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The effect of pro-inflammatory cytokines on immunophenotype, differentiation capacity and immunomodulatory functions of human mesenchymal stem cells

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ABSTRACT

Mesenchymal stem cells (MSCs), as cells with potential clinical utilities, have demonstrated preferential incorporation into inflammation sites. Immunophenotype and immunomodulatory functions of MSCs could alter by inflamed-microenvironments due to the local pro-inflammatory cytokine milieu. A major cellular mediator with specific function in promoting inflammation and pathogenicity of autoimmunity are IL-17-producing T helper 17 (Th17) cells that polarize in inflamed sites in the presence of pro-inflammatory cytokines such as Interleukin-1β (IL-1β), IL-6 and IL-23. Since MSCs are promising candidate for cell-based therapeutic strategies in inflammatory and autoimmune diseases, Th17 cell polarizing factors may alter MSCs phenotype and function. In this study, human bone-marrow-derived MSCs (BM-MSC) and adipose tissue-derived MSCs (AD-MSC) were cultured with or without IL-1β, IL-6 and IL-23 as pro-inflammatory cytokines. The surface markers and their differentiation capacity were measured in cytokine-untreated and cytokine-treated MSCs. MSCs-mediated immunomodulation was analyzed by their regulatory effects on mixed lymphocyte reaction (MLR) and the level of IL-10, TGF-β, IL-4, IFN-γ and TNF-α production as immunomodulatory cytokines. Pro-inflammatory cytokines showed no effect on MSCs morphology, immunophenotype and co-stimulatory molecules except up-regulation of CD45. Adipogenic and osteogenic differentiation capacity increased in CD45+ MSCs. Moreover, cytokine-treated MSCs preserved the suppressive ability of allogeneic T cell proliferation and produced higher level of TGF-β and lower level of IL-4. We concluded pro-inflammatory cytokines up-regulate the efficacy of MSCs in cell-based therapy of degenerative, inflammatory and autoimmune disorders.

1. Introduction

Inflammatory abnormalities comprise a large set of disorders that trigger a vast variety of human diseases. Autoimmune diseases and organ transplant rejection are example of immune-mediated inflammations. Autoimmunity, including type 1 diabetes, multiple sclerosis, inflammatory bowel disease, rheumatoid arthritis and other immune-inflammatory diseases results from the dysregulation of the immune system leading to tissue damage [1]. CD4+ helper T (Th) lymphocytes including, Th1 and Th17 cells are known to be cellular mediators of inflammation in autoimmunity and Th17 cells play a critical role in pathogenesis of inflammatory autoimmune disorders [2,3]. Th17 cells were identified on the basis of their ability to produce Interleukin (IL)-17, IL-21, IL-22, IL-23 and IL-6. As all of these cytokines have pro-inflammatory properties, it is not surprising that Th17 cells are implicated in a wide range of inflammatory disorders [4]. A clear association is reported between severity of inflammatory conditions and Th17 response in human and mice [5,6]. The differentiation of human Th17 cells requires a combination of pro-inflammatory cytokines including IL-1β, IL-6 and IL-23 [7,8] which their concentration obviously enhances in inflammatory environments.

Mesenchymal stem cells (MSCs) are a population of adult multipotent stem cells and stromal cells that migrate to and proliferate within damaged, inflamed and malignant tissues as part of the tissue regenerating process, also displaying immunomodulatory properties [9,10]. MSCs exist in most tissues in the body for instance bone-marrow, adipose tissue, peripheral blood, dental

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pulp, lung, skeletal muscle and many other tissues [11]. Human MSCs are defined according to three criteria: (1) Property to adhere to plastic, (2) their phenotype: CD44+, CD73+, CD90+, CD105+, CD11b−, CD34−, CD45−, HLA-DR−, they also do not express the co-stimulatory molecules CD40, CD80, CD83 and CD86, and (3) their capacity to be differentiated into three mesodermal lineages: chondrocyte, osteoblast and adipocyte [12]. Due to lacking the expression of co-stimulatory molecules and HLA-II, MSCs are regarded as non-immunogenic cells and therefore, their transplantation into allogeneic host may not require immunosuppressive treatments [13]. Moreover, MSCs have immunomodulatory properties as well and can suppress and inhibit the activation, maturation and proliferation of innate and adaptive immune cells (B cells, T cells, NK cells, dendritic cells and macrophages) [14]. MSCs show their immunomodulatory functions through direct cell-cell contact and secretion of soluble factors such as indoleamine-2,3-dioxygenase (IDO), nitric oxide (NO), prostaglandin-E2 (PGE-2), IL-10 and transforming growth factor-β (TGF-β) and can be clinically applied as a new therapeutic for treating a variety of immune-mediated inflammations such as graft-versus-host reaction (GVHD) [15], autoimmune diseases [16] and organ transplant rejection [17,18]. In addition, MSC therapy is a novel approach in cell-based therapy of degenerative and inflammatory disorders.

