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Original Article

Interleukin-25 primed mesenchymal stem cells achieve better therapeutic effects on dextran sulfate sodium-induced colitis via inhibiting Th17 immune response and inducing T regulatory cell phenotype

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Abstract: Aim: This study aimed to investigate the anti-inflammatory mechanism of IL-25 mediated mesenchymal stem cells (MSC) treatment for inflammatory bowel disease (IBD) in a DSS-induced rat colitis model. Methods: Rats with DSS-induced colitis were divided into control and treatment groups: normal control group (rats fed with water), DSS group (rats fed with DSS solution), MSC group (DSS-treated rats injected intravenously with GFP-MSCs), IL-25-MSC group (DSS-treated rats injected intravenously with IL-25 primed GFP-MSCs), and mesalazine group (DSS-treated rats fed with mesalazine). Results: In IL-25-MSC group, therapeutic efficacy (clinical symptoms) was better than in MSC group, but comparable to mesalazine group. In IL-25-MSC group and mesalazine group, fewer infiltrating inflammatory cells and lower pathological score were observed in the intestine. The $FOXP3^+$ cells and $IL\text{-}4^+$ cells decreased, but $IL\text{-}17A^+$ cells and $IFN\text{-}\gamma^+$ cells increased in the peripheral blood and colonic mucosa after DSS induced colitis, and these phenomena were reversed by MSC or mesalazine treatment. $IL\text{-}17A^+$ cells reduced and $FOXP3^+$ cells increased in IL-25-MSC group as compared with MSC group. The expressions of Ki67 and LGR5 were significantly elevated in MSC treatment groups as compared with normal control group, DSS group, and mesalazine group. Definite GFP positive cells were not observed in the intestine of MSC-treated rats. Conclusion: IL-25 primed MSCs exert improved therapeutic effects on the intestinal inflammation of IBD rats which may be related to the inhibition of Th17 immune response and induction of T Regulatory cell phenotype. Thus, IL-25 may be an attractive candidate for MSC-based therapy of IBD.

Keywords: Inflammatory bowel disease, mesenchymal stem cells, interleukin-25, colitis, therapeutic effect

Introduction

Inflammatory bowel diseases (IBD) include two types of chronic intestinal disorders including Crohn's disease (CD) and ulcerative colitis (UC), which differ in disease distribution and histological features of the affected bowel segments. Although the etiology of IBD is still poorly understood, dysregulation of the mucosal immune response toward intestinal flora together with genetic and environmental factors has been found to play important roles in the pathogenesis of human IBD [1, 2]. Accumulating evidence has demonstrated that the imbalance between effector and regulatory T cells (Th1, Th2, Th17 and Treg) may induce pathological responses in the IBD intestinal mucosa, which is characterized by the elevated expression of adhesion molecules, chemokines, and other pro-inflammatory cytokines [3, 4]. Some drugs have been used for the treatment of human IBD including 5-aminosalicylic acid, glucocorticoids, immunosuppressants and monoclonal antibodies, but none is specific for IBD and may result in some adverse effects.

Mesenchymal stem cells (MSCs) are non-hematopoietic, multipotent, stromal precursor cells resident in most adult tissues. MSCs possess the capabilities of differentiation and regeneration, and can migrate to the injured site to repair the damaged tissues [5]. In addition, MSCs are

also able to modulate immune response by regulating the recruitment, function, and fate of cells in the innate and adaptive immune system [6, 7]. Numerous animal and clinical studies have suggested that MSCs can suppress T cell proliferation and maintain them in a quiescent state by releasing related mediators and directing cell-cell contact [8-10]. Therefore, MSCs are emerging as a candidate for treatment of immune-mediated diseases, including IBD. In 1993 Drakos et al. first used autologous blood stem cell to treat a hematological malignancy patient with CD, and the symptoms of IBD were improved simultaneously [11]. Subsequent studies have also demonstrated the efficacy of MSC treatment in Crohn's perianal fistula and refractory luminal CD [12, 13]. In recent years, investigators find that MSCs have better immunomodulatory potential after being primed with cytokines such as interferon-γ (IFN-γ) and tumor necrosis factor-α (TNF-α) [14, 15], and granulocyte colonystimulating factor (G-CSF) treated MSCs showed enhanced therapeutic effects on UC via an anti-inflammatory mechanism [16]. Available findings suggest the local microenvironment may affect immune related behaviors and therapeutic efficacy of MSCs.

Interleukin (IL)-25, a member of the cytokine IL-17 family, has been shown to stimulate the Th2 cell-mediated immune responses, resulting in epithelial cell hyperplasia and enhanced recruitment of inflammatory cells into injured tissues [17-19]. By decreasing the syntheses of several Th1 or Th17 related cytokines, IL-25 also attenuates the destructive inflammation in several autoimmune diseases [19-21]. Our previous study has proven that IL-25 was markedly decreased in the injured mucosa of IBD and could inhibit the activation and differentiation of IBD $CD4^+$ T cells into Th1/Th17 cells in an IL-10-dependent manner [22]. In a mouse model of intestinal helminth infection, Saenz et al. found that IL-25 could promote the accumulation of a multipotent progenitor cell population in the gut-associated lymphoid tissue through inducing Th2 response [23]. In this study, a DSS-induced colitis model was established in rats and the therapeutic effects of IL-25 primed MSCs on the intestinal inflammation were further evaluated, in which the expression of Th-associated cytokines was measured and the underlying immunologic mechanism in MSC treatment of IBD was explored. Our study may provide evidence for the treatment of IBD and the development of new targets for IBD therapy.

Materials and methods

Animals

Sprague-Dawley rats (6-8 weeks, weighing 150-170 g) were purchased from Shanghai Laboratory Animal Research Center (Shanghai, China). Rats were maintained under specific pathogen-free conditions with controlled temperature (20-25°C) and 12-12 hour light-dark cycle and given *ad libitum* access to food and water. All animal experiments were approved by the Ethical Committee and Institutional Animal Care and Use Committee of Xiamen University.

MSC isolation, culture and characterization

Isolation and culture of MSCs were performed as described previously [24]. In brief, rats were sacrificed by cervical dislocation, and the tibiae was collected and flushed with Dulbecco's modified Eagle's medium (DMEM)-low glucose (Gibco Invitrogen, Carlsbad, USA). The bone marrow cells were harvested, then seeded into flasks and cultured at 37°C in an environment with 5 % CO_2. After 3-day culture, non-adherent cells were removed and the medium was refreshed every 2-3 days. Once the cell confluence reached approximately 80%, cells were treated with 0.25% trypsin-EDTA (HyClone, Utah, USA) and used in following experiments. Immunophenotyping of MSCs was done by flow cytometry using specific antibodies (CD44, CD90, CD73, CD34, CD29, CD45 and CD11b; BD Biosciences, San Diego, USA). After passaging twice, MSCs were transfected with lentiviral eGFP vector which uses a CMV promoter. The transfection efficiency and fluorescence intensity were determined by inverted fluorescent microscopy and flow cytometry.

