Biomedicine & Pharmacotherapy 100 (2018) 426–432

Contents lists available at ScienceDirect

Biomedicine & Pharmacotherapy

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

Intraperitoneal administration of mesenchymal stem cells ameliorates acute dextran sulfate sodium-induced colitis by suppressing dendritic cells

Aleksandar Nikolic[a], Bojana Simovic Markovic[a], Marina Gazdic[b], C. Randall Harrell[c], Crissy Fellabaum[c], Nemanja Jovicic[a], Valentin Djonov[d], Nebojsa Arsenijevic[a], Miodrag L Lukic[a], Miodrag Stojkovic[b,e], Vladislav Volarevic[a,*]

[a] Department of Microbiology and Immunology, Center for Molecular Medicine and Stem Cell Research, Faculty of Medical Sciences, University of Kragujevac, Kragujevac, Serbia
[b] Department of Genetics, Faculty of Medical Sciences, University of Kragujevac, Kragujevac, Serbia
[c] Regenerative Processing Plant, LLC, Palm Harbor, Florida, United States
[d] Institute of Anatomy, University of Bern, Bern, Switzerland
[e] Spebo Medical, Leskovac, Serbia

ARTICLE INFO

Keywords:
Dendritic cells
DSS-induced colitis
Mesenchymal stem cells

ABSTRACT

Dendritic cells (DCs) have important pathogenic role in the induction and progression of ulcerative colitis (UC), but their role in mesenchymal stem cells (MSCs)-mediated suppression of colon injury and inflammation is not revealed. By using dextran sodium sulfate (DSS)-induced colitis, a well-established murine model of UC, we examined effects of MSCs on phenotype and function of colon infiltrating DCs. Clinical, histological, immunophenotypic analysis and passive transfer of MSCs-primed DCs were used to evaluate capacity of MSC to suppress inflammatory phenotype of DCs in vivo. Additionally, DCs:MSCs interplay was also investigated in vitro, to confirmed in vivo obtained findings. Intraperitoneally administered MSCs (2×10^6) significantly reduced progression of DSS-induced colitis and reduced serum levels of inflammatory cytokines (IL-1β, IL-12, and IL-6). Passive transfer of in vivo MSCs-primed DCs reduced severity of colitis while passive transfer of MSCs-non-primed DCs aggravated DSS-induced colitis. Through the secretion of immunomodulatory Galectin 3, MSCs, in paracrine manner, down-regulated production of inflammatory cytokines in DCs and attenuated expression of co-stimulatory and major histocompatibility complex class II molecules on their membranes. Taken together, these results indicate that MSCs achieved their beneficial effects in DSS-induced colitis by suppressing inflammatory phenotype of DCs in Gal-3 dependent manner. Therapeutic targeting of DCs by MSCs should be explored in future studies as a useful approach for the treatment of UC.

1. Introduction

Since etiology and pathogenesis of ulcerative colitis (UC) is not completely revealed, several animal models have been developed in the last few decades [1–3]. Among them, because of the high degree of uniformity and reproducibility as well as some similarities to human UC, dextran sodium sulfate (DSS)-induced colitis has been usually used to elucidate molecular and cellular pathways involved in pathogenesis of UC and for the evaluation of new diagnostic and therapeutic approaches [2,3].

Among colon infiltrating immune cells, dendritic cells (DCs) have a central role in maintaining tolerance in the gut [4,5]. Their localization in the intestine allows them to be one of the crucial factors in preservation of tissue homeostasis [5]. Pathogenic role of DCs in development and progression of UC is well described [4–7]. Activation of gut-infiltrated DCs results with the induction of T cell-mediated immune response resulting with the progression of colon inflammation and development of UC [4,5].

Mesenchymal stem cells (MSCs) are self-renewing cells with significant regenerative potential [8–10]. By modulating immune response in juxtacrine or paracrine manner [8,11–14], MSCs attenuate inflammation and promote tissue regeneration representing promising therapeutic tool for various inflammatory diseases including UC [15–21]. MSC may polarize DCs towards inflammatory or tolerogenic phenotype in juxtacrine or paracrine manner in dependence of DCs maturation state and DCs:MSCs ratio in the gut microenvironment

* Correspondence author at: Center for Molecular Medicine and Stem Cell Research, Faculty of Medical Sciences University of Kragujevac, 69 Svetozara Markovica Street, 34000 Kragujevac, Serbia.
E-mail address: drvolarevic@yahoo.com (V. Volarevic).

https://doi.org/10.1016/j.biopha.2018.02.060
Received 1 December 2017; Received in revised form 2 February 2018; Accepted 13 February 2018

[11,22,23]. Although several studies demonstrated the efficacy and therapeutic potential of MSCs for treatment of DSS-induced colitis in mice [15–21,24], none of them explored the influence of MSCs on DCs in colitis. Therefore, the aim of this study was to examine effects of MSCs on phenotype and function of colon infiltrating DCs in the pathogenesis of DSS-induced colitis.

Herewith, we present the first evidence that MSCs achieved their beneficial effects in DSS-induced colitis by suppressing inflammatory phenotype of DCs in Galectin-3 (Gal-3) dependent manner, suggesting MSCs-based therapeutic targeting of DCs as a new and useful approach for the treatment of UC.

2. Materials and methods

2.1. Animals

Wild type C57BL/6 mice, 6–8 week old, 19–21 g weight, which were born and housed during experiments in animal breeding facility into Center for Molecular Medicine and Stem Cell Research, Faculty of Medical Sciences, University of Kragujevac, Serbia were used. Mice maintained at 12-h day/night cycle in a temperature-controlled environment, accessing food and water ad libitum. All experimental procedures were approved and conducted according to Guidelines of the Ethics Committee of the Faculty of Medical Sciences, University of Kragujevac, Serbia.

2.2. Induction of DSS-induced colitis

Acute colitis in C57BL/6 mice was induced by 3% (w/v) DSS (m.w. 40 kDa,TdB Consultancy, Uppsala, Sweden) added to drinking water up to 7 days. Control mice have access only to DSS-free water [21,25]. During experiments, mice were fed with commercial pelleted food.

2.3. Application of MSCs and vehicle control

Syngenic MSCs used in these experiments were purchased from Gibco (Cat.No S10502-01) as cells isolated from bone marrow of C57BL/6 mice. MSCs were cultured in a humid atmosphere at 37 °C and 5% CO_2, in T75 flasks with complete medium, consisted DMEM (Dulbecco's Modified Eagle Medium), heat-inactivated 10% FBS (fetal bovine serum), 100 IU/mL penicillin and 100 µg/mL streptomycin (all purchased from Sigma-Aldrich, St. Louis, MO).

MSCs were daily administrated (2×10^6, i.p.), divided in 3 daily doses dissolved in 0.3 ml PBS (phosphate buffer saline) during 7 days of experiment. The first dose was applied 12 h after induction of colitis. Prior each application, MSCs were trypsinized, counted using Trypan blue exclusion test and diluted, and as fresh solution applied. Control group received vehicle only (PBS) in a same way and volume as MSCs-treated group.

2.4. Assessment of colitis

Clinical scoring of colitis has performed according to previously published studies [21,25]. The weight of mice and monitoring of clinical signs of disease progression (presence or absence of diarrhea and rectal bleeding) as parameters for calculating disease activity index (DAI) was done daily by two independent researchers.

2.5. Histopathological analysis

Animals were sacrificed, colons were removed from caecum and the length was measured. Colons were flushed with PBS, sliced longitudinally, rolled by "Swiss rolls" method, and placed in tube with 4% formalin. After 24 h of fixation, 5 µm paraffin-embedded colon sections were stained by haematoxylin and eosin (H&E). Histological score was determined summing two parameters (damaged tissue and leucocytes infiltration) by two investigators in a blind manner [21,25].

2.6. Isolation of dendritic cells and passive transfer

To investigate the interactions between DCs and MSCs in DSS-induced colitis under in vivo conditions, DCs were isolated from spleen of mice treated with DSS or DSS and MSC for two days. Isolation of DCs was performed using commercial kit for immunomagnetic sorting of $CD11c^+$ DCs (CD11c MicroBeads, Miltenyi Biotec GmBH, Germany, Cat. No 130-052-001). Passive transfers of 2×10^5 DCs dissolved in 0.2 ml PBS were conducted i.p. into DSS-treated recipients at day 5 of continuous DSS administration [4].

2.7. In vitro stimulation of DCs and interaction with MSCs

DCs were isolated from spleen of healthy mice using commercial kit for immunomagnetic sorting of $CD11c^+$ DCs (CD11c MicroBeads, Miltenyi Biotec GmBH, Germany). MSCs were seeded in a 6.5 mm transwell chamber with a 0.4 µm pore size of 24-well plate in co-culture with DCs on the bottom at a 10:1 DCs/MSCs ratio [26,27], in complete medium alone or in addition of 3% (w/v) DSS for a 2 days. All cases were performed in quadruplicate. After 48 h, collected supernatants were frozen at −20 °C until cytokine analysis, while scraped cells were immediately analyzed using flow cytometry.

2.8. Measurement of cytokines

To analyze concentration of cytokines, commercial ELISA kits for IL-1β, IL-6, IL-12, PGE2 (R&D Systems, Inc. Minneapolis, MN), and IDO (Neobiolab, Cambridge, MA) were used. Supernatants were analyzed on following cytokines: TNFα, IL-12, HGF, IL-1β, and Galectin 3 (Gal-3) (R &D Systems, Inc. Minneapolis, MN) according to manufacture prescription.

2.9. Flow cytometry analysis

Scraped cells were stained with immunofluorences antibodies against CD11c, CD80, CD86, and I-A surface markers and IL-6 and TNFα cytokines (BioLegend, San Diego, CA). Acquiring data performed on BD FACSCalibur, (BD Biosciences), while data were analyzed using Flowing Software 2.5.1 (Cell Imaging Core, Turku Centre for Biotechnology, University of Turku, Finland).

2.10. Statistical analysis

All results were presented as mean ± standard error of mean (SEM). Results were analyzed by two-tailed Student's *t*-test for independent sample. All statistical analyses were performed using the SPSS (Statistical Package for Social Sciences, IBM, Chicago, IL) version 20 for Windows. Statistical differences have been considered at $p \leq 0.05$.

3. Results

3.1. Intraperitoneally administrated MSCs significantly alleviate acute DSS-induced colitis

Daily administration of MSCs to DSS-treated mice significantly ($p < 0.001$) alleviate disease activity index (DAI) (Fig. 1A). Daily monitoring showed that DSS + PBS-treated mice develop more severe clinical parameters of UC compared to DSS + MSCs-treated animals. The first appearance of blood in the feces or loose stool in DSS + PBS mice were detected from day 4, with daily gaining in the intensity leading to gross bleeding and diarrhea with the presence of mucus, while in DSS + MSCs mice gross bleeding and diarrhea was not observed until the end of experiment. Significant differences in the weight