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Mesenchymal stem cells regulate the proliferation of T cells via the growth-related oncogene/CXC chemokine receptor, CXCR2

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ABSTRACT

Mesenchymal stem cells (MSCs) have known to induce immunosuppressive properties by preventing T cell proliferation. However, it is remains unclear how MSCs inhibit T cell proliferation. To identify the factor that inhibits T cell proliferation, we conducted a cytokine array analysis of culture medium from a co-culture of MSCs and T cells and found that the chemokines, CXCL1, 2 and 3, were induced in T cells. MSCs also induced the expression of the CXCR2 receptor on T cell surface. Particularly, CXCL3 inhibited proliferation and increased apoptosis in T cells, which were reversed by CXCR2 inhibitor treatment. Moreover, CXCL3 decreased JAK2, STAT3, and AKT phosphorylation and these responses were also abolished by CXCR2 inhibitor treatment. MSCs suppressed the proliferation of T cells into tumor tissue. Collectively, these data demonstrate that MSCs directly regulate T cell proliferation by induction of CXCL3 chemokine and its receptor, CXCR2 on the surface in T cells.

1. Introduction

Mesenchymal stem cells (MSCs) have multipotential differentiation ability into chondrocytes, tenocytes, skeletal myocytes, and neuronal cells, and are also able to improve the engraftment, survival, and differentiation of human hematopoietic stem cells [1–4]. MSCs is known to have immunomodulatory activity; they suppress the T cell-mediated response by inducing apoptosis of activated T cells and contribute to the successful control of severe graft-versus-host disease [5,6]. The main suppressive reactions by MSCs were involved in the enhancement of the regulatory T cells, such as $CD4^+CD25^{hi}$, $CD4^+$, and $CTLA4^+$, and eventually the modulation of the cytokine or allogeneic T cell response [7,8]. MSCs also attenuated $CD8^+$ T cell-mediated lysis in mixed lymphocyte reactions [9]. Moreover, the MSCs-induced inhibition of T cell-mediated immune responses were also caused by several factors, such as interferon(IFN)-γ, interleukin(IL)-10, tumor necrosis factor(TNF)-α, IL-2, inoleamin 2,3-dioxygenase(IDO), and prostaglandin E [10–12]. It is demonstrated that MSCs can inhibit almost all immune responses in immune cells including B lymphocytes, natural killer cells, dendritic cells, and monocytes [7,13,14]. Therefore, it is assumed that MSCs may act as an important regulator in immune responses induced by T cells.

Chemokines are cytokine-like proteins that selectively regulate the recruitment and trafficking of leukocyte subsets into inflammatory site by chemoattraction [15]. CXC chemokine family has been found to be associated with tumorigenesis, angiogenesis, and metastasis [16–18]. Moreover, growth-related oncogene (GRO) is known as a member of the CXC chemokine subfamily and plays a major role in inflammation and wound healing [18]. GRO chemokine is composed of CXCL1, 2, and 3 (called as GROα, β, and γ, respectively) and binds to its common receptor CXCR2. The ligands of this receptor are also reported to be several chemokines such as IL-8, CXCL5(ENA-78), CXCL6(GCP2), and CXCL7(NAP2) [19]. Signaling via the CXCR2 chemokine receptor is mediated by various signaling pathways, including extracellular signal-regulated kinase [18–21]. Moreover, GRO chemokines inhibited the ability of downstream signals of the CXCR2 chemokine receptor to suppress monocyte arrest and also played a pivotal role in metastasis of several tumor cell lines and in engraftment of hematopoietic stem cells [22–24]. Previous reports demonstrated that MSCs downregulated the expression of chemokine receptor

Abbreviations: MSC, mesenchymal stem cells; GRO, growth-related oncogene; NOD/SCID, non-obese diabetic severe combine immunodeficient disease; CB-MNCs, cord blood-derived mononuclear cells.

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CXCR4, CXCR5, and CCR7B, and reduced chemotaxis into CXCL12, a CXCR4 ligand, CXCL13, and the CXCR5 ligand [25–29], suggesting that MSCs may affect chemotactic properties via chemokine receptors in immune cells.

Although MSCs is known to exert inhibitory effect on T cell proliferation induced by various stimuli [30,31], it remains unclear how MSCs inhibit T cell proliferation. In the present study, to define the immunosuppressive effect of MSCs, we explored the factors that modulate the ability of MSCs involved in T cell proliferation, using both an in vivo proliferative animal model and an in vitro cytokine microarray technique. We here showed that MSCs induced the expression of GRO, CXCL1, CXCL2, and CXCL3 and the expression of the CXCR2 receptor on T cells when co-cultured with T cells and that MSCs regulated immunosuppressive activity through CXCL3 signaling by reducing the activation of AKT, Janus kinase (JAK) 2, and signal transducer and activator of transcription (STAT) 3 signaling via the CXCR2 receptor in T cells.