Experimental colitis induction and MSC transplantation

Colitis was induced by drinking water containing 5% DSS (MW: 36,000-50,000 Da; MP Biochemicals, Shanghai, China) for 7 days. All rats were divided into five groups: control group (healthy rats drunk water alone), DSS plus PBS group (rats drunk DSS and were intravenously treated with PBS), DSS plus MSC group (rats

drunk DSS and were intravenously treated with MSCs), DSS plus IL-25-MSC group (rats drunk DSS and were intravenously treated with IL-25 primed MSCs), and DSS plus mesalazine group (rats drunk DSS and were intravenously treated with mesalazine). MSCs at a density of 5×10^6 cells/1 ml PBS or PBS were injected through the tail vein on days 1, 2 and 3. IL-25 primed MSCs was prepared by treatment of MSCs with 50 ng/ml IL-25 (ProSpec, NJ, USA) for 24 h. Mesalazine (SinoPharm, Shanghai, China) was administered intragastrically at 1000 mg/kg. Rats in each group were sacrificed on Day 8, and the peripheral blood and colon tissues were harvested for further detections.

Disease activity evaluation

During the study, the changes in the hair, mental status, and activity of these rats were observed daily. The weight loss and characteristics of the stool (including bloody stool) were recorded to determine the disease activity index (DAI) [25]. The scoring was performed by two investigators who were blind to the grouping.

Histological examination

The colon samples were fixed in 4% paraformaldehyde, embedded in paraffin, and sliced into 5-μm sections. Histological evaluation was performed after hematoxylin and eosin (H&E) staining based on the extent of edema, ulceration, crypt loss and infiltration of immune cells as previously described [26]. Total score was defined as the sum of each parameter. The investigators responsible for the histological examination were blind to the grouping. For immunohistochemistry, sections were incubated with Envision Flex Peroxidase-Blocking Reagent for 10 min, and then with rabbit anti-rat LGR-5 mAb (1:100, Novus, CO, USA), anti-rat Ki67 mAb (1:100, Abcam, MA, USA) or anti-rat GFP mAb (1:150, Abcam, MA, USA) overnight at 4°C. After washing in PBS, sections were treated for 30 min with biotin-labeled goat anti-rabbit IgG (Dako; Glostrup, Denmark) at 1:400. Visualization was done with 3,3'-diaminobenzidine. As negative controls, sections were treated with isotype-matched mouse IgG1 or PBS instead of primary antibody. To determine the proportion of positive cells, 5-10 high-power fields of intestinal mucosa were randomly selected, and the positive cells and total stromal cells were subsequently counted. The percentage of positive cells was calculated as follow: [(positive cells)/(total cell number)] × 100.

Immunofluorescence staining

$CD4^+FOXP3^+$, $CD4^+IL\text{-}4^+$, $CD4^+IL\text{-}17^+$ and $CD4^+IFN\text{-}\gamma^+$ cells in the intestinal mucosa were detected by immunofluorescence staining. Briefly, 5-μm sections were obtained, then fixed in cold acetone for 30 min and blocked with normal goat serum in PBS for 1 h at room temperature. The sections were incubated with primary antibodies (CD4 Ab, Santa Cruz, TX, USA; FOXP3 Ab, Abcam, MA, USA; IL-4 Ab, Abcam, MA, USA; IL-17 Ab, Abcam, MA, USA; IFN-γ Ab, Novus, CO, USA) at 4°C overnight and then with the fluorescent secondary antibodies (Invitrogen, Carlsbad, USA). After mounting with glycerol, sections were observed under a fluorescent microscope (Olympus BX43, Japan). To determine the proportion of positive cells, 5-10 high-power fields of the intestinal mucosa were randomly selected, and the positive cells and total stromal cells were counted. The percentage of positive cells was calculated as follow: [(positive cells)/(total cell number)] × 100%.

Flow cytometry

Rat peripheral blood mononuclear cells (PBMCs) were isolated from each group as previously described [27]. The cells were then treated for 4 hours with 50 ng/ml phorbolmyristate acetate (PMA) and 1 μg/ml ionomycin (Sigma-Aldrich, St. Louis, USA), followed by addition of 10 μg/ml brefeldin A (eBiosciences, CA, USA). Then, cells were stained with CD4-FITC (eBiosciences, CA, USA) and incubated for 20 min at 4°C in dark. After washing twice, intracellular staining was performed for IFN-γ-eFluor660, IL-17-PE, IL-4-eFluor660 or FoxP3-APC (Santa Cruz, TX, USA). Cytofix/Cytoperm (BD Biosciences, CA, USA) was used to wash and permeabilize the cells for intracellular staining. After incubation, washing buffer was added to each sample, followed by centrifugation at 500 g for 5 min at 4°C. Finally the samples were fixed in 0.5 ml of 3% paraformaldehyde buffer, and subjected to flow cytometry on a FACS Calibur instrument (BD Biosciences, CA, USA). Data were analyzed with CellQuest software according to our previously reported.

