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Comparative analysis of the immunomodulatory capacities of human bone marrow– and adipose tissue–derived mesenchymal stromal cells from the same donor

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Abstract

Background aims. The immunomodulatory properties of mesenchymal stromal cells (MSCs), together with their tissue regenerative potential, make them interesting candidates for clinical application. *Methods.* In the current study, we analyzed the *in vitro* immunomodulatory effects of MSCs derived from bone marrow (BM-MSCs) and from adipose tissue (AT-MSCs) obtained from the same donor on both innate and acquired immunity cells. BM-MSCs and AT-MSCs were expanded to fourth or fifth passage and co-cultured with T cells, monocytes or natural killer (NK) cells isolated from human peripheral blood and stimulated *in vitro*. The possible differing impact of MSCs obtained from distinct sources on phenotype, cell proliferation and differentiation, cytokine production and function of these immune cells was comparatively analyzed. *Results.* BM-MSCs and AT-MSCs induced a similar decrease in NK-cell proliferation, cytokine secretion and expression of both activating receptors and cytotoxic molecules. However, only BM-MSCs significantly reduced NK-cell cytotoxic activity, although both MSC populations showed the same susceptibility to NK-cell-mediated lysis. AT-MSCs were more potent in inhibiting dendritic-cell (DC) differentiation than BM-MSC, but both MSC populations similarly reduced the ability of DCs to induce $CD4^+$ T-cell proliferation and cytokine production. BM-MSCs and AT-MSCs induced a similar decrease in T-cell proliferation and production of inflammatory cytokines after activation. *Conclusions.* AT-MSCs and BM-MSCs from the same donor had similar immunomodulatory capacity on both innate and acquired immunity cells. Thus, other variables, such as accessibility of samples or the frequency of MSCs in the tissue should be considered to select the source of MSC for cell therapy.

Key Words: *adipose tissue, bone marrow, dendritic cell, human mesenchymal stromal cell, immunomodulation, NK cell, T cell*

Introduction

Mesenchymal stromal cells (MSCs) are multipotent cells capable of differentiating into various mesodermal lineages including adipogenic, osteogenic and chondrogenic cells [1]. An important property of MSCs is their immunomodulatory capacity [2–4]. MSCs are able to suppress the proliferation of T cells [5–7], B cells [8] and natural killer (NK) cells [9,10]; inhibit the differentiation and maturation of dendritic cells (DCs) [11,12]; and promote the generation of regulatory T cells [13,14].

These abilities have been the subject of many studies over the past few years and make MSCs an attractive therapeutic approach for a number of diseases. The use of MSCs has been shown to modulate immune responses both in experimental disease models, including experimental autoimmune

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encephalomyelitis [15] and graft-versus-host disease (GvHD) [16], and in clinical trials, including promotion of engraftment after hematopoietic stem cell transplantation [17–19] treatment of GvHD [20] and Crohn's disease [21], among others.

Although bone marrow (BM) has been the main source of MSCs and BM-MSCs have been most widely used for clinical applications, the finding of alternative sources is currently under investigation. The frequency of MSCs in BM is low and other cell sources including adipose tissue (AT), placenta, umbilical cord blood, and dental pulp are being evaluated. AT is a particularly interesting alternative source of MSCs because it generally yields a higher amount of MSCs [22–24].

Studies comparing MSCs derived from different sources have been focused on their phenotype, transcription profiling and differentiation potential [25–31] but also on their immunomodulatory properties [28,30,32–35], with controversial results. Whereas some authors reported that BM-MSCs and AT-MSCs have similar immunomodulatory capacities [16,30,33,36], others described a greater potential for AT-MSCs [28,32,34,35]. These differences could be in part due to different culture media or expansion protocols [31]. Additionally, some degree of heterogeneity can be explained by donor-to-donor variation, with donor age an important variable because the composition of the MSC population and the functional properties of MSCs change with age [37–40]. Furthermore, MSCs derived from BM and AT of the same donor have been only minimally tested. Finally, most studies focused on the immunosuppressive effects of MSCs on a unique immune cell type: T cells, B cells, NK cells or DCs.

The aim of this study is to clarify the controversy surrounding BM- and AT-MSC immunomodulatory potency and to determine the advantages and disadvantages of BM versus AT as sources of MSCs for cell therapy. Thus, we analyzed for the first time (to our knowledge) the immunomodulatory capacities of BM-MSCs and AT-MSCs obtained from the same donor and compared the effects not on a single cell type, but on both innate and acquired immunity cells. Our results demonstrated that AT-MSCs and BM-MSCs from the same donor had similar immunomodulatory capacities on both innate and acquired immunity cells.

Methods

MSC isolation, expansion and characterization

BM and AT samples were obtained from 11 volunteer donors (5 men/6 women) undergoing lumbar orthopedic surgery without other comorbidities. Median age was 51.7 years (range 31–67 years). Five to 10 mL of BM were obtained by iliac crest aspiration under local anesthesia. AT was obtained by surgical resection. All samples were collected after informed consent was obtained. All procedures were approved by the Clinical Research Ethics Committee of the Health Area of Salamanca and were in accordance with the Helsinki Declaration of 1975 (revised in 2000).

To obtain MSCs from BM, low-density mononuclear cells (MNCs) were isolated by Ficoll-Paque (GE Healthcare Bio-Sciences) density gradient centrifugation and cultured in Dulbecco's Modified Eagle's Medium—low glucose (DMEM; Gibco, Life Technologies) supplemented with 5% human platelet lysate, obtained as previously described [41], 2 U/mL heparin (Biochrom), 100 U/mL penicillin and 100 µg/mL streptomycin (Gibco), referred to as DMEM complete medium.

To isolate MSCs from AT, 3 g of fat were digested at 37°C and shaken for 1 h with 0.2% collagenase type I (Merck) in phosphate buffered saline (PBS) 2% bovine serum albumin. The digested sample was filtered through a 40-µm nylon mesh and centrifuged 10 min at 1800 rpm. The pellet was then treated with ACK lysis buffer (Gibco) for 15 min on ice and washed twice with PBS, and the obtained MNCs were cultured in DMEM-complete medium.

Both BM- and AT-derived MNCs were seeded at an initial density of 10^6 cells/cm^2 in culture flasks (Corning) and maintained in DMEM-complete medium in a humidified incubator at 37°C with 5% CO_2. Twice a week, adherent cells were fed by complete replacement of the medium (and non-adherent cells subsequently washed out). When the layer was 80% confluent, the culture was trypsinized, and cells were passed to new culture flasks at a cell density of 5×10^3 cells/cm^2. Cells underwent three passages in culture to ensure that all contaminating hematopoietic cells had been totally washed out of the flask. Cells were then frozen in fetal calf serum (FCS, Gibco)/10% dimethyl sulfoxide at –80°C until use.

After the third passage, MSCs were immunophenotypically characterized by flow cytometry using the following fluorochrome-conjugated monoclonal antibodies: CD34-PE, CD90-PE, CD117-PE, CD73-FITC, CD166-FITC and CD29-PE (BD Biosciences); CD10-FITC and CD105-FITC (BioLegend); CD45-FITC, CD44-FITC and CD80-FITC (Beckman-Coulter); CD31-PE (eBioscience). Cells were acquired on a FORTESSA flow cytometer (BD Biosciences). Data were analyzed with FlowJo Analysis Software (FlowJo).

Isolation of human NK cells, T cells and monocytes

Peripheral blood mononuclear cells (PBMCs) were isolated from 20 buffy coats of volunteer healthy donors by density gradient centrifugation using Ficoll-Paque solution. Buffy coats were provided by the Centro de Hemodonación de Castilla y León