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Potential therapeutic utility of mesenchymal stem cells in inflammatory bowel disease in mice

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ABSTRACT

Mesenchymal stem cells (MSCs) were found to provide an effective therapeutic role in inflammatory diseases by modulating inflammatory responses and tissue regeneration by their differentiation ability. The present work sought to demonstrate the potential therapeutic use of MSCs in treating chronic inflammatory bowel disease (IBD) in mice. A new model to induce chronic IBD based on alternative administration periods of Dextran Sodium Sulfate (DSS). Mice were divided into 2 groups; one was treated with MSCs and the other was treated with phosphate-buffered saline (PBS). Assessment of therapeutic efficacy of MSCs was by measuring weight, stool scoring, histopathologic examination, and measuring the gene expression of inflammatory markers: Interleukin-23 (IL-23), Tumor necrosis factor-α (TNF-α), Interferon-γ (IFN-γ), and Intercellular adhesion molecule-1 (ICAM-1). The results showed that DSS administration causes bloody and watery stool, weight loss, and altered histopathologic picture. MSC treated mice showed a significant improvement in stool condition, weight gain, and normal histopathologic picture compared to the PBS treated mice. Moreover, gene expressions of inflammatory markers in the intestines of the MSC treated mice were also significantly lower than those of the PBS treated mice. In conclusion, the data here showed that MSCs have a clear potential efficacy in the treatment for IBD, as their immune modulation effects include inhibition in the expression of key inflammatory markers that each plays an important role in the pathogenesis of IBD.

1. Introduction

Inflammatory bowel disease (IBD) is a chronic idiopathic disease mainly represented by two major forms, ulcerative colitis (UC) and Crohn's disease (CD) [1]. While the precise etiology of IBD is still unknown, possible etiologies include the role of environmental factors [2], genetics [3], microbial factors [4], and mucosal immune defects [5]. Based on these etiologies, many treatment lines were developed. The current range of treatments for IBD covers both conventional and biological therapies. Conventional therapy includes the use of anti-inflammatory drugs, immunosuppressive agents, antibiotics, and probiotics; biological therapies mainly include the use of different anti-TNF-α agents, and a plethora of other novel biological agents [6].

The mechanism of DSS-induced colitis is mainly due to the direct toxicity to the colonic epithelial cells, subsequently increasing the permeability of the intestinal mucosa and allowing the transport of luminal bacterial products from the bowel lumen to the submucosal tissue [7]. This hypothesis is bolstered by the work of Leung et al. [8], who demonstrated that activated T cells in the colonic mucosa play a role in modulating the immunological reaction in DSS induced colitis in mice by interacting with lysed bacterial cell wall lipopolysaccharide and activated macrophages. It has been shown that $CD4^+$ T cells play an important role in the development of DSS induced experimental colitis [9]. According to Yan et al. [10], DSS administration increases the gene expression of TNF-α, IFN-γ and IL-12 in the area of the distal colon due to massive infiltration of macrophages, T cells, B cells and neutrophils. Moreover, DSS causes an increase in the level of ICAM-1 that leads to the migration of leukocytes to the site of inflammation [7]. According to Dieleman et al. [11], acute DSS induced colitis is predominantly macrophage-driven colitis, while, Dieleman et al. [12] reported that chronic DSS induced colitis is mediated by Th1 and Th2 activation. Thus, it appears that activated macrophages alone lead to an acute colitis but that only activated macrophages interacting with activated T-cells lead to chronic disease [13].

Bone marrow transplantation (BMT) has improved not only hematological diseases such as leukemia, but also autoimmune diseases. However, BMT significantly affects the systemic immune system of recipients; whether it is effective for IBD remains controversial [14]. Bone marrow contains hematopoietic stem cells and non-hematopoietic

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MSCs. These MSCs are isolated from the adult bone marrow and expanded ex vivo in order to differentiate into several types of cells, such as osteocytes, chondrocytes, adipocytes, hematopoietic supporting stroma, and endothelial cells [15]. Therefore, these MSCs may be useful in tissue regeneration [16]. Moreover, MSCs have shown to modulate allogeneic immune cell responses from a pro-inflammatory toward an anti-inflammatory state by affecting dendritic cells, T-lymphocytes, and natural killer (NK) cells [17]. MSCs also appear to regulate the immune function in inflamed tissues by affecting the formation and secretion of pro-inflammatory cytokines and chemokines. In fact, the in vivo exogenous administration of MSCs imparts an anti-inflammatory action and improves the health of tissues that had become inflamed in response to injury induction [18]. Although, MSCs have shown an anti-inflammatory effect, their cellular mechanisms to pursue such function should be examined.

In the present study, we sought to examine the anti-inflammatory potential of MSC exogenously administered to mice that were suffering from DSS induced colitis. Assessment of therapeutic efficacy of MSCs was by measuring weight, stool scoring, histopathologic examination, and measuring the gene expression of inflammatory markers: IL-23, TNF-α, IFN-γ, and ICAM-1 in colonic tissues recovered from the mice.