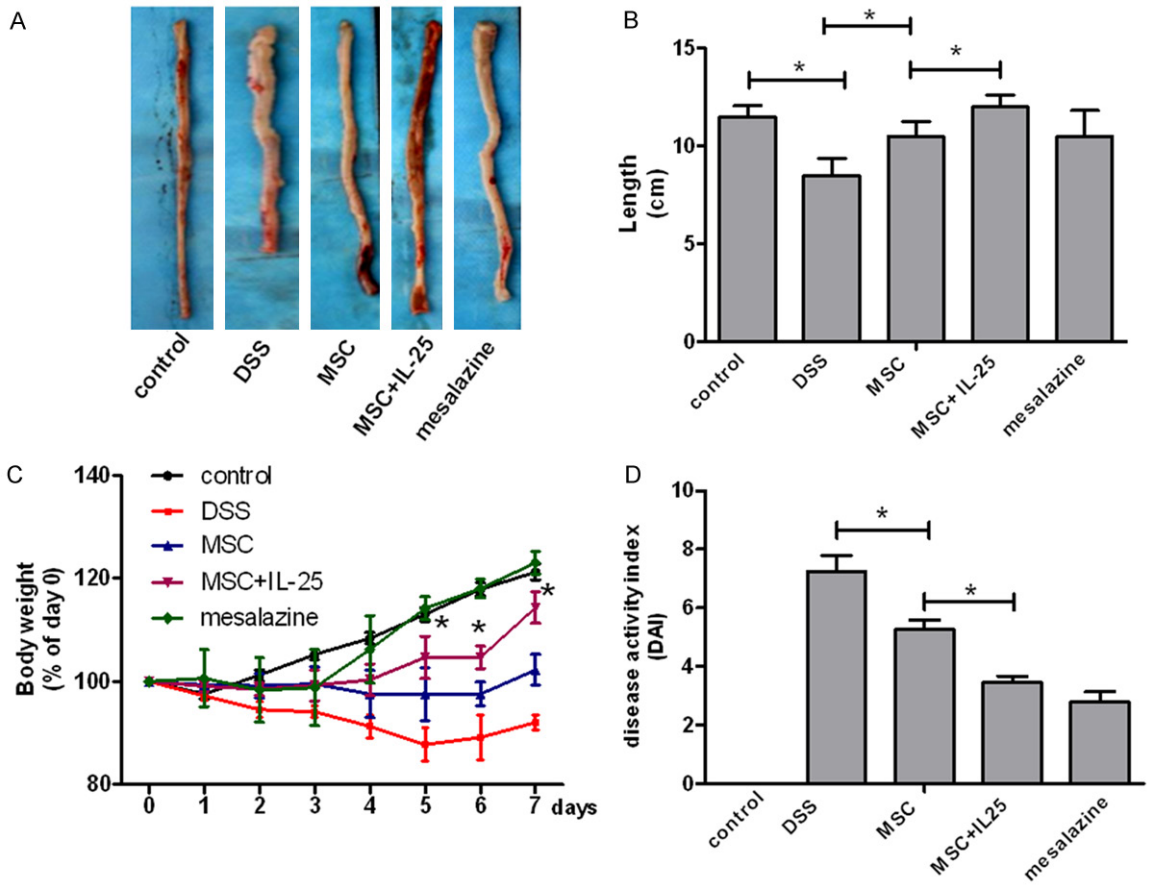

Figure 1. Clinical status and DAI score in DSS induced colitis rats. Colitis was induced by drinking water containing 5% DSS for 7 days. Rats received intravenous injection of 5×10^6 MSCs, 5×10^6 IL-25 primed MSCs or oral mesalazine at 1000 mg/kg on days 1, 2 and 3. Rats from each group were sacrificed on Day 8. Data were representatives from at least three independent experiments and expressed as mean ± SEM. (A) Macroscopic images of representative colons on Day 8 (B) The colon length in each group (*$P < 0.05$) (C) Loss of body weight in each group (%), *$P < 0.05$ vs MSCs group). (D) Disease activity index (DAI) determined on Day 8 (*$P < 0.05$).

Statistical analysis

Statistical analysis was done with SPSS statistical software 17.0 (Chicago, IL, USA). Data were expressed as mean ± standard error (SEM). Differences between means were determined using the Student's t test. A value of $P < 0.05$ was considered statistically significant.

Results

Clinical status and DAI scores in different groups

The immunophenotype of isolated MSCs was consistent with previously reported [13, 28]. Then, the therapeutic efficacy of MSCs was evaluated in rats with 5% DSS induced acute colitis. DSS resulted in severe colitis that was characterized by ruffled hair, loss of appetite, weight loss and bloody diarrhea, and the DAI score increased significantly (7.23±0.56). Compared with DSS group, rats in MSC group showed a slight improvement of symptoms and a small decrease in DAI score (5.26±0.33). However, in IL-25-MSC group, a better therapeutic efficacy (significant improvement of clinical symptoms) and a significant decrease in DAI score (3.45±0.22, $P < 0.05$) were observed, and the DAI score was comparable to mesalazine treatment group (2.78±0.36). Moreover, macroscopic examination revealed that inflammation-related shortening of the colon was reduced after treatment with MSCs or mesalazine (3.45 cm±0.22 cm,10.5 cm±0.22 cm vs 8.5 cm±0.85 cm, $P < 0.05$), but IL-25-MSC treatment significantly attenuate the colon shortening (12.00 cm±0.62 cm). Data are shown in **Figure 1**.

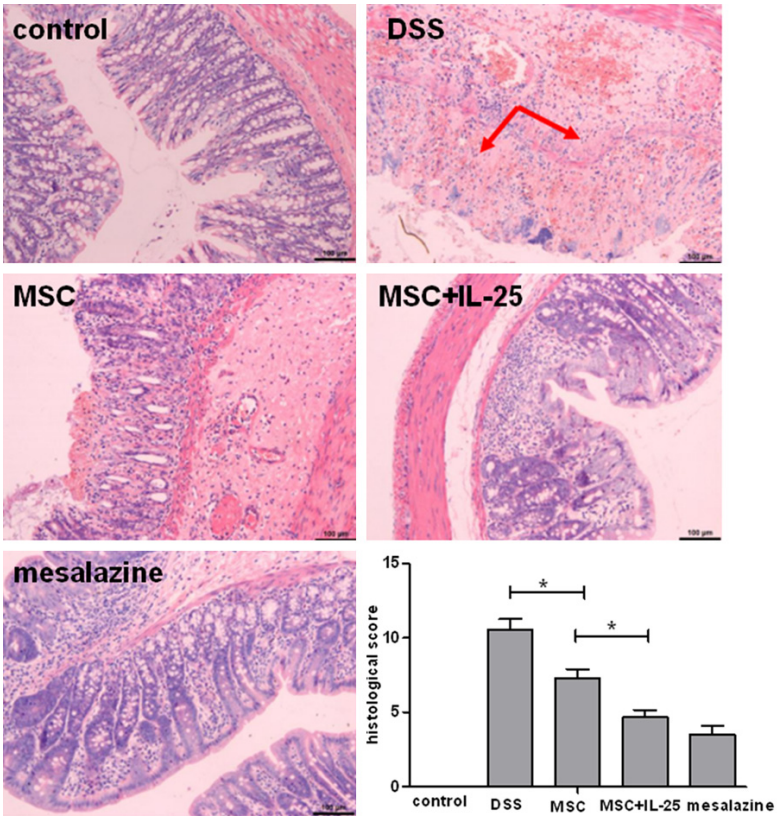

Figure 2. Histology of the colon in DSS induced colitis rats. Histological examination of the colon was performed after H&E staining (100 ×). The histological damage was scored based on the extent of edema, ulceration, crypt loss and infiltration of immune cells. Data were expressed as mean ± SEM (*$P < 0.05$).

Histological changes in different treatment groups

Then, the histopathology of the colon was examined in each group and the histological score was obtained. DSS-treated rats showed severe disruption of the crypt architecture and infiltration of inflammatory cells, including neutrophils, lymphocytes, and macrophages. The inflammatory lesions were located in the mucosa and sub-mucosa. Compared with DSS group, colitis rats treated with MSCs exhibited protective effects on DSS-induced histological damage, including fewer inflammatory infiltrates and milder crypt structure injury. The intestinal injury was also significantly improved in mesalazine treatment group. In addition, in IL-25 -MSC group, a better therapeutic efficacy was observed, which displayed reduction of total histological score compared with DSS group or MSC group (4.67±0.48 vs 10.54±0.74, 7.28±0.61, $P < 0.05$). There was no significant difference in the histological score between IL-25 -MSC group and mesalazine treatment group (4.67±0.48 vs 3.52±0.58, $P>0.05$) (**Figure 2**).

Transcription factor/cytokine expressions in peripheral blood and lamina propria $CD4^+$ cells of rats

Increasing evidence shows that IL-25 plays an important role in the initiation of Th2-mediated immune response and the control of Th1/Th17-mediated inflammation [29, 30]. To investigate the potential role of IL-25 in the regulation of MSC-based IBD treatment, the $IL\text{-}17A^+CD4^+$ cells, $IFN\text{-}\gamma^+CD4^+$ cells, $FOXP3^+CD4^+$ cells and $IL\text{-}4^+CD4^+$ cells in the peripheral blood and intestinal mucosa were detected by flow cytometry and immunofluorescence staining, respectively. As shown in **Figures 3** and **4**, the $FOXP3^+CD4^+$ cells and $IL\text{-}4^+CD4^+$ cells decreased, but $IL\text{-}17A^+CD4^+$ cells and $IFN\text{-}\gamma^+CD4^+$ cells increased in rats with DSS induced colitis. This phenomenon was reversed by MSC treatment or mesalazine treatment. Interestingly, compared with MSC treatment, the IL-25 primed MSCs markedly lowered $IL\text{-}17A^+CD4^+$ cells and elevated $FOXP3^+CD4^+$ cells in the peripheral blood- and lamina propria-$CD4^+$ cells of DSS-treated rats. The $IL\text{-}4^+CD4^+$ cells and $IFN\text{-}\gamma^+CD4^+$ cells were comparable between MSC group and IL-25-MSC group. These findings indicated that IL-25 could enhance the immunomodulatory ability of MSCs mainly via inhibiting Th17 immune response and promoting the immune regulation of Treg cells.

