), transforming growth factor-β (TGF-β), interleukin (IL)-10, prostaglandin E2 (PGE2) and human leukocyte antigen (HLA) G5 [13]. In addition to T-lymphocytes, MSCs also target other immune cells, including B-lymphocytes, natural killer (NK) cells and dendritic cells. The regulation of these cells by MSCs is promoted by several other mechanisms [14].

Although the suppression of lymphocyte proliferation by AT- and WJ-MSCs have been reported [15], their effect on T-cell activation as well as on T-cell subpopulations were not defined. Our study aimed to compare these immunomodulatory effects and to investigate the molecular mechanisms involved in AT- and WJ-derived MSC immunosuppression.

2. Materials and methods

2.1. MSC isolation

BM was obtained from the sternum or iliac crest of 15 healthy donors. All individuals gave written consent and the study was approved by the local ethical committee. The mean age of the donors was 33 ± 2 years (range 16–41 years). MSCs were isolated using the classical adhesion method. Briefly, mononuclear cells (MNCs)

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doi:10.1016/j.cellimm.2010.06.006

were isolated by density-gradient centrifugation (LinfoSep, Biomedics, Madrid, Spain), washed in Hanks' Balanced Salt Solution (HBSS, Lonza Europe, Verviers, Belgium) and seeded at 2×10^4 cells/cm^2 in Dulbecco's Modified Eagle's Medium with low glucose (DMEM-LG, Lonza) supplemented with 15% fetal bovine serum (FBS, Sigma–Aldrich, Bornem, Belgium), 2 mM L-glutamine and 50 U/ml penicillin (both from Lonza).

After informed consent was obtained from the mother, umbilical cords (n = 20) were collected after full-term deliveries and were processed according to a previous protocol [16]. Immediately after obtention, cords were transferred to an aseptic saline buffer. Then, they were washed in HBSS (Lonza) and cut into small pieces that were sectioned longitudinally to expose the WJ under the amniotic membrane. MSCs were isolated based on their migratory and adhesive properties. WJ matrix was completely immersed in DMEM-LG culture medium for 15 days to allow the MSCs to exit and attach to the plastic surface. At the end of the culture period, umbilical cord fragments were removed from the wells, and cells were cultured until confluence.

Adipose tissue was obtained from patients undergoing liposuction procedure, after informed consent. Fresh lipoaspirates (n = 15) used to isolate AT-MSCs were processed according to a previous protocol [3]. Briefly, lipoaspirates were washed intensively with an equal volume of Dulbecco's Phosphate-Buffered saline (DPBS, Lonza), and the extracellular matrix was digested with 0.075% collagenase A (Roche Applied Science, Vilvoorde, Belgium) at 37 °C for 30 min. The samples were centrifuged at 1200 g for 10 min in complete culture medium.

After discarding the supernatants containing oil, primary adipocytes and collagenase solution, the stromal-vascular fraction pellet was then cultured.

2.2. MSC culture and expansion

Cell cultures were incubated at 37 °C in a 5% CO_2 humidified atmosphere. After 48 h, non-adherent cells were removed by washing, and the medium was changed twice a week. When subconfluence (80–90%) was achieved, adherent cells were trypsinized (Lonza) and expanded by replating at a lower density (1000 cells/cm^2).

2.3. Characterization of MSCs

MSCs were characterized according to the International Society for Cellular Therapy (ISCT) criteria [17] and following previously described protocols [18]. Briefly, MSCs were immunophenotypically defined by flow cytometry using conjugated monoclonal antibodies. The Colony Forming Units–Fibroblast (CFU-F) assay was performed to estimate the number of mesenchymal progenitors in the culture. To confirm their multilineage potential, MSCs were cultured in the appropriate induction media to assess adipogenic, osteogenic and chondrogenic differentiation.

