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MSCs inhibit bone marrow-derived DC maturation and function through the release of TSG-6

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ABSTRACT

Dendritic cells (DCs) are potent antigen-presenting cells (APCs) that are characterized by the ability to take up and process antigens and prime T cell responses. Mesenchymal stem cells (MSCs) are multipotent cells that have been shown to have immunomodulatory abilities, including inhibition of DC maturation and function *in vivo* and *in vitro*; however, the underlying mechanism is far from clear. In this study we found that MSCs can inhibit the maturation and function of bone marrow-derived DCs by releasing TSG-6. In the presence of MSCs, lower expression of mature DC surface phenotype (CD80, CD86, MHC-II, and CD11c) was observed. In addition, typical DC functions, such as the production of IL-12 and the ability to prime T cells, were decreased when co-cultured with MSCs. In contrast, knockdown of TSG-6 reduced the inhibitory effect of MSCs on DC. Moreover, we found that TSG-6 can suppress the activation of MAPKs, and NF-κB signaling pathways within DCs during Lipopolysaccharides (LPS) stimulation. In conclusion, we suggest that TSG-6 plays an important role in MSCs-mediated immunosuppressive effect on DC.

1. Introduction

Mesenchymal stem cells (MSCs) possess the immunomodulatory activity, which has attracted considerable attention in recent years. MSCs regulate a wide range of immune cells [1]. For example, MSCs inhibit proliferation of T and B lymphocytes [2,3], prevent differentiation of monocytes into dendritic cells (DCs), and inhibit DC maturation [4,5]. Due to the immunosuppressive properties, MSC-based therapy has been successfully applied in various immune-related diseases, such as graft versus host disease (GvHD), systemic lupus erythematosus (SLE), autoimmune encephalomyelitis, and multiple sclerosis (MS) [6,7,1,8–10].

DCs are the most potent antigen-presenting cells, and play a key role in the initiation of primary immune responses and the induction of tolerance. DCs have the ability to take up and process antigens, up-regulating several immune-related molecules, such as co-stimulatory, adhesion, and integrin molecules to prime naive T cells [11]. Once activated by DCs, these T cells can complete the immune response by interacting with other cells, such as B cells for antibody formation, macrophages for cytokine release, and targets for lysis [12]. In addition to the capacity to stimulate naive T cells, DCs can also interact directly with B [13] and natural killer cells [14]. Given the critical role in the initiation of primary immune responses, DCs are becoming a vital target for immunosuppression to prevent allograft rejection, autoimmune diseases, and other immune-related diseases.

Recent studies have focused on the influence of MSCs on DCs. Many reports have demonstrated that MSCs exert a potent suppressive effect on the maturation and function of myeloid or monocyte DCs. For example, Djouad et al. [15] observed that MSCs can produce IL-6, which may be involved in reversing the maturation of DCs into a less mature phenotype, and in the partial inhibition of bone marrow progenitor differentiation into DCs. Spaggiari et al. [16] demonstrated that PGE2 secreted by MSCs plays a major role in MSC-mediated inhibitory effects on DCs during the progression from monocytes to immature DCs (iDCs). In brief, the soluble factors released by MSCs may be involved in the mechanism by which MSCs exert their inhibitory effect on DC maturation.

TNFa-stimulating gene (TSG)-6, an anti-inflammatory protein produced by MSCs in response to inflammatory cytokines [17], has been revealed to produce immune modulating effects in several animal models [18–20]. Research has demonstrated that TSG-6 secreted by MSCs endowed the ability of suppressing

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NF-κB signaling in resident macrophages induced by zymosan in the peritoneum [21]. Because of the importance of NF-κB signal transduction pathways in regulating expression of functionally important immune molecules expressed by DCs [22], therefore, we sought to determine whether TSG-6 produced by MSCs has an inhibitory effect on DCs maturation and function.

In the current study we observed that MSCs can affect DC maturation and function by secreting TSG-6. Knockdown of TSG-6 reduce the inhibitory effect of MSCs on DCs. Furthermore, our data showed that TSG-6 suppressed MAPKs and NF-κB signaling activation during the progression from iDCs to mDCs induced by LPS. Therefore, we demonstrated that TSG-6 play an important role in MSCs-mediated inhibition of DCs maturation and function.

2. Materials and methods

2.1. Ethics statements

Six-to-eight-week-old female C57BL/6 and naive BALB/c mice were purchased from the Laboratory Animal Center of Southern Medical University (Guangzhou, China). All animal experiments were carried out in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publication No. 80-23, 1996 revision). All of the experimental procedures were approved by the Southern Medical University Ethics Committee.

2.2. MSC isolation and culture

MSCs were prepared from mouse bone marrow (BM) cells, as previously described [23] with minor modifications. MSCs were isolated from the BM of male C57BL/6 mice tibias and femurs. MSCs were assessed by flow cytometry for the expression of the typical markers (CD9, CD44, and Sca-1), and negative for CD11b, CD34, and CD45. MSCs were used in the experiments only from passages 3–8.