Transplanted MSCs in the colon of rats

IHC was performed to identify transplanted MSCs and their influence on the intestinal epithelial cells and intestinal stem cells in the colonic mucosa on day 8. The expressions of Ki67 and LGR-5 were determined by IHC. Results indicated that the expressions of Ki67

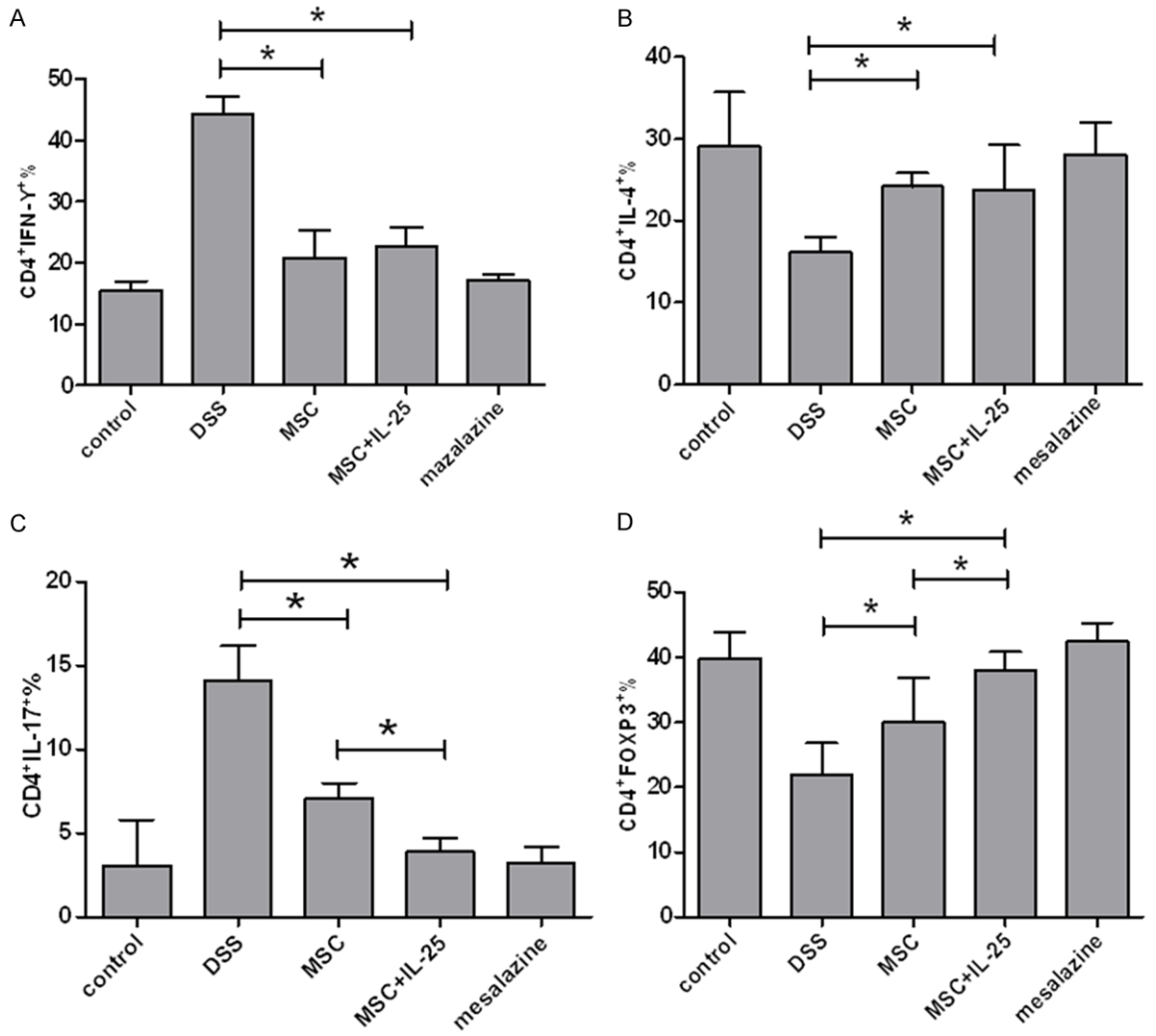

Figure 3. Transcription factor/cytokine expression in peripheral blood $CD4^+$ cells of rats. Transcription factor/cytokine levels of peripheral blood cells were determined with flow cytometry. Results were obtained from three independent experiments. Data were presented as the mean ± SEM (*P < 0.05).

and LGR-5 were significantly elevated in MSC group compared with control group, DSS group, and mesalazine group ($P < 0.05$), but there was no marked difference between MSC group and IL-25-MSC group ($P < 0.05$) (**Figure 5**). Moreover, no definite GFP positive cells were observed in the colon of MSC-treated colitis rats. These findings suggest that IL-25 has no significant effect on the capability of MSCs to induce the differentiation of intestinal stem cells and the proliferation of intestinal epithelial cells.

Discussion

IL-25 is a member of the structurally related IL-17 cytokine family, possesses the lowest degree of homology to IL-17A, does not share biological functions with other members of the IL-17 cytokine family, but has been implicated in the promotion of Th2 immunity. Traditionally, IL-25 can induce the expression of IL-4, IL-5, and IL-13, thereby contributing to allergic diseases and host defense against helminthic parasites [31-33]. On the other hand, IL-25 has been found to suppress the development of Th1/Th17 immune response, and plays an anti-inflammatory role in autoimmune diseases, such as IBD, rheumatoid arthritis, and multiple sclerosis [34-36]. Recent report shows that IL-25 can induce a lineage negative multipotent progenitor (MPP) cell population at the inflammatory mucosa in a mouse model of Trichuris