The specific cytokine milieu within the site of inflammation is vital in determining the fate of immune responses and cell behaviors. Concentration of pro-inflammatory cytokines e.g. IL-1, TNF-α, IFN-γ, IL-2, IL-6 and other inflammatory factors increases in microenvironment of inflammation, e.g. site of transplant rejection and autoimmune-mediated injuries [19]. Following this, migratory immune cells differentiate into effector cells. Th17 cells as an inflammatory subset of helper T cells polarize in the site of inflammation in the presence of such pro-inflammatory factors IL-1β, IL-6 and IL-23. Since the MSCs recruit and migrate into injury and inflammatory areas, it is supposed that their phenotype and immunomodulatory functions alter following exposure to the inflammatory situation [20]. Indeed it is well established that MSCs exposed with IFN-γ, as a well-known inflammatory cytokine, express more IDO [21], TGF-β and IL-10 [22] and demonstrating more efficient immunomodulatory activity compare with MSCs that were not exposed to inflammatory condition [23]. Pre-treatment of MSCs with TNF-α and IFN-γ also excite MSCs to produce more IDO and PGE-2 [24]. It is also reported that T cell proliferation could be suppressed by MSCs treated with concomitant addition of either TNF-α, IL-1α or IL-1β along with IFN-γ [25]. Pro-inflammatory cytokines which are released by inflamed-tissues, up-regulate the expression of chemokine receptors and IL-8, that are needed for homing of MSCs and angiogenesis, respectively [26]. Taken together, it is well accepted that cohabitation of MSCs with pro-inflammatory cytokines can modulate their behaviors in situ of inflamed and injured tissues.

As mentioned above, Th17 cells are one of the main cellular components of inflammatory conditions especially chronic form, which are derived from their precursor cells in situ and under influence of special combination of cytokines which are present in inflammatory microenvironment. It is expected that the same microenvironment alters the immunomodulatory properties of MSCs which are used for cell therapy of inflammatory and chronic diseases such as autoimmune disorders. The outcome of each particular combination of cytokines effects on MSCs could be down- or up-regulation of their immunomodulatory and regenerative properties which need to be clarified. This study was set-up to demonstrate the probable effects of human Th17 cell polarizing factors (IL-1β, IL-6 & IL-23) as pro-inflammatory cytokines on the morphology, immunophenotype, multilineage differentiation capacity and immunomodulatory function of human bone-marrow-derived (BM-MSCs) and adipose tissue-derived (AD-MSCs) mesenchymal stem cells.

2. Materials and methods

2.1. Mesenchymal stem cell isolation and culture

Human bone-marrow-derived MSCs (BM-MSCs) and adipose tissue-derived MSCs (AD-MSCs) were obtained from bone-marrow and adipose tissues of persons at ROYAN Institute's Cell Therapy Center. Ethical approval was obtained from Royan Institute Ethic Committee prior to any experimental work. Briefly, according to ROYAN Institute standard protocol for purification of MSCs from bone-marrow, 5 ml of bone-marrow aspirates were diluted with 5 ml PBS loaded over Lymphodex (Inno-Train, Sweden), and centrifuged at 400*g* for 20 min at room temperature. The mononuclear cells were separated based on density gradient. The cells were collected from the media-Lymphodex interface were washed twice with PBS/EDTA and plated at 1×10^6 cells/ml in alpha-Minimum Essential Medium (α-MEM; Sigma-Aldrich, USA) supplemented with heat-inactivated 10% fetal bovine serum (FBS, Gibco, USA), 100 IU/ml penicillin and 100 μg/ml streptomycin (Gibco, USA), 2 mM L-glutamine (Gibco, USA) and 1% non-essential amino acids (NEAA; Sigma-Aldrich, USA). Cultures were maintained at 37 °C in a humidified 5% CO_2. Three days after initial culture, the fresh medium was replaced and non-adherent cells were removed. The medium was changed every 3 days to achieve 70–80% cell-confluency. Additional passages were performed to obtain sufficient cells which were achieved at passage 3–5.

Human AD-MSCs were obtained from adipose tissues of persons at ROYAN Institute's Cell Therapy Center. In brief, according to ROYAN Institute standard protocol for purification of MSCs, the lipo-aspirate was extensively washed and the red cells were lysed. The floating adipose tissue was digested with 0.2% collagenase for 30–60 min to break down the extracellular matrix. The cells were then filtered and centrifuged. After spinning down, the resulting pellet is known as the stromal vascular cell fraction (SVF). Cells within the SVF were cultured in α-MEM supplemented with heat-inactivated 10% fetal bovine serum (FBS, Gibco, USA), 100 IU/ml penicillin and 100 μg/ml streptomycin (Gibco, USA), 2 mM L-glutamine (Gibco, USA) and 1% non-essential amino acids (NEAA; Sigma-Aldrich, USA). Hereafter, the culture condition is the same as BM-MSCs to obtain purified AD-MSCs.

2.2. Treatment of MSCs with pro-inflammatory cytokines

MSCs were treated for 96 h with human recombinant cytokines IL-1β (20 ng/ml), IL-6 (40 ng/ml) and IL-23 (20 ng/ml) as pro-inflammatory factors require for polarization of human Th17 lymphocytes, as an inflammatory component of T helper cells. All recombinant cytokines were purchased from Peprotech (London, U.K.). Cultured but cytokine-untreated MSCs (BM-MSCs and AD-MSCs) were used as controls.

2.3. Phenotypic characterization of MSCs

Cytokine-treated and untreated adherent MSCs were trypsinized using trypsin-EDTA solution for 2–3 min at 37 °C, resuspended in α-MEM and washed after single cell harvesting. The cells were then assessed for expression of surface markers CD11b (PE-conjugated Mouse Anti-Human CD11b; BD Pharmingen), CD34 (FITC-conjugated Mouse Anti-Human CD34; BD Pharmingen), CD44 (FITC-conjugated Mouse Anti-Human CD44; BD Pharmingen), CD45 (FITC-conjugated mouse Anti-Human CD45; BD Pharmingen), CD73 (PE-conjugated Mouse Anti-Human CD73; BD Pharmingen), CD90 (FITC-conjugated Mouse Anti-Human CD90; BD Pharmingen), CD105 (PE-conjugated Mouse Anti-Human CD105; R&D Systems) and HLA-DR (PE-conjugated Mouse