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Human adipose tissue−derived mesenchymal stromal cells promote B-cell motility and chemoattraction

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Abstract

Background aims. Mesenchymal stromal cells hold special interest for cell-based therapy because of their tissue-regenerative and immunosuppressive abilities. B-cell involvement in chronic inflammatory and autoimmune pathologies makes them a desirable target for cell-based therapy. Mesenchymal stromal cells are able to regulate B-cell function; although the mechanisms are little known, they imply cell-to-cell contact. *Methods.* We studied the ability of human adipose tissue−derived mesenchymal stromal cells (ASCs) to attract B cells. *Results.* We show that ASCs promote B-cell migration through the secretion of chemotactic factors. Inflammatory/innate signals do not modify ASC capacity to mediate B-cell motility and chemotaxis. Analysis of a panel of B cell−related chemokines showed that none of them appeared to be responsible for B-cell motility. Other ASC-secreted factors able to promote cell motility and chemotaxis, such as the cytokine interleukin-8 and prostaglandin E_2, did not appear to be implicated. *Conclusions.* We propose that ASC promotion of B-cell migration by undefined secreted factors is crucial for ASC regulation of B-cell responses.

Key Words: *B cells, chemotaxis, mesenchymal stromal cells, migration*

Introduction

Mesenchymal stromal cells (MSCs) are multipotent adult stem cells able to differentiate into distinct cell types. They are present in practically all tissues and are a key element in tissue repair and regeneration [1]. In addition to their differentiation potential, MSCs can modulate the function of several immune cell types through secretion of soluble factors and/or cell contact−dependent mechanisms [1,2]. This feature makes them an attractive potential tool for cell-based therapy for chronic inflammatory and autoimmune pathology. The MSC-mediated immunosuppressive effects on T-cell responses are well established [3,4]; there is also evidence that they inhibit natural killer cell function [5], regulate dendritic cell maturation and trafficking [6,7] and modulate macrophage differentiation [8]. In contrast, little is known of the MSC effect on B-cell function.

B cells are central to the adaptive immune response; they specialize in antigen recognition and the production of highly specific antibodies. They also have an important role as antigen-presenting cells for T cells and regulate immune responses through interleukin (IL)-10 secretion. Nevertheless, B cells participate in the development of numerous autoimmune disorders [9−11] and are crucial mediators in allograft rejection [12]. The beneficial effects of B-cell−depleting therapies on chronic inflammatory (Crohn's disease) and autoimmune pathologies (rheumatoid arthritis) stress the relevance of targeting B cells for immunosuppression in such diseases. Some reports point to a suppressive effect of MSCs on antigen-triggered B-cell activation and differentiation [13−18]. The extent of B-cell suppression appears to depend on the MSC source [15] and the need for B cell−MSC contact [14,18]. We studied the ability of human MSCs derived from adipose tissue (ASCs) to

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promote B-cell migration and chemoattraction to facilitate ASC–B-cell contact.

Methods

Cell isolation and culture

Human adult MSCs were obtained from adipose tissue of 3 donors (donors 10, 14 and 16), as described [19]. Briefly, lipoaspirates of human adipose tissue from healthy donors were washed with phosphate-buffered saline (PBS), digested with 0.075% collagenase type-I (Invitrogen, Camarillo, CA, USA; 37°C, 30 min), washed with PBS/10% fetal bovine serum (FBS) and treated with ammonium chloride to eliminate residual erythrocytes. Cells were cultured in Dulbecco's modified Eagle's medium/10% FBS and passaged when they reached 90% confluence. Cells were characterized phenotypically, as described [19]. ASCs from donors 10, 14 and 16 were used at population doubling (pd) 14, pd 12 and pd 14, respectively. Primary human foreskin fibroblasts were obtained from a healthy donor and expanded *in vitro*. The human endothelial cell line Eahy derived from human umbilical vein endothelial cells was kindly provided by Dr J. Millán (Centro de Biología Molecular Severo Ochoa, Madrid). The human fibrosarcoma cell line HT1080 from the American Type Culture Collection was kindly provided by Dr J.M. Rodríguez-Frade (CNB-CSIC, Madrid).