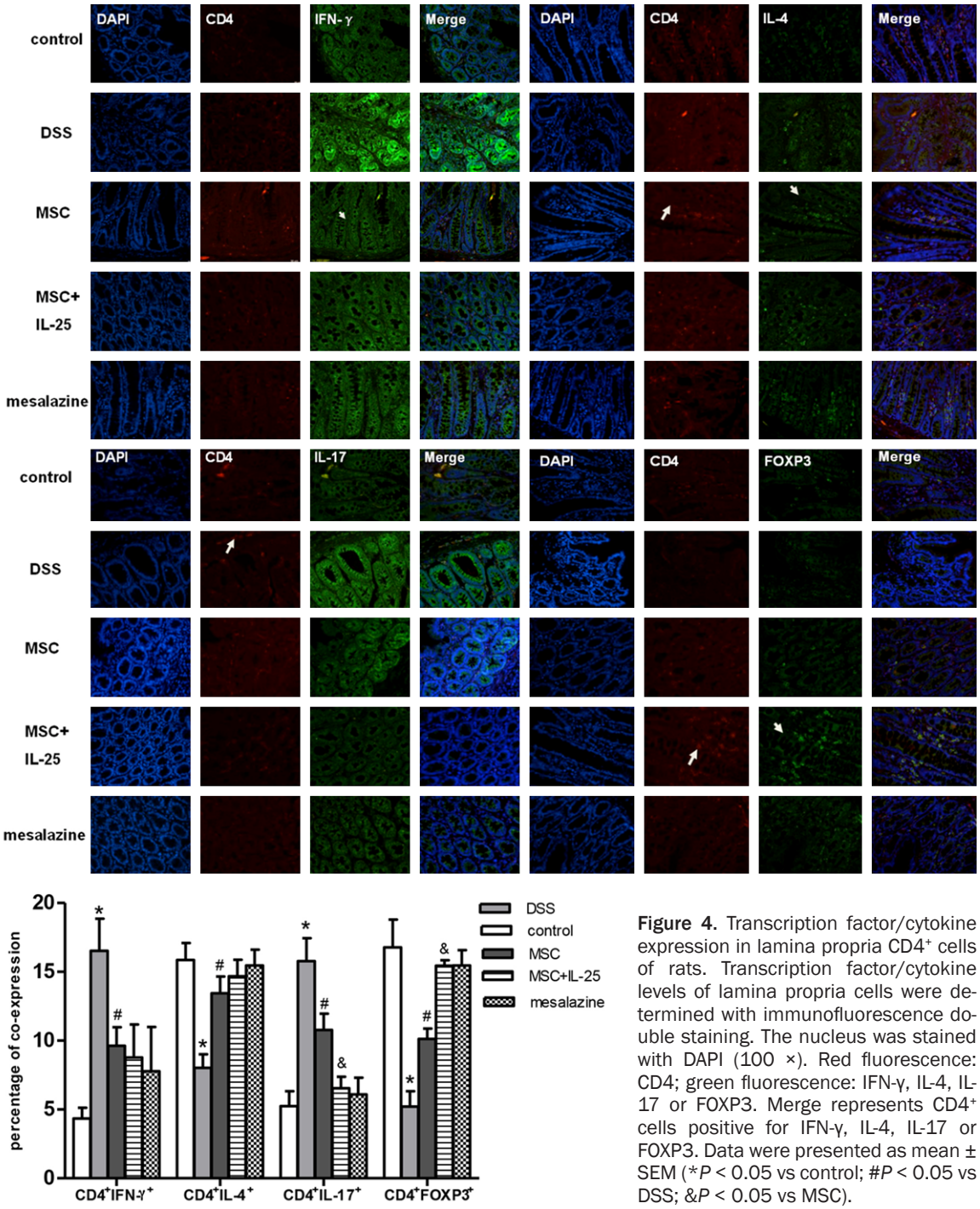

Figure 4. Transcription factor/cytokine expression in lamina propria $CD4^+$ cells of rats. Transcription factor/cytokine levels of lamina propria cells were determined with immunofluorescence double staining. The nucleus was stained with DAPI (100 ×). Red fluorescence: CD4; green fluorescence: IFN-γ, IL-4, IL-17 or FOXP3. Merge represents $CD4^+$ cells positive for IFN-γ, IL-4, IL-17 or FOXP3. Data were presented as mean ± SEM (*$P < 0.05$ vs control; #$P < 0.05$ vs DSS; &$P < 0.05$ vs MSC).

muris infection, and MPP cells have multipotent activity, giving rise to cells of monocytes/macrophages and granulocyte lineages both *in vitro* and *in vivo* [23]. Our previous study indicated that IL-25 was deficient in the sera and inflamed mucosa of IBD rats, and it inhibited the differentiation of IBD $CD4^+$ T cells into Th1/Th17 cells but did not interfere with Th2-associated transcription factor expression [22]. In the present study, a well-established colitis model was established with DSS in rats, whether IL-25 could influence the therapeutic effects of MSCs on IBD was investigated, and then the potential mechanism was further explored. Our results showed that IL-25 primed MSC had a better protective effects on the intestinal symp-

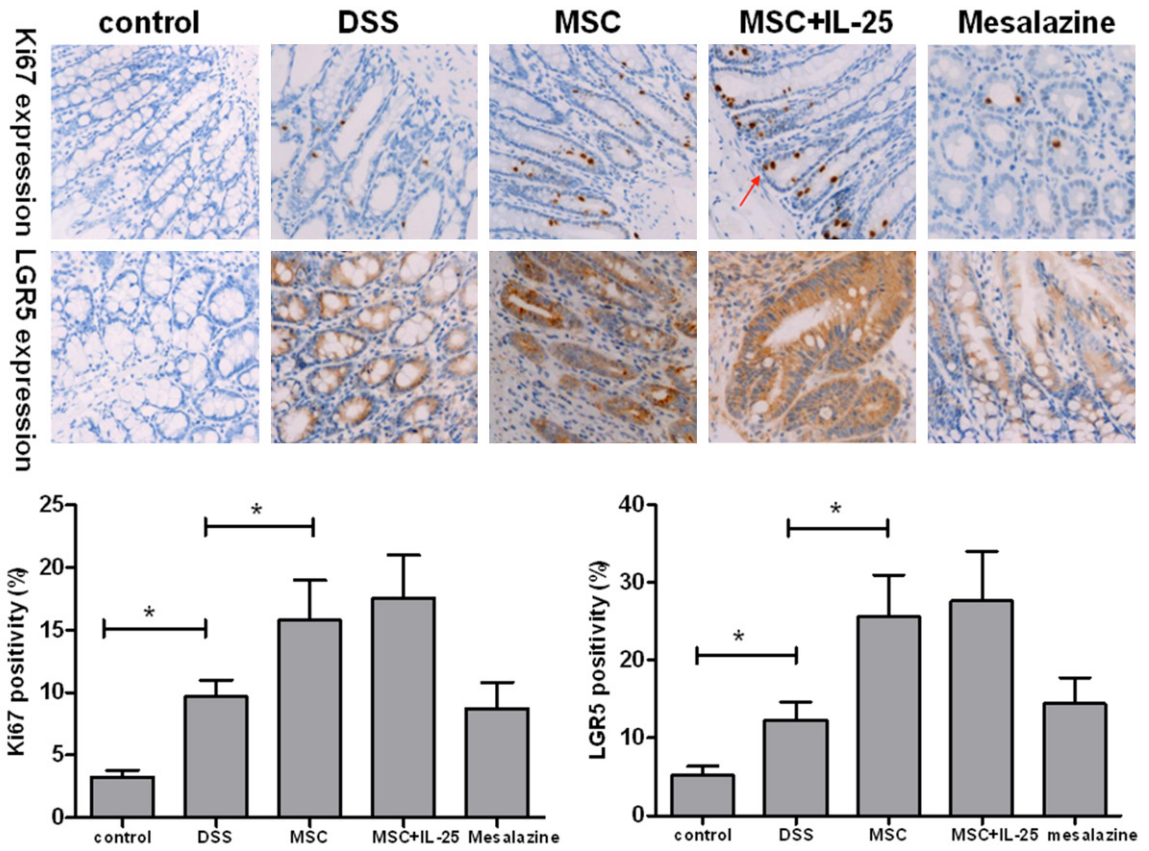

Figure 5. Immunohistochemistry for Ki67 and LGR-5 in the colonic mucosa of rats (× 200). Ki67 and LGR-5 positive cells were mainly located in the colonic villus and intestinal epithelium. The percentage of positive cells was calculated as follow: [(positive cells)/(total cell number)] × 100%. Results were obtained from three independent experiments. Data were presented as the mean ± SEM (**P* < 0.05).

