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Human umbilical cord blood-derived mesenchymal stem cells ameliorate psoriasis-like skin inflammation in mice

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ABSTRACT

Mesenchymal stem cells (MSCs) inhibit the proliferation or activation of lymphocytes, and their inhibitory effects do not require human leukocyte antigen (HLA)-matching because MSCs express low levels of HLA molecules. Therefore, MSCs may be able to regulate immune responses. In this study, we determined whether MSCs could inhibit psoriasis-like skin inflammation in mice. After induction of psoriasis-like skin inflammation using intradermal injection of IL-23 or topical application of imiquimod with or without treatment with MSC, mouse skins were collected, and H & E staining and real-time PCR were performed. IL-23-induced skin inflammation was inhibited when MSCs were injected on day −1 and day 7. The expression of proinflammatory cytokines such as IL-6, IL-17, and TNF-α was inhibited by MSC injection, and the expression of chemokines such as CCL17, CCL20, and CCL27 was also decreased in mouse skin. We also determined whether MSCs could not only prevent but also treat psoriasis-like skin inflammation in mice. Furthermore, in vitro experiments also showed anti-inflammatory effects of MSCs. Dendritic cells which are co-cultured with MSCs suppressed CD4+ T cell activation and differentiation, which are important for the pathogenesis of psoriasis. These results suggest that MSCs could be useful for treating psoriasis.

1. Introduction

Mesenchymal stem cells (MSCs) have inhibitory effects on innate and adaptive immune cells. It has been shown that MSCs inhibit $CD4^+$ T cell proliferation and differentiation and dendritic cell (DC) maturation and induce regulatory T (Treg) cell differentiation [1–4]. Therefore, MSCs could be used for the treatment of many immune cell-mediated diseases because of their regulatory effects on immune cells. Indeed, some experimental results show that MSCs can prevent or treat autoimmune diseases, such as experimental autoimmune encephalomyelitis (EAE) [5] and collagen-induced arthritis [6]. However, the mechanisms of immune suppression by MSCs are not well understood. Even though many immuno-suppressive molecules such as IL-10 [7], transforming growth factor (TGF)-β [8], nitric oxide [9], indoleamide 2,3-deoxygenase [10], and prostaglandin (PG) E2 [11] are involved in MSC-mediated immune suppression, it has been reported that human umbilical cord blood-derived MSC produces PGE2 and PGE2 might be important factor to inhibit colitis in mice [12]. However, further experiments are necessary to determine whether there are other mediators are required to inhibit colitis by hUCB-MSCs.

MSCs can be isolated from bone marrow, umbilical cord blood, and adipose tissue. Although many researchers have used bone marrow-derived (BM)-MSC to determine their immuno-suppressive effects and their possible use for the treatment of diseases, human umbilical cord blood-derived (hUCB)-MSCs have recently been regarded as an another source for MSCs [13,14].

Similar to BM-MSCs, hUCB-MSCs do not express Major Histocompatibility Complex class II (MHCII), CD40, CD80, and CD86, which are involved in T cell activation for transplant rejection. Thus, it was suggested that hUCB-MSCs could be used for stem cell therapy because of their low immunogenicity and it was demonstrated

Abbreviations: hUCB-MSC, human umbilical cord blood-derived mesenchymal stem cell; IL, interleukin; BMDC, bone marrow-derived dendritic cell; IDO, indoleamine 2,3-dioxygenase

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that hUCB-MSCs are effective in modulating immune cells and treating diseases [12,15]. Furthermore, hUCB-MSCs do not raise ethical issue for clinical applications. Thus, hUCB-MSCs have many advantages for the treatment of immune cell-mediated diseases.

Psoriasis is a chronic skin inflammatory disorder, and its histological features are characterized by epidermal hyperplasia, increased angiogenesis and immune cell infiltration [16]. Although the pathogenesis of psoriasis is not fully understood, numerous evidences suggest that Th17 cell is a major player in the pathogenesis of psoriasis [17,18]. Therefore, it has been proposed that targeting IL-17 or its related cytokines may be an effective therapy for the psoriasis. Indeed, anti-IL-12/23p40 antibody down-regulates psoriasis-related cytokine and chemokine gene expressions in psoriasis patients [19]. It has also been reported that human anti-IL-17A antibody can effectively treat psoriasis, confirming that the IL-17/IL-23 axis is a good target for psoriasis treatment [20].

Th17 cells are involved not only in psoriasis but also in other autoimmune diseases, such as EAE, collagen-induced arthritis, inflammatory bowel disease, and uveitis [21–24]. Therefore, the pathogenesis of psoriasis is similar to that of other autoimmune diseases and treatment methods for psoriasis might be applied to other autoimmune diseases.

MSC can be used to treat Th17-mediated autoimmune diseases, and psoriasis is an autoimmune disease with similar pathogenesis to that of other autoimmune diseases. Therefore, we hypothesized that hUCB-MSCs could be used to effectively treat psoriasis. In this study, we demonstrated that hUCB-MSCs ameliorate psoriasis-like skin inflammation in mice and have regulatory effects on immune cells, including $CD4^+$ T cells and DCs.