2.3. Generation of mouse BM-derived DCs

BM-derived DCs were generated as previously described with minor modifications [24]. BM cells were extracted from marrow cavities of femurs and tibias. The erythrocytes were lysed using erythrocyte lysis buffer (R&D Systems, Minneapolis, MN, USA), then washed three times in serum-free RPMI-1640 medium and cultured in 6-well plates (Costar, Cambridge, MA, USA) at 1×10^6 cells/well containing 10% FBS, 20 ng/mL recombinant murine granulocyte–macrophage colony-stimulating factor (GM-CSF; Peprotech, Rocky Hill, NJ, USA), and 20 ng/mL recombinant murine interleukin-4 (IL-4; Peprotech) at 37 °C in a humidified 5% CO_2 humidified atmosphere. DC maturation was induced at day 5 with 200 ng/mL of LPS (Sigma–Aldrich, St. Louis, MO, USA) stimulation for another 2 days. Cytofluorimetric analysis was performed to evaluate the DC maturation phenotype (CD80, CD86, MHC-II, and CD11c).

2.4. Transfection of MSCs with TSG-6 siRNA

A total of 2×10^5 MSCs were plated in 6-well dishes and cultured for 24 h; then, the cells were transfected with TSG-6 siRNA (sc-39820; Santa Cruz Biotechnology Inc, Paso Robles, USA) using Lipofectamine 2000 according to the manufacturer's instructions (Invitrogen). To confirm the silencing effect of the TSG-6 siRNA, after 48 h, RNA was extracted from aliquots of the cells and analyzed for TSG-6 expression using real-time RT-PCR.

2.5. DC and MSC co-cultures

A 6-well transwell system (0.3-mm pore size membrane; Corning, Cambridge, MA, USA) was used to assess the effect of MSCs on DCs without cell-to-cell contact. At day 5, 10^6 iDCs (cultured alone with GM-CSF and IL-4) were placed in the lower chamber and stimulated for another 48 h with 200 ng/mL of LPS in the presence or absence of MSCs or TSG-6-siRNA MSCs in the upper chamber at a 10:1 ratio.

2.6. Flow cytometry

For phenotypic analysis of the cell surface marker expression, cells were harvested, resuspended in PBS, and incubated for 20 min with phycoerythrin- or FITC-conjugated monoclonal antibodies (mAbs) on ice. For MSCs, cells were stained with antibodies against CD9, CD44, Sca-1, CD11b, CD34, and CD45. For DCs, cells were stained with antibodies against CD11c, CD80, CD86, and MHC-II (eBioscience, San Diego, CA, USA). Mouse IgG1 isotype-control antibodies were used in parallel as negative controls. Stained cells were then washed twice and resuspended in cold buffer and analyzed with flow cytometry (FACS Calibur; BD Biosciences), and the results were processed using FlowJo software (Tree Star, Inc). The results are expressed as the percentage of positively stained cells relative to the total cell number.

2.7. Quantitative reverse transcription-polymerase chain reaction (qRT-PCR)

Levels of IL-12 mRNA in DCs were quantified by real-time RT-PCR. Total RNA was isolated from DCs using TRIzol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. Briefly, the total RNA of each sample was reversely transcribed with oligo dT and SuperScript III RT (Invitrogen). PCR was carried out using an ABI 7500HT fast real-time PCR System (Applied Biosystems). GAPDH was used as the endogenous control. The primers which were used are listed in Supplementary Table 1.

2.8. Cytokine analysis

The concentration of IL-12p70 was determined by an enzyme-linked immunosorbent assay (ELISA) using commercially-available kits (R&D Systems) on supernatants derived from 48 h DC cultures and transwell co-cultures with MSCs, according to the manufacturer's protocol. Interferon-gamma (IFN-γ) secreted from T cells were assayed by ELISA. Quantitative analysis of TSG-6 was performed by ELISA on supernatants derived from MSC cultures for 12, 24, and 48 h in the presence or absence of DCs stimulated by LPS according to the manufacturer's instructions. TSG-6 concentrations were determined with a standard curve constructed by titrating standard TSG-6.

2.9. T-cell proliferation assay

Purified $CD3^+$ T cells (responder cells) from naive BALB/c mice spleens were added to round-bottom 96-well plates (Costar) at 1×10^5 cells/well and incubated at 37 °C in 5% CO_2. The iDCs, mDCs, and DCs conditioned by MSCs or TSG-6-siRNA MSCs were incubated with mitomycin C (50 μg/mL; Sigma–Aldrich) for 1 h at 37 °C to inhibit cell proliferation. These cells were washed 3 times with PBS as stimulator cells and co-cultured with T cells at various ratios (1:40, 1:20, 1:10, and 1:5). T-cell proliferation was detected using a cell counting kit (CCK)-8, according to the manufacturer's instructions (Dojindo Laboratories, Tokyo, Japan) after co-culture with DCs for 5 days.