Biochemical and Biophysical Research Communications xxx (2018) 1–8

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Adipose-derived stem cells ameliorate colitis by suppression of inflammasome formation and regulation of M1-macrophage population through prostaglandin E2

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ARTICLE INFO

Article history:
Received 12 March 2018
Accepted 13 March 2018
Available online xxx

Keywords:
Inflammatory bowel disease
Mesenchymal stem cells
Macrophages
Prostaglandin E2

ABSTRACT

Inflammatory bowel disease (IBD) is an idiopathic disease caused by a dysregulated immune response to intestinal microbes in an individual with a genetic predisposition. Therefore, alleviation of inflammation is very important to treat IBD. Mesenchymal stem cells (MSCs) have been highlighted as new candidates for treating autoimmune disease based on their immunomodulatory properties. In this study, we investigated the anti-inflammatory mechanism and therapeutic effects of adipose tissue-derived MSCs (ASCs) using THP-1 macrophages and dextran sodium sulfate (DSS)-induced mice with chronic colitis. LPS-treated THP-1 cells expressed mRNA of CD11b, an M1 macrophage marker, at day 2. However, THP-1 co-cultured with ASCs expressed mRNA of CD206, CD68, CCL18, legumain, and IL-10, markers of M2 macrophages. In THP-1 cells co-cultured with ASCs, precursor (pro)-IL-1β, Cox-2, and NLRP3 increased dramatically compared to LPS-treated THP-1 cells. Secretion of IL-1β and IL-18 was significantly inhibited by ASCs, but PGE2 production was highly increased in co-culture conditions of THP-1 and ASCs. IL-18 secretion was inhibited by PGE2 treatment, and PGE2 inhibited inflammasome complex (ASC/Cas-1/NLRP3) formation in THP-1 cells. In the DSS-induced chronic colitis model, ASCs ameliorated colitis by decreasing the total number of macrophages and the M1 macrophage population. Our results suggest that ASCs can suppress the inflammatory response by controlling the macrophage population, and ASCs may be therapeutically useful for the treatment of IBD.

1. Introduction

Inflammatory bowel disease (IBD) is an idiopathic disease caused by dysregulated immune response to intestinal microbes in an individual with a genetic predisposition [1,2]. The two major types of IBD are ulcerative colitis and Crohn's disease. Innate immune cells (neutrophils, macrophages, dendritic cells, and natural killer T cells) and adaptive immune cells (B cells and T cells) strongly infiltrate the lamina propria [3], resulting in mild to severe symptoms including abdominal pain, vomiting, diarrhea, rectal bleeding, internal cramps/muscle spasms in the pelvis, and weight loss. In the United States, about 1–1.3 million people currently suffer from IBD [4,5].

The goals of IBD treatment include symptom relief, long-term remission, and reduced risks of complications, which are achieved by alleviating inflammation. Anti-inflammatory drugs such as corticosteroids, azathioprine, 6 mercaptopurine (6-MP), and antibodies against tumor necrosis factor (TNF)-α (infliximab, adalimumab, certolizumab, and golimumab) or α4β7 integrin (vedolizumab) have been used to reduce symptoms [6–17]. Recently, mesenchymal stem cell (MSC) therapy has been

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https://doi.org/10.1016/j.bbrc.2018.03.096
0006-291X/

suggested as an effective alternative therapy for immune diseases including IBD based on the immunomodulatory properties of MSCs [18–20].

MSCs have been shown to migrate to sites of tissue injury/inflammation [21,22]. At injured sites, MSCs are be stimulated by inflammatory cytokines such as interferon (IFN)-γ, TNF-α, or interleukin (IL)-1β to express various immunosuppressive factors, including indoleamine 2,3-dioxygenase (IDO), prostaglandin E2 (PGE2), TNF-α-stimulated protein/gene 6 (TSG-6), nitric oxide (NO), IL-6, IL-10, and HLA-G [23, 24]. PGE2 has multiple roles in proliferation, apoptosis, tissue repair, angiogenesis, inflammation, immune surveillance, and tumor growth [25–27]. PGE2 can induce acute local inflammation and activate immune reaction at the entry site of pathogens, but it has suppressive roles in the proliferation and differentiation of T cells, macrophages, and monocytes, as well as in the cytotoxic activity of NK cells and cytotoxic T lymphocytes [28–30]. Moreover, PGE2 augments the synthesis of anti-inflammatory cytokine IL-10 and decreases production of the pro-inflammatory cytokines TNF-α, IFN-γ, and IL-12 by DCs and macrophages. Therefore, PGE2 secreted by MSCs can regulate inflammation and ameliorate immune disease including IBD. In this study, we analyzed whether MSCs co-cultured with activated THP-1 cells secrete PGE2. We also evaluated the anti-inflammatory roles of PGE2 on THP-1 and the therapeutic effects of PGE2 secreted by MSCs in dextran sodium sulfate (DSS)-induced chronic colitis in mice.