2. Materials and methods

2.1. Mice

C57/BL6 male mice were housed in an environmentally controlled room with a 12:12-h light-dark cycle and free access to laboratory chow and water. Mice between 8 and 12 weeks of age were used. The protocol for mouse use was approved by the Catholic Research Institute of the Medical Science Committee.

2.2. Isolation and culture of hUCB-MSCs

hUCB-MSCs were isolated and maintained as previously described [12]. The stem cell characteristics of hUCB-MSCs were verified by determination of their differentiation, proliferation, and immunological phenotypes as previously described [25]. The study was conducted according to the principles of the Declaration of Helsinki and IRB was approved from Seoul National University (approved number; 1109/001-006).

2.3. IL-23-mediated psoriasis-like skin inflammation in mice

IL-23-mediated psoriasis-like skin inflammation was induced as previously described [26]. Phosphate buffered saline (20 μl) or recombinant mouse IL-23 (500 ng/20 μl) was injected intradermally into the ears of wild type mice every other day for 15 days. On day 15, mouse ears were collected and stored at −80 °C until use for real-time PCR experiments or fixed in 4% paraformaldehyde for paraffin section.

2.4. Imiquimod-induced psoriasis-like skin inflammation in mice

Imiquimod-induced psoriasis-like skin inflammation was induced as described previously [27]. Briefly, 62.5 mg of imiquimod cream (5%; 3.125 mg of the active compound) was applied on the shaved back skin of C57BL/6 mice every day for 5 days with or without subcutaneous injection of hUCB-MSCs on day −1. The mice were euthanized on day 6 for analysis.

2.5. Flow cytometry

For the single cell suspensions, the ears or back skins were collected and single cell suspensions were prepared as previously described [26]. Spleens and draining lymph nodes were collected and minced through 70 μm mesh for single cell suspensions. The cells were collected, washed and stimulated with plate-bound anti-CD3 (BD Pharmingen) and soluble anti-CD28 (eBioscience) antibodies in the presence of GolgiStop (BD Biosciences) for 5 h. Cells were harvested and intracellular staining was performed according to the manufacturer's instructions (BD Pharmingen). The cells were acquired on a flow cytometer (FACS Calibur, BD Bioscience) and analyzed using FlowJo software (Ashland, OR).

2.6. Generation of bone marrow-derived DC (BMDC) cells

$CD11c^+$ cells were generated from bone marrow using a lineage negative cell isolation kit (Miltenyi Biotec) as previously described [28]. The lineage negative cells were grown with Dulbecco's Modified Eagle's Medium supplemented with 10% fetal bovine serum, granulocyte-macrophage colony-stimulating factor (10 ng/ml), and IL-4 (10 ng/ml) for 5 days. For activation of DCs, TNF-α (20 ng/ml) or lipopolysaccharide (1 μg/ml) was treated for 16 h.

2.7. Co-culture of DC and CD4+ T cells

A transwell plate (Corning, Acton, MA) was used to prevent MSCs from DC contact. MSCs and immature DCs were placed in the upper and lower chambers of the transwell plate, respectively, at a 1:10 ratio. Each well in a 6-well transwell co-culture plate contained 1×10^6 DCs in RPMI 1640 medium, supplemented with 10% fetal bovine serum, 1% penicillin and streptomycin, recombinant mouse GM-CSF (20 ng/ml), and IL-4 (10 ng/ml). lipopolysaccharide (1 μg/ml) was added to the DC layer for 1 d to stimulate immature DCs. After stimulation, DCs were co-cultured with MSC in a transwell plate for 16 h and MSCs were removed. Naïve $CD4^+$ T cells were added for contact co-culture of DCs and cultured under Th17 or Treg cell conditions for 4 days. The $CD4^+$ T cells were collected, and total RNA was isolated.

2.8. RNA isolation, cDNA synthesis, and quantitative real-time PCR

Total RNA was isolated from CD4+ T cells or mouse skin with the TRIzol reagent (Invitrogen) as previously described [29]. The primer sets for IL-6, IL-10, IL-17, TNF-α, IL-22, IL-20, CXCL1, CCL17, CCL20, and CCL27 were purchased (Qiagen, Hilden, Germany). PCR was performed using Rotor-Gene 6000 (Corbett, AUS) and the QuantiTect SYBR Green PCR Kit (Qiagen). The amplification program consisted of 1 cycle of 95 °C for 10 min, followed by 35 cycles of 95 °C for 20 s, 55 °C for 20 s, and 72 °C for 20 s. For relative quantification of gene expressions, delta-delta Ct method was used and GAPDH mRNA level was used as an endogenous normalization control.

2.9. Carboxyfluorescein succinimidyl ester (CFSE) dilution assay

CFSE dilution assay was performed using CellTrace CFSE Cell Proliferation kit (Molecular Probes, Inc., Eugene OR) according to the manufacturer's instruction. Briefly, purified naïve $CD4^+$ T cells were incubated with CFSE at 37 °C for 10 min. After quenching the staining by the addition of ice-cold culture media, the cells were incubated for 5 min on ice followed by the collection and washing of cells. The cells were co-cultured with DCs and anti-CD3 antibody for 4 days. Graphical displays showing each cell division were obtained using Flow Jo software.