toms and histology of colitis rats compared with untreated colitis rats. Further analysis demonstrated that IL-25 acted via inhibiting Th17 and inducing Treg immune response. However, our study failed to confirm that IL-25 affected the migratory and regenerative capacities of MSCs *in vivo*. These finding indicate that IL-25 can augment the therapeutic efficacy of MSCs in the treatment of IBD.

MSCs can be easily separated from the bone marrow, where they provide a support for the growth and differentiation of hematopoietic progenitor cells in the bone marrow environment [37]. MSCs have multipotent differentiation potential, which allows them to differentiate into other cell types. In addition, they also have immunomodulatory capacity by controlling inflammation and modifying the proliferation and cytokine production of immune cells [38]. In 2002-2003, the observation that MSCs inhibited T-cell proliferation *in vitro* identified by three independent investigators opened a door for the use of MSCs in the treatment of autoimmune disorders [39-41]. The pathogenesis of IBD also has involvement of misregulation of T-cell subsets, which is accompanied by aberrant Th1- or Th17-mediated immune responses in CD, and a Th2-driven aberrant immune response in UC. Therefore, numerous reports have provided evidence that the effectiveness of MSC-based immunotherapy in IBD is associated with the decrease in inflammatory $CD4^+$ T cells [42, 43]. The inhibitory effect of MSCs on pro-inflammatory T cells seems to depend on many parameters such as culture condition (an inflammatory environment). Thus, modification of culture condition for MSCs can enhance their immunomodulatory effects. Duijvestein et al. found that IFN-γ-stimulated MSCs, but not untreated MSCs, significantly attenuated DSS-induced colitis and TNBS-induced colitis via

inhibiting Th17 response [14]. Another study also indicated that IL-37 transfected MSCs could attenuate the histology in DSS-induced colitis mice though inducing Th2-related cytokines and suppressing Th1-related cytokines produced by splenic $CD4^+$ T cells [44]. In our study, results showed that IL-25 primed MSCs had enhanced anti-inflammatory activity, achieving better therapeutic efficacy (improvements of clinical symptoms and intestinal damages) in a DSS-induced colitis rat model. In addition, flow cytometry and immunofluorescence staining revealed that IL-25 primed MSCs exhibited enhanced capability to inhibit Th17 response in $CD4^+$ T cells of colitis rats, but had no marked effect on Th1 or Th2 response. These finding suggest that IL-25 primed MSCs seem to reduce immune response mainly via suppressing Th17 cell activity rather than inducing Th2-related immunity or suppressing Th1-related immunity, finally avoiding the amplification of inflammatory cascade and exerting an anti-inflammatory effect.

Regulatory T cells play a key role in the treatment of inflammatory diseases (such as IBD), mainly by inhibiting pro-inflammatory cytokine production, down-regulating costimulatory molecules on antigen presenting cells, and modulating T-cell proliferation and differentiation. Luz-Crawford et al. found that the suppressive effect of MSCs was associated with the increased functional $CD4^+CD25^+Foxp3^+$ regulatory T cells and elevated IL-10 secretion [45]. In line with previous studies, the percentage of Tregs in peripheral blood- and lamina propria-$CD4^+$ T cells increased significantly in rats after treatment with IL-25-primed MSCs as compared with MSC treated colitis rats and untreated colitis rats, suggesting that IL-25 helps MSCs ameliorate DSS-induced colitis by upregulating Tregs. The MSCs mediated induction of regulatory T cells depends on local conditions. Under different inflammatory environments, MSC constitutively secrete transforming growth factor-β (TGF-β), indoleamine 2,3 deoxygenase (IDO) or IL-2, which acts as a growth factor for regulatory T cells [46]. Furthermore, MSC can induce the differentiation of type 2 macrophages, which produce IL-10, CCL-18 or PGE2 to induce regulatory T cells [47]. However, how IL-25 improves the ability of MSCs to induce regulatory T cells remains to be further identified.

In response to inflammation or tissue injury, MSCs are able to migrate to the injured sites, replace dysfunctional cells and differentiate into various tissues. The migration of MSCs to such sites may be dependent on the chemotactic signals derived from injured or inflamed tissues. Park et al. employed confocal imaging analysis to investigate the direct migration of MSCs in a DSS-induced colitis model, and they found that a larger amount of MSCs was detectable in either colon or regional mesenteric lymph nodes of the DSS-induced colitis group, most in the distal part of the colon, but few in the proximal part [13]. However, in our study, results failed to identify the migration of infused MSCs in the inflamed colonic mucosa in two MSC treatment groups on Day 8. We speculated that the absence of infused MSC was associated with the survival time of MSCs in the inflammatory tissues. Some studies have revealed that *in vitro* expanded MSCs have a short lifespan after in vivo administration [48]. Another study displays that the infused MSCs are detectable in the inflamed intestine until day 5, but not on day 7 in an experimental colitis model [49]. In mice suffering from TNBS-induced colitis, intravenous injection of MSCs leads to the increased expressions of Ki67 (a cell proliferation marker) and LGR5 (an intestinal stem cell markers) in damaged mucosa, indicating that MSCs induce the differentiation of intestinal stem cells and the proliferation of intestinal epithelial cells [50]. Our results are consistent with previous findings that MSCs upregulate the expressions of Ki67 and LGR5, but those were comparable between MSC group and IL-25 primed MSC group in our study. Therefore, we postulate that IL-25 has no significant effect on the capability of MSCs to induce the differentiation of intestinal stem cells and the proliferation of intestinal epithelial cells.

In summary, our study demonstrates that IL-25 primed MSCs achieve better therapeutic effects on intestinal inflammation of IBD through inhibiting Th17 immune response and inducing T regulatory cell phenotype. Thus, IL-25 may become an attractive candidate for the MSC-based therapy of IBD.

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Disclosure of conflict of interest

None.

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References

[1] Fakhoury M, Negrulj R, Mooranian A and Al-Salami H. Inflammatory bowel disease: clinical aspects and treatments. J Inflamm Res 2014; 7: 113-120.

[2] Zhang YZ and Li YY. Inflammatory bowel disease: pathogenesis. World J Gastroenterol 2014; 20: 91-99.

[3] Geremia A, Biancheri P, Allan P, Corazza GR and Di Sabatino A. Innate and adaptive immunity in inflammatory bowel disease. Autoimmun Rev 2014; 13: 3-10.