2. Materials and methods

2.1. Primary cells and cell lines

Cord blood (CB) samples from human were obtained from umbilical and placental tissues according to the institutional guidelines of CHA General Hospital (Seoul, Korea). Mononuclear cells (MNCs) from CB were isolated by density gradient centrifugation in Ficoll-Paque™ Plus (Amersham Biosciences, Uppsala, Sweden). Purified cells were washed and suspended in phosphate buffered saline (PBS; Gibco, Gaithersburg, MD, USA) containing 2% fetal bovine serum (FBS) (Gibco). After washing twice, CB-derived MNCs (CB-MNCs) were placed on ice until transplantation into 1–3 day old neonate non-obese diabetic-severe combined immunodeficient (NOD/SCID) mice (KKIBB, Seoul, Korea). human cervical tumor (HeLa) cell lines were purchased from the Korean Cell Line Bank (KCLB, Seoul, Korea) and cultured in Dulbecco's modified eagle's medium (Gibco) medium supplemented with Eagle's minimal essential medium (Gibco) and 10% heat-inactivated FBS. Human MSCs were purchased from Chambrex Bioscience (Walkersville, MD, USA). MSCs were cultured in minimum essential α-medium (α-MEM; Gibco), 10% FBS, and 2 mM L-glutamine (Gibco). Media were changed every three days, and cells were sub-cultured at the 70–80% confluent stage. MSCs were used at passage 4–8 for all experiments.

2.2. Animal model mice

NOD/SCID mice were purchased from the Animal Laboratory of KKIBB and maintained in an animal facility at the CHA Stem Cell Institute at CHA University. Six- to ten-week-old mice were used for transplantation of CB-MNCs and the tumor formation experiment. To reconstitute NOD–SCID mice-T cells, we injected 1×10^7 CB-MNCs into 1–3 days old NOD/SCID mice. Treatment with 1×10^6 MSCs was initiated simultaneously with injection of CB-MNCs into NOD/SCID mice. To investigate the effects of the MSCs on human cervical tumorgenesis, 2×10^6 HeLa cells were subcutaneously injected into the right back-pad without MSCs and into the left back-pad with MSCs (4×10^5) of NOD/SCID mice-T cells that had been pre-injected with CB-MNCs for 4 weeks according to the above-mentioned method. The volume and weight of tumors were determined when the animals were sacrificed five weeks post injection with tumor cells with or without MSCs to determine tumor growth and MSC-mediated inhibition of T cells in vivo. The tumor volume was measured before after excision (volume = length × with × height). All animal experiments in this study were approved by the CHA University Ethical Committee for Animal Experiment Regulation.

2.3. Flow cytometry analysis

To determine the chimerism of human hematopoietic cells in peripheral blood (PB), engrafted human hematopoietic cells were investigated at four, six, and eight weeks after injection of CB-MNCs. Mice were sacrificed and the bone marrow, spleen, and lymph nodes were collected to confirm the presence of human hematopoietic cells in organs. The tissues were teased apart and passed through a nylon filter to remove debris. Samples were prepared as single cell suspensions in staining medium with PBS and 2% FBS. Cells were stained with the following labeled antibodies: Fluorescein isothiocyanate (FITC)-conjugated anti-human CD45 (HI30), CD4 (RPA-T4), CD45RO (UCHL1), CD8 (HIT8a), CD45RA (HI100), and CD182 (CXCR2); PE-conjugated anti-human CD34 (581), CD33 (WIM53), CD19 (HIB19), CD3 (UCHT1), CD4 (RPA-T4), CD8 (HIT8a), and CD182 (CXCR2); and APC-conjugated anti-human CD56 (B159) and CD3 (UCHT1). Activated and isotypic control antibodies were purchased from BD Pharmingen, (San Diego, CA, USA). Stained cells were analyzed with a fluorescence-activated cell sorter (FACS) VantageSE flow cytometer (BD Biosciences, CA, USA). Data were live-gated by forward and side scatter and by lack of propidium iodide (PI) uptake. The frequencies in quadrant corners are given as percentages of gated cells. Collected data were analyzed with CELLQUEST software (BD Biosciences).

2.4. Human T cell isolation

To isolate purified T cells, human $CD3^+$ T cells were selectively isolated from splenocytes of NOD/SCID mice injected with CB-MNCs for four to six weeks prior to sacrifice or from human PB. Splenocytes were resuspended in PBS supplement with 2% FBS and stained with anti-human FITC-conjugated anti-CD3 (UCHT1) antibody (BD Bioscience), and the EasySep FITC Selection Kit (StemCell Technologies, Seattle, WA, USA) was used to isolate CD3-positive T cells. Enrichment of purified cells was confirmed by flow cytometric analysis, which showed a >90% positive cell population, and these purified cells were used for further experiments.

2.5. Proliferation assays

To determine the anti-proliferative effects of MSCs, 2×10^4 MSCs/well were seeded in 96-well dishes containing α-MEM with 10% FBS. After a 24 h culture period, the medium was removed and the MSCs were washed with PBS and then further cultured in α-MEM with 10% FBS and human CD3+ T cells (1×10^5/well) purified from CB-MNCs, human PB leukocytes, and splenocytes from NOD/SCID mice pre-established by intraperitoneal injection with CB-MNCs. To induce T cell proliferation, cells were stimulated with 10 ng/ml IL-2 (BD Bioscience) for two days and cell proliferative ability was measured using a CCK Solution Kit (Dojindo, kumamoto, Japan). To investigate the effect of chemokines on T cells, MSCs were cultured for 24 h and then washed twice with PBS. Purified CD3+ T cells obtained from human PB leukocytes or NOD–SCID mice pre-established with CB-MNCs for five to seven weeks were co-cultured with MSCs and anti-CD3/CD28 antibody. After two days of culture, T cells were isolated and transferred into 96-well plates in serum-free medium containing 100 ng/ml each of CXCL1, CXCL2, and CXCL3 chemokine (R&D, Minneapolis, MN, USA) for two days. The inhibition test of CXCR2 signaling was conducted using 200 nM of CXCR2-specific antagonist, SB225002 (Calbiochem, San Diego, CA, USA) [25,32], before a 30 min treatment with CXCL3 in serum-free culture medium. After two days of treatment,