Combined Rg1 and adipose-derived stem cells Alleviate DSS-induced colitis in a mouse model

Rui Zhang (✉ 18101597871@163.cm)  
Southeast University Zhongda Hospital  https://orcid.org/0000-0002-3760-2174

Qingqing Zhang  
Nanjing Medical University

Yanni Chen  
Southeast University Zhongda Hospital

Qing Zhao  
Nanjing Medical University

Bo Zhang  
Southeast University Zhongda Hospital

Ling Wang  
Nanjing University of Chinese Medicine

Chungen Zhou  
Nanjing University of Chinese Medicine

Qi Zhang  
Nanjing University of Chinese Medicine

Kun Chen  
Southeast University Zhongda Hospital

Yuqing Zhang  
Nanjing University of Chinese Medicine

Xiaotao Hou  
Southeast University Zhongda Hospital

Hao Chen  
Southeast University Zhongda Hospital

Xingyin Liu  
Nanjing Medical University

Min Ni  
Nanjing University of Chinese Medicine

Bin Jiang  
Nanjing University of Chinese Medicine

Research Article
Abstract

Background

Inflammatory bowel diseases (IBDs) including Crohn's disease and ulcerative colitis are chronic inflammatory disorders that can affect the entire gastrointestinal tract and the colonic mucosa, no medical or surgical cure for IBD, and all have side effects that limit their use, exhibit a high necessity for new therapeutic strategies. Adipose-derived stem cells (ADSC) therapy represents a promising option for the treatment of IBD. Rg1 Previous study indicated that ginsenoside (Rg1) can ameliorate inflammatory disease such as colitis by inhibiting the binding of LPS to TLR4 on macrophages and restoring the Th17/Treg imbalance [1]. In this study, we investigated whether Rg1 can enhance the effect of ADSC on DSS-induced colitis in a mouse model.

Methods

Mice with dextran sulfate sodium-induced colitis were injected intravenously with ADSC and administered with Rg1 by gavage. Body weight change, colon length, H&E staining were used to evaluate colon inflammation severity in a DSS-induced colitis Serum were collected for Cytokine detection by ELISA. The proportion and FMI of immune cells in spleen were analyzed by flow cytometry. Stool DNA was extracted for 16S rRNA