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Mesenchymal stem cell expression of interleukin-35 protects against ulcerative colitis by suppressing mucosal immune responses

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Abstract

Background. Interleukin-35 (IL-35) has recently been identified as an immunosuppressive cytokine that has been used as a potential therapy for chronic inflammatory and autoimmune diseases. However, there remains a paucity of data regarding its potential benefits after integration into mesenchymal stem cells (MSCs). *Methods.* We used a dextran sulfate sodium (DSS)–induced colitis mice model and treated them with IL-35-MSCs, MSCs or saline. The body weight was recorded daily and inflammatory processes were determined. Cytokine secretion by lamina propria lymphocytes (LPLs) and percentage of regulatory T cells (Tregs) were also measured. *Results.* The data showed that mice in the two treated groups recovered their body weight more rapidly than mice treated with saline in the later stage of colitis. The colon lengths of IL-35-MSC–treated mice were markedly longer than those in the other two groups and the inflammation reduced significantly. Furthermore, the percentage of Foxp3 + Tregs increased significantly and the level of proinflammatory cytokines produced by LPLs decreased significantly in the IL-35-MSC–treated group. *Discussion.* The results demonstrate that IL-35-MSCs could ameliorate ulcerative colitis by down-regulating the expression of pro-inflammatory cytokines.

Key Words: *interleukin-35, lentivirus, mesenchymal stem cell, regulatory T cells, ulcerative colitis*

Introduction

The etiology of ulcerative colitis (UC) remains unclear although considerable progress has been made in this field. A complex interaction of genetic factors, environmental factors and autoimmune factors are considered to contribute to disease initiation and progression [1]. There is still no widely accepted effective treatment for UC, but several experimental treatments, including aminosalicylic acids [2], corticosteroids [3], thiopurines [4] and anti-tumor necrosis factor (TNF)-α treatment [5], have been developed for patients with UC. More recent data suggest that therapies administered during the early course of the disease may modify its progression [6]. The goal of these therapies is to induce and maintain disease remission. Newly emerging therapies, in particular, mesenchymal stem cell (MSC) therapy, are being investigated as a treatment strategy for UC. Data have shown this to be a promising strategy to improve disease control, especially in refractory patients [7]. Besides the capability to differentiate into different cell types [8], transdifferentiate into ectodermal and endodermal cells [9] and secrete cytokines or chemokines to exert their different functions, MSCs can both repair tissue damage [10] and suppress the immune response of the gastrointestinal tract. Although studies have indicated the safety of MSC therapy, efficacy data remain elusive and conflicting clinical benefits have been reported so far [11,12]. Further research, for example, into the genetic modification of MSCs, is necessary to improve the efficacy of MSC therapy.

Interleukin (IL)-35 is a novel anti-inflammatory cytokine and is encoded by two separate genes: EBV-induced gene 3 (EBI3) and the p35 subunit of IL-12 [13]. Since it was identified in 2007, IL-35 has been described as an immunosuppressive factor in different autoimmune diseases by several authors. Previous findings have shown that increased serum IL-35 levels suppress the inflammatory response in collagen-induced arthritis [14], acute pancreatitis [15], periodontal inflammation [16], asthma and allergic rhinitis [17]. In particular, it has been reported that IL-35 could reduce colonic gene expression of pro-inflammatory cytokines and Th1/Th17–associated

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transcription factors [18,19], which indicates that IL-35 may have anti-inflammatory activity in inflammatory bowel disease. Based on these reports, in the present study, we aimed to investigate whether increasing the expression of IL-35 would lead to a suppression of inflammation in UC [20].

Although in our previous study we observed that the injection of an IL-35–expressing plasmid inhibited the function of the immune system [21], the side effects of systemic immunosuppression may cause infection and tumorigenesis. To address these problems, it is important to increase the expression of IL-35 locally to suppress mucosal immune responses. MSCs not only have immunosuppressive activity, they also possess the ability of homing and infusion into inflamed sites [22]. Therefore, in this study, we engineered MSCs to overexpress IL-35. We then examined whether IL-35-gene–modified MSCs (IL-35-MSCs) are effective in treating chemically induced colitis and explored their mechanism of action.