[4] Hisamatsu T, Kanai T, Mikami Y, Yoneno K, Matsuoka K and Hibi T. Immune aspects of the pathogenesis of inflammatory bowel disease. Pharmacol Ther 2013; 137: 283-297.

[5] Eggenhofer E, Luk F, Dahlke MH and Hoogduijn MJ. The life and fate of mesenchymal stem cells. Front Immunol 2014; 5: 148.

[6] de Witte SF, Franquesa M, Baan CC and Hoogduijn MJ. Toward development of imesenchymal stem cells for immunomodulatory therapy. Front Immunol 2015; 6: 648.

[7] Gao F, Chiu SM, Motan DA, Zhang Z, Chen L, Ji HL, Tse HF, Fu QL and Lian Q. Mesenchymal stem cells and immunomodulation: current status and future prospects. Cell Death Dis 2016; 7: e2062.

[8] Lee HK, Kim HS, Kim JS, Kim YG, Park KH, Lee JH, Kim KH, Chang IY, Bae SC, Kim Y, Hong JT, Kehrl JH and Han SB. CCL2 deficient mesenchymal stem cells fail to establish long-lasting contact with T cells and no longer ameliorate lupus symptoms. Sci Rep 2017; 7: 41258.

[9] Rozenberg A, Rezk A, Boivin MN, Darlington PJ, Nyirenda M, Li R, Jalili F, Winer R, Artsy EA, Uccelli A, Reese JS, Planchon SM, Cohen JA and Bar-Or A. Human mesenchymal stem cells impact Th17 and Th1 responses through a prostaglandin E2 and myeloid-dependent mechanism. Stem Cells Transl Med 2016; 5: 1506-1514.

[10] Yoo HS, Lee K, Na K, Zhang YX, Lim HJ, Yi T, Song SU and Jeon MS. Mesenchymal stromal cells inhibit CD25 expression via the mTOR pathway to potentiate T-cell suppression. Cell Death Dis 2017; 8: e2632.

[11] Drakos PE, Nagler A and Or R. Case of Crohn's disease in bone marrow transplantation. Am J Hematol 1993; 43: 157-158.

[12] Duijvestein M, Vos AC, Roelofs H, Wildenberg ME, Wendrich BB, Verspaget HW, Kooy-Winkelaar EM, Koning F, Zwaginga JJ, Fidder HH, Verhaar AP, Fibbe WE, van den Brink GR and Hommes DW. Autologous bone marrow-derived mesenchymal stromal cell treatment for refractory luminal Crohn's disease: results of a phase I study. Gut 2010; 59: 1662-1669.

[13] Park JS, Yi TG, Park JM, Han YM, Kim JH, Shin DH, Tak SJ, Lee K, Lee YS, Jeon MS, Hahm KB, Song SU and Park SH. Therapeutic effects of mouse bone marrow-derived clonal mesenchymal stem cells in a mouse model of inflammatory bowel disease. J Clin Biochem Nutr 2015; 57: 192-203.

[14] Duijvestein M, Wildenberg ME, Welling MM, Hennink S, Molendijk I, van Zuylen VL, Bosse T, Vos AC, de Jonge-Muller ES, Roelofs H, van der Weerd L, Verspaget HW, Fibbe WE, te Velde AA, van den Brink GR and Hommes DW. Pretreatment with interferon-gamma enhances the therapeutic activity of mesenchymal stromal cells in animal models of colitis. Stem Cells 2011; 29: 1549-1558.

[15] Jin P, Zhao Y, Liu H, Chen J, Ren J, Jin J, Bedognetti D, Liu S, Wang E, Marincola F and Stroncek D. Interferon-gamma and tumor necrosis factor-alpha polarize bone marrow stromal cells uniformly to a Th1 phenotype. Sci Rep 2016; 6: 26345.

[16] Tang Y, Chen Y, Wang X, Song G, Li Y and Shi L. Combinatorial intervention with mesenchymal stem cells and granulocyte colony-stimulating factor in a rat model of ulcerative colitis. Dig Dis Sci 2015; 60: 1948-1957.

[17] Bredo G, Storie J, Shrestha Palikhe N, Davidson C, Adams A, Vliagoftis H and Cameron L. Interleukin-25 initiates Th2 differentiation of human CD4(+) T cells and influences expression of its own receptor. Immun Inflamm Dis 2015; 3: 455-468.

[18] Kempuraj D, Frydas S, Conti P, Kandere- Grzybowska K, Boucher W, Letourneau R, Madhappan B, Huang SH, Sugimoto K, Papadopoulou NG, Christodoulou S and Theoharides TC. Interleukin-25 (or IL-17E): a new IL-17 family member with growth factor/inflammatory actions. Int J Immunopathol Pharmacol 2003; 16: 185-188.

[19] Saadoun D, Terrier B and Cacoub P. Interleukin-25: key regulator of inflammatory and autoimmune diseases. Curr Pharm Des 2011; 17: 3781-3785.