2. Materials and methods

2.1. Animal studies

Female C57BL/6 mice (5- to 8-week-old) were purchased from Orient Bio, Inc. (Seongnam, Korea). Mice were maintained in a 12-h light/12-h-dark cycle under specific pathogen free (SPF) conditions at 25 °C. All animal care and experiments were conducted in accordance with The Guide for Animal Experiments published by the Korea Academy of Medical Sciences, after approval of the Institutional Animal Care and Use Committee of Yonsei University, Wonju College of Medicine. To induce chronic colitis, mice were administered drinking water with 3% DSS (Molecular weight 36–50 kDa; MP Biomedicals, Santa Ana, CA, USA) for 5 days ad libitum, followed by 5 days of normal water without DSS. The DSS administration cycle was repeated once. Mice were divided into four groups: control group that did not drink DSS, colitis group administered with 3% DSS, colitis + ASCs group infused with ASCs (1×10^6 cells) by intraperitoneal injections twice on days 6 and 16, and colitis + ASCs + celecoxib group that was administered celecoxib (3 mg/kg; Sigma, San Diego, CA, USA) once a day for the first 3 days after ASC infusion on days 6 and 16. Animal weights were monitored and recorded daily. Mice were sacrificed on day 20 to isolate the colon, and the entire colon length was measured.

2.2. Cell culture

Human adipose tissue (AT) from three healthy donors (age, 24–38 years) was obtained from Wonju Severance Christian Hospital (Wonju, Korea) by elective liposuction procedures under anesthesia according to procedures approved by the Institutional Review Board of Yonsei University Wonju College of Medicine. Informed consent was obtained from all donors. Mononuclear cells were isolated using a modified protocol described by Zuk et al. [31]. Briefly, lipoaspirates were extensively washed with phosphate-buffered saline (PBS, Welgene, Gyeongsan, Korea) to remove contaminating blood cells and local anesthetics. Mononuclear cells were obtained from the digested lipoaspirates with 0.075% type IA collagenase (Sigma) in PBS. A total of 5×10^6 mononuclear cells were seeded in 100-mm culture dishes with low glucose Dulbecco's minimal essential medium (LG-DMEM, Gibco BRL, Rockville, MD, USA) containing 10% FBS and penicillin/streptomycin. After 2 days, the medium was changed to remove non-adherent cells. Thereafter, the cell culture medium was changed twice weekly, and cells were passaged with 0.25% trypsin/0.1% EDTA (Gibco BRL) until reaching 90% confluency.

The human monocytic cell line THP-1 was maintained in complete RPMI-1640 (Gibco BRL: supplemented with 10% FBS, penicillin/streptomycin and 2 mM L-glutamine). Macrophage differentiation of THP-1 was induced with 100 nM of phorbol ester 12-O-tetradecanoylphorbol-13-acetate (TPA, Sigma) for 2 days and was co-cultured with or without ASCs in transwell plates (Corning, Lowell, MA, USA) while treating with 1 μg/ml lipopolysaccharide (LPS, Sigma). To analyze cytokines or PGE2, conditioned medium was collected, filtered (0.45 μm), and stored at −80 °C until needed.

2.3. Immunohistochemistry

After measuring the entire colon length, colons were fixed in 10% formalin, embedded in paraffin, and sectioned at 5 μm slices with a microtome. For immunohistochemical staining of macrophages, deparaffinized slices were blocked with 0.3% Triton-X 100 and 5% normal horse serum in TBS (20 mM Tris pH 7.6, 137 mM NaCl) and incubated with anti-F4/80 antibodies (Abcam, Cambridge, MA, USA) overnight at 4 °C. After washing three times in TBS, slices were incubated with secondary antibody (Abcam) for 30 min. Slides were counterstained with hematoxylin. To assess macrophages, digital images at 200× magnification were obtained (Zeiss Axioimager M1, Göttingen, Germany). Both positive cells and total cells were counted from three random fields, with over 200 cells counted per field.

2.4. IL-1β and PGE2 measurement

Human IL-1 beta/IL-1F2 Quantikine Kit (R&D Systems, Minneapolis, MN, USA) and PGE2 Assay Kit (R&D) were used to analyze the secretion levels of IL-1β and PGE2 in conditioned media obtained by co-culturing THP-1 with ASCs according to the manufacturer's instructions.

2.5. Reverse transcription-polymerase chain reaction (RT-PCR)

Total RNA was extracted from 1×10^5 cells using TRIzol Reagent according to the manufacturer's instructions (Gibco BRL). Total RNA (2 μg) was reverse-transcribed with M-MLV reverse transcriptase (Bioneer, Daejeon, Korea) for one hour at 42 °C in the presence of oligo-dT primer. PCR was performed using *Taq* DNA polymerase (Bioneer). Specific primers used for PCR assays are listed in Table 1. Amplified products were electrophoresed on a 2% agarose gel and photographed using a ChemiDoc XRS + system (Bio-Rad, Hercules, CA, USA).

2.6. Immunoblotting

Cells were lysed in sodium dodecyl sulfate/polyacrylamide gel electrophoresis (SDS–PAGE) sample buffer (62.5 mM Tris-HCl (pH 6.8), 1% SDS, 10% glycerol, and 5% β-mercaptoethanol), boiled for 5 min, subjected to SDS–PAGE, and transferred to an Immobilon membrane (Millipore, Darmstadt, Germany). The membrane was blocked with 5% skim milk in TBST (Tris-HCl buffered saline containing 0.1% Tween 20) and then incubated with primary antibodies against IL-1β, NLRP3 (1:1000, purchased from Cell Signaling Technology, Danvers, MA, USA), F4/80 (1:1000, purchased from Abcam),

Please cite this article in press as: H.J. Park, et al., Adipose-derived stem cells ameliorate colitis by suppression of inflammasome formation and regulation of M1-macrophage population through prostaglandin E2, Biochemical and Biophysical Research Communications (2018), https://doi.org/10.1016/j.bbrc.2018.03.096