Human B cells were obtained from buffy coats of healthy donors, kindly provided by the National Transfusion Centre of the Comunidad Autonoma de Madrid. Briefly, each blood sample was diluted 1:1 in PBS and centrifuged on a Ficoll density gradient (GE Healthcare Madrid, Spain; 1800 rpm, 30 min, 25°C). The peripheral blood mononuclear cell interface was recovered, washed in Roswell Park Memorial Institute medium (RPMI)/10% FBS, and the B-cell population was purified with the use of the Dynabeads Untouched Human B cells isolation kit (Invitrogen); purity was confirmed by CD19 staining and flow cytometry (≥98%).

Experimentation with human tissue samples was approved by the CNB-CSIC Bioethics Committee and conforms to institutional, national and European Union regulations.

Flow cytometry analysis

Human B cells (2×10^5) were stained with the use of fluorescently labeled antibodies to CD19, CD27, immunoglobulin (Ig)M, IgD, C-X-C-motif chemokine receptor 4 (CXCR4) and C-C-motif chemokine receptor 2 (CCR2) (BioLegend, San Diego, CA, USA) or with antibodies to CXCR1, CXCR2 (Biosource, Invitrogen) and CXCR3 (R&D Systems, Minneapolis, MN, USA), followed by fluorescently labeled Fab anti-mouse IgG (Jackson Immunoresearch Labs, West Grove, PA, USA) in PBS/1% bovine serum albumin/1% FBS (20 min, 4°C); B cells were washed and analyzed in a FACScalibur flow cytometer (BD Biosciences, San Jose, CA, USA).

B-cell migration assays

ASCs (3×10^4) were seeded in p24 multiwell plates (24 h), and stimuli were added (interferon [IFN]-γ, 30 ng/mL, Peprotech (London, UK); the Toll-like receptor [TLR]4 ligand lipopolysacharide [LPS], from *Salmonella typhymurium*, 0.1 μg/mL, List Biological Laboratories (Campbell, CA, USA); the TLR3 ligand poly I:C, 0.1 μg/mL, Invivogen, San Diego, CA, USA) in a final volume of 500 μL RPMI/10% FBS per well. Supernatants were collected 72 h after stimulation and used immediately in the B-cell migration assay. Eahy cells, primary fibroblasts and HT1080 cells (3×10^4), were seeded in p24 multiwell plates in a final volume of 500 μL RPMI/10% FBS per well; supernatants were collected at 72 h and used immediately in the B-cell migration assay.

Freshly purified B cells (3×10^5) were loaded into the top insert of Boyden chambers (5-μm pore size, Corning, Tewksbury, MA, USA) in a final volume of 100 μL RPMI/10% FBS; the bottom well was filled with 600 μL supernatant (obtained from ASCs, Eahy, fibroblast or HT1080 cell culture), RPMI/10% FBS alone or with recombinant human C-X-C-motif ligand 12 (CXCL12), C-C-motif ligand 2 (CCL2), CXCL8, CXCL10 (Peprotech) or prostaglandin E_2 (PGE_2; Sigma-Aldrich, St Louis, MO, USA) at specified concentrations, in duplicate or triplicate. In assays with CCL2, the top insert was precoated with fibronectin (20 μg/mL, 1 h, 37°C; Sigma) to facilitate B-cell migration. Plates were incubated for 3 h (37°C, 5% CO_2). Where indicated, B cells were pre-incubated with anti-CXCR4 blocking antibody (clone 12G5, BioLegend; 10 μg/mL, 20 min, 37°C, with shaking) or with the isotype control (mouse IgG2a, BioLegend) and loaded into the Boyden chamber; the antibodies remained present throughout the assay. B cells that migrated to the bottom well were counted for 1 min at high flow rate in the flow cytometer; B-cell input was estimated similarly, with 3×10^5 B cells added to 600 μL RPMI/10% FBS. Migration frequency in each condition was calculated as the ratio between the number of B cells in the bottom well and total B-cell input and multiplied by 100.

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