2.4. Lymphocyte proliferative

Peripheral blood (PB) samples were collected from healthy donors after informed consent was obtained. Mononuclear cells were isolated as described for BM-MNCs. CD3 T-lymphocytes were purified by positive selection using the MACS system (Miltenyi Biotec GmbH, Bergisch, Germany) according to the manufacturer's instructions. The purity of the selected cells was always above 95%, as determined by flow cytometry. CD3 T-lymphocytes (1×10^5) were activated by allogeneic or mitogenic stimulation. For the mitogenic assay, CD3 T-cells were stimulated with 5 μg/ml phytohemagglutinin (PHA, Remel Europe, Kent, UK) and 20 U/ml IL2, (Biotest AG, Dreieich, Germany). For the allogeneic assay, we performed mixed leukocyte reactions (MLRs) using irradiated allogeneic PBMCs as stimulating cells (1×10^5) to activate CD3 T-lymphocytes. Activated T-lymphocytes were added to plated, irradiated MSCs (1-day-ahead co-cultures) in 96-well plates and incubated for 5 days. We tested different MSC concentrations (MSC/T-cell ratios from 1/40 to 1/1) to investigate their importance in the MSC-mediated effects.

Lymphocyte proliferation was assessed by 5-bromo-2-deoxyuridine (BrdU) incorporation or after carboxyfluorescein diacetate-succinimidyl ester (CFDA-SE) labeling. For BrdU incorporation (Roche Applied Science, Mannheim, Germany), 50 μM BrdU was added to the co-cultures at day four. T-cell proliferation was evaluated by measuring BrdU incorporation in a colorimetric assay according to the manufacturer's instructions. Data were expressed as the percentage of allogeneic proliferation.

For CFDA-SE labeling (CellTrace™ CFSE Cell Proliferation Kit, Invitrogen, Molecular Probes, Eugene, OR, USA), 10 μM of CFDA-SE dye was used to stain 10^7 T-cells before co-incubation with MSCs. After 5 days of co-culture, CFSE fluorescence was visualized by flow cytometry and the results were expressed as the percentage of positive T-cells. Samples were run on a FACS Calibur (BD Biosciences, CA, USA) and analyzed using CellQuest software (BD Biosciences).

In order to assess whether the MSCs antiproliferative effect targets specific T-cell subpopulations and whether this effect was associated with the inhibition of T-cell activation, we evaluated the impact of MSCs on CD4 and CD8 positive T-lymphocyte as well as on the lymphocyte activation marker expression such as CD38. Anti-CD4-FITC, anti-CD8-PE and anti-CD38-PC5 antibodies (Immunotech, Marseille, France) were used for these experiments and data analyzed by flow cytometry.

2.5. Quantitative real-time PCR

We conducted quantitative real-time PCR (qRT-PCR) to assess COX1 and COX2 gene expression in MSCs derived from the three sources. For qRT-PCR, total RNA was extracted with Trizol reagent according to the manufacturer's guidelines (Invitrogen, Merelbeke, Belgium), and first-strand cDNAs were synthesized by standard reverse transcription (Superscript First-strand Synthesis System for RTPCR kit; Invitrogen). We used 25 ng of cDNA in qRT-PCR reactions with SYBR® Green PCR Master Mix (Applied Biosystems, Rotterdam, The Netherlands) and 0.32 μM of gene-specific forward and reverse primers (Invitrogen). The amplification was performed on an ABI Prism 7900 HT Sequence Detection System (Applied Biosystems) using 40 cycles of a two-step PCR (15 s at 95 °C and 60 s at 60 °C) after an initial activation step (95 °C for 10 min). Melting curves from 60 to 99 °C were assessed to evaluate PCR specificity. Serial dilutions of purified amplicons served to generate standard melting curves. Amplification of β-actin mRNA (as an endogenous control) was used to standardize the amount of sample added to the reactions. Each sample was examined in triplicate, and a mean value was calculated. The data are presented as the relative mRNA levels.

The following primers were used:
COX1 (forward) 5′- GAGTTTGTCAATGCCACCT-3′
(reverse) 5′- CAACTGCTTCTTCCCTTTG-3′
COX2 (forward) 5′- TCCTTGCTGTTCCCACCCATG-3′
(reverse) 5′- CATCATCAGACCAGGCACCAG-3′
β-actin (forward) 5′- CTGGCACCCAGCACAATG -3′
(reverse) 5′- CCGATCCACACGGAGTACTTG -3′

2.6. Cytokines quantification assay

PGE2 and HGF levels were measured in cell culture supernatants using the ELISA technique, according to the manufacturer's

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