[20] Javan MR, Seyfizadeh N, Aslani S, Farhoodi M and Babaloo Z. Molecular analysis of interleukin-25 exons 1 and 2 and its serum levels in Iranian patients with multiple sclerosis. Am J Clin Exp Immunol 2014; 3: 91-96.
[21] Shi T, Xie Y, Fu Y, Zhou Q, Ma Z, Ma J, Huang Z, Zhang J and Chen J. The signaling axis of microRNA-31/interleukin-25 regulates Th1/Th17-mediated inflammation response in colitis. Mucosal Immunol 2017; 10: 983-995.
[22] Su J, Chen T, Ji XY, Liu C, Yadav PK, Wu R, Yang P and Liu Z. IL-25 downregulates Th1/Th17 immune response in an IL-10-dependent manner in inflammatory bowel disease. Inflamm Bowel Dis 2013; 19: 720-728.
[23] Saenz SA, Siracusa MC, Monticelli LA, Ziegler CG, Kim BS, Brestoff JR, Peterson LW, Wherry EJ, Goldrath AW, Bhandoola A and Artis D. IL-25 simultaneously elicits distinct populations of innate lymphoid cells and multipotent progenitor type 2 (MPPtype2) cells. J Exp Med 2013; 210: 1823-1837.
[24] Bruder SP, Kurth AA, Shea M, Hayes WC, Jaiswal N and Kadiyala S. Bone regeneration by implantation of purified, culture-expanded human mesenchymal stem cells. J Orthop Res 1998; 16: 155-162.
[25] Murano M, Maemura K, Hirata I, Toshina K, Nishikawa T, Hamamoto N, Sasaki S, Saitoh O and Katsu K. Therapeutic effect of intracolonically administered nuclear factor kappa B (p65) antisense oligonucleotide on mouse dextran sulphate sodium (DSS)-induced colitis. Clin Exp Immunol 2000; 120: 51-58.
[26] Williams KL, Fuller CR, Dieleman LA, DaCosta CM, Haldeman KM, Sartor RB and Lund PK. Enhanced survival and mucosal repair after dextran sodium sulfate-induced colitis in transgenic mice that overexpress growth hormone. Gastroenterology 2001; 120: 925-937.
[27] Liu Z, Feng BS, Yang SB, Chen X, Su J and Yang PC. Interleukin (IL)-23 suppresses IL-10 in inflammatory bowel disease. J Biol Chem 2012; 287: 3591-3597.
[28] Song SU, Kim CS, Yoon SP, Kim SK, Lee MH, Kang JS, Choi GS, Moon SH, Choi MS, Cho YK and Son BK. Variations of clonal marrow stem cell lines established from human bone marrow in surface epitopes, differentiation potential, gene expression, and cytokine secretion. Stem Cells Dev 2008; 17: 451-461.
[29] Franze E, Rizzo A, Caruso R, Pallone F and Monteleone G. Interleukin-25 negatively controls pathogenic responses in the gut. Inflamm Allergy Drug Targets 2011; 10: 187-191.
[30] Wang C, Liu Q, Chen F, Xu W, Zhang C and Xiao W. IL-25 Promotes Th2 immunity responses in asthmatic mice via nuocytes activation. PLoS One 2016; 11: e0162393.
[31] Beale J, Jayaraman A, Jackson DJ, Macintyre JD, Edwards MR, Walton RP, Zhu J, Ching YM, Shamji B, Edwards M, Westwick J, Cousins DJ, Hwang YY, McKenzie A, Johnston SL and Bartlett NW. Rhinovirus-induced IL-25 in asthma exacerbation drives type 2 immunity and allergic pulmonary inflammation. Sci Transl Med 2014; 6: 256ra134.
[32] Howitt MR, Lavoie S, Michaud M, Blum AM, Tran SV, Weinstock JV, Gallini CA, Redding K, Margolskee RF, Osborne LC, Artis D and Garrett WS. Tuft cells, taste-chemosensory cells, orchestrate parasite type 2 immunity in the gut. Science 2016; 351: 1329-1333.
[33] Vannella KM, Ramalingam TR, Borthwick LA, Barron L, Hart KM, Thompson RW, Kindrachuk KN, Cheever AW, White S, Budelsky AL, Comeau MR, Smith DE and Wynn TA. Combinatorial targeting of TSLP, IL-25, and IL-33 in type 2 cytokine-driven inflammation and fibrosis. Sci Transl Med 2016; 8: 337ra365.
[34] Caruso R, Stolfi C, De Nitto D, Pallone F and Monteleone G. The dual role of interleukin-25 in the control of immune-mediated pathologies. Curr Mol Med 2011; 11: 26-30.
[35] Su X, Huang Q, Chen J, Wang M, Pan H, Wang R, Zhou H, Zhou Z, Liu J, Yang F, Li T and Liu L. Calycosin suppresses expression of pro-inflammatory cytokines via the activation of p62/Nrf2-linked heme oxygenase 1 in rheumatoid arthritis synovial fibroblasts. Pharmacol Res 2016; 113: 695-704.
[36] Zare L, Sheikh Fathollahi M, Kazemi Arababadi M, Shamsizadeh A, Daneshpajouh B, Zainodini N and Allahtavakoli M. The association between C424c/A polymorphism within the IL-25 gene and multiple sclerosis. Iran Red Crescent Med J 2016; 18: e25995.
[37] Lv FJ, Tuan RS, Cheung KM and Leung VY. Concise review: the surface markers and identity of human mesenchymal stem cells. Stem Cells 2014; 32: 1408-1419.
[38] Bernardo ME and Fibbe WE. Mesenchymal stromal cells: sensors and switchers of inflammation. Cell Stem Cell 2013; 13: 392-402.
[39] Bartholomew A, Sturgeon C, Siatskas M, Ferrer K, McIntosh K, Patil S, Hardy W, Devine S, Ucker D, Deans R, Moseley A and Hoffman R. Mesenchymal stem cells suppress lymphocyte proliferation in vitro and prolong skin graft survival in vivo. Exp Hematol 2002; 30: 42-48.
[40] Di Nicola M, Carlo-Stella C, Magni M, Milanesi M, Longoni PD, Matteucci P, Grisanti S and Gianni AM. Human bone marrow stromal cells suppress T-lymphocyte proliferation induced by cellular or nonspecific mitogenic stimuli. Blood 2002; 99: 3838-3843.
[41] Le Blanc K, Tammik L, Sundberg B, Haynesworth SE and Ringden O. Mesenchymal stem

cells inhibit and stimulate mixed lymphocyte cultures and mitogenic responses independently of the major histocompatibility complex. Scand J Immunol 2003; 57: 11-20.

[42] Ozdemir AT, Ozgul Ozdemir RB, Kirmaz C, Sariboyaci AE, Unal Halbutogllari ZS, Ozel C and Karaoz E. The paracrine immunomodulatory interactions between the human dental pulp derived mesenchymal stem cells and CD4 T cell subsets. Cell Immunol 2016; 310: 108-115.

[43] Wang D, Huang S, Yuan X, Liang J, Xu R, Yao G, Feng X and Sun L. The regulation of the Treg/Th17 balance by mesenchymal stem cells in human systemic lupus erythematosus. Cell Mol Immunol 2017; 14: 423-431.

[44] Wang WQ, Dong K, Zhou L, Jiao GH, Zhu CZ, Li WW, Yu G, Wu WT, Chen S, Sun ZN, Wang YM, Liu WT, Zhang J, Wang BM and Feng XM. IL-37b gene transfer enhances the therapeutic efficacy of mesenchumal stromal cells in DSS-induced colitis mice. Acta Pharmacol Sin 2015; 36: 1377-1387.

[45] Luz-Crawford P, Kurte M, Bravo-Alegria J, Contreras R, Nova-Lamperti E, Tejedor G, Noel D, Jorgensen C, Figueroa F, Djouad F and Carrion F. Mesenchymal stem cells generate a CD4+CD25+Foxp3+ regulatory T cell population during the differentiation process of Th1 and Th17 cells. Stem Cell Res Ther 2013; 4: 65.

[46] Yagi H, Soto-Gutierrez A, Parekkadan B, Kitagawa Y, Tompkins RG, Kobayashi N and Yarmush ML. Mesenchymal stem cells: mechanisms of immunomodulation and homing. Cell Transplant 2010; 19: 667-679.

[47] Braza F, Dirou S, Forest V, Sauzeau V, Hassoun D, Chesne J, Cheminant-Muller MA, Sagan C, Magnan A and Lemarchand P. Mesenchymal stem cells induce suppressive macrophages through phagocytosis in a mouse model of asthma. Stem Cells 2016; 34: 1836-1845.

[48] Eggenhofer E, Benseler V, Kroemer A, Popp FC, Geissler EK, Schlitt HJ, Baan CC, Dahlke MH and Hoogduijn MJ. Mesenchymal stem cells are short-lived and do not migrate beyond the lungs after intravenous infusion. Front Immunol 2012; 3: 297.

[49] Gonzalez MA, Gonzalez-Rey E, Rico L, Buscher D and Delgado M. Adipose-derived mesenchymal stem cells alleviate experimental colitis by inhibiting inflammatory and autoimmune responses. Gastroenterology 2009; 136: 978-989.

[50] Chen QQ, Yan L, Wang CZ, Wang WH, Shi H, Su BB, Zeng QH, Du HT and Wan J. Mesenchymal stem cells alleviate TNBS-induced colitis by modulating inflammatory and autoimmune responses. World J Gastroenterol 2013; 19: 4702-4717.