Materials and methods

Mice

Six- to eight-week-old, male, C57BL/6 wild type mice were purchased from the Laboratory Animals Center in the Institute of Radiation Medicine Laboratory, Chinese Academy of Medical Sciences, Tianjin, China. All animals were housed in specific pathogen-free conditions at Tianjin Surgery Institute, Tianjin General Hospital, China. To minimize suffering, all injections and animal surgeries were performed under anesthesia. All procedures were approved by the Ethics Committee of Tianjin Medical University (number TMUaMEC2014063).

Colitis model induced by dextran sulfate sodium

Dextran sulfate sodium (DSS; Sigma-Aldrich; 5%) was drank freely by mice for 7 days to induce UC. Body weight and disease activity index (DAI) were observed and recorded daily. DAI is a series of qualifiers about the symptoms of UC, which includes body weight loss, stool property and rectal bleeding [23]. Each of these items is given a number from 0 to 4, with 4 being the highest rating for disease activity (Table I). On day 8, colons from experimental mice were harvested for further experiments.

Table I. DAI score of UC mice.

Body weight loss (%)	Stool	Bloody stool	Score
None	Normal	Normal	0
1–5	Soft	Guaiac test (+)	1
6–10	Soft but formed	Guaiac test (++)	2
11–15	Liquid	Guaiac test (+++)	3
>16	Liquid	Blood visible	4

IL-35 gene cloning and lentivirus production

Murine IL-35 complementary DNA (cDNA) was amplified from the pSecTag2A-IL35 plasmid (a gift from the Glasgow Biomedical Research Centre), and subcloned into pCCS-Lv201 to generate the recombinant vector pCCS-IL35-Lv201. Lentivirus generation was performed using GeneCopoeia 293Ta cells, according to the instructions of the Lenti-Pac HIV lentivirus packaging kit (GeneCopoeia). Cell culture supernatants were collected and concentrated with the **Lenti-Pac** concentration kit (GeneCopoeia).

Adipose-derived mesenchymal stem cells (Ad-MSCs) purification and transfection

Subcutaneous adipose tissues were isolated from C57/BL6J mice under aseptic conditions. After tissue mincing, type I collagenase (1 mg/mL) was added for 1 h at 37°C with shaking. The digested adipose tissue was filtered through a 70-μm mesh and centrifuged at 1500 rpm for 10 min. The resulting pellet was washed twice with phosphate-buffered saline (PBS), and resuspended in Dulbecco's Modified Eagle's Medium (DMEM)-F12 supplemented with 10% fetal bovine serum (FBS) and 1% antibiotic cocktail. Cells were plated at a density of 10^7 cells/cm^2 and incubated at 37°C in a humidified environment containing 5% CO_2. The culture medium was replaced every 48 h. Confluent third-passage cells were used for lentivirus transfection. A multiplicity of infection (MOI) of 10 was selected and 5 μg/mL polybrene (Sigma-Aldrich) was added. Six hours later, the medium was refreshed. Green fluorescent protein-positive transduced Ad-MSCs were sorted using a BD FACS Aria II (BD Biosciences) instrument for further experiments.

Treatment

Mice were divided into three groups and treated with 1×10^6 IL-35-MSCs, MSCs or saline intravenously, respectively, on the second, fourth and sixth day of DSS feeding. Body weight and disease DAI were observed and recorded daily. All the mice were euthanized on day 8, colon lengths were measured and colon tissues were fixed for hematoxylin and eosin (H-E) staining to determine disease severity.

Colon lamina propria lymphocyte isolation

Colon tissue was cut into 2-cm pieces and digested for 1.5 h in 6 mL RPMI medium with 100 U/mL collagenase VIII (Sigma-Aldrich) and 150 μg/mL DNase in non-tissue culture coated six-well plates. The tissue and digestion mix was filtered through a 70-μm mesh