2. Materials and methods

2.1. Animals

Eight week female albino mice with an average weight of 18–25 g (provided from The Nile Company for Pharmaceutical Industries) were housed in the animal house facility at Misr International University and kept at 25 °C with normal light/dark cycle with free access to food and water for 7 days. 8 week male mice were housed in the same conditions and used for flushing of the bone marrow for isolation and culture of MSCs.

2.2. Development of DSS-induced colitis model in mice (pilot study)

According to Wirtz et al. [21], the appropriate cycle for the induction of chronic IBD was 5 ml DSS/day/mouse for 7 days, followed by replacing DSS on Day 8 with DSS-free drinking water for another 14 days, for three cycles. This approach was recommended because prolonged administration of DSS causes high mortality rates, and DSS-induced colitis (being a reversible model) requires administration of DSS and normal water alternatively for a specific period of time. Thus, a pilot study was undertaken in two parts: the first to determine the appropriate concentration of DSS to induce IBD, and the second to determine the proper DSS/water cycle for our experimental purposes.

2.2.1. Part A: assessment of determination of appropriate DSS concentration

To determine the appropriate DSS concentration, 150 mice were placed in individual cages and randomly divided into three groups. In the first group, each mouse was given 5 ml of 2% DSS, in the second group 5 ml of 4% DSS, and in the third group 5 ml of 5% DSS for 7 days.

2.2.2. Part B: determination of appropriate DSS/water cycle

To determine the appropriate DSS/water cycle to be used, 90 mice were placed in individual cages and randomly divided into three groups. In the first group, mice were administered 5 ml of 4% DSS for 7 days followed by 14 days of water administration. In the second group, mice were administered 5 ml of 4% DSS for 7 days followed by 10 days of water administration. In the third group, mice were administered 5 ml of 4% DSS for 7 days followed by 5 days of water administration.

The suitable model to induce chronic colitis in mice using DSS is by administration of 4% DSS for 7 days followed by administration of water for 5 days for 3 consecutive cycles.

2.3. Preparation of BM-derived MSCs

Bone marrow was harvested by flushing the tibiae and femurs of 8-wk-old male albino mice (18–25 g) with Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% fetal bovine serum (each from Gibco, Grand Island, NY). Nucleated cells were isolated with a density gradient [Ficoll/Histopaque (Pharmacia, Uppsala, Sweden)] and re-suspended in a complete culture medium supplemented with 1% penicillin–streptomycin (Gibco). Cells were incubated at 37 °C in 5% humidified CO_2 for 12–14 days; media were changed every 2–3 days. When large colonies developed (80–90% confluence), cultures were washed twice with PBS (pH 7.4) and cells were trypsinized with 0.25% trypsin/1 mM EDTA solution for 5 min at 37 °C. After centrifugation, cell pellets were re-suspended with serum-supplemented medium and incubated in a 50-cm^2 culture flask (Falcon, Cairo, Egypt). The resulting cultures were referred to as first-passage cultures [19].

2.4. Administration of MSCs

After 7 days of 4% DSS administration, mice were divided into two groups; the first group, injected with PBS, and the second group, injected intraperitoneally with MSCs [20] .

2.5. Efficacy of MSC in treating IBD

The treatment efficacy of the MSC was evaluated by three methods: physical examination, histopathological examination, and measuring the gene expression of inflammatory markers.

2.5.1. Macroscopic/microscopic evaluation of therapeutic efficacy of MSC on DSS-colitis

The following indices of experimental colitis were monitored daily among the various experimental mice: (a) body weight, (b) food and water intake, and (c) stool condition. Daily, the mice were removed from their individual cages and weighed. The amount of food present and the level of water in their cage bottle were also noted. Loss of food, due to powdering, and water, due to any seeping/forced loss (i.e., if bedding was pushed up at the bottle), was noted when apparent. Q15 Stool condition was subjectively scored from grades 0 to 3 as described: grade 0 indicates a normal-to-semi-solid stool and no blood; grade 1 Q16 indicates a normal-to-semi-solid stool and blood-tinged; grade 2 indicates a semi-solid-to-fluid stool with definite evidence of blood; and, grade 3 indicates a bloody fluid stool [20].

2.5.2. Histopathological examination of intestine tissue

Necropsy samples were taken from the intestine of mice in different groups and fixed in 10% formalin for 24 h. After rinsing in tap water, the samples were serially dehydrated in alcohol (methanol, ethanol, and then absolute ethanol). Specimens were then cleared in xylene, and embedded in paraffin. Samples were then sectioned at 4-μm thickness using a microtome. The tissue sections were collected on glass slides, de-paraffinized, stained with hematoxylin and eosin, and then subjected to blinded examination under a light microscope [20].

2.5.3. Inflammation by measuring the gene expression of inflammatory markers

The mRNA expression of inflammatory mediators such as TNF-α, IFN-γ, IL-23, and ICAM-1 in the colon was measured by real-time reverse transcription-polymerase chain reaction (RT-PCR). Local mRNA expression was measured in the rectum and distal colon (slightly orally to the rectum) samples. Total RNA was extracted from the tissues using QIAamp RNA mini kit (Qiagen, Cairo, Egypt) according to the manufacturer's instructions. PCR primers and Taqman probes for each mediator were used. Real-time quantitative RT-PCR analyses were performed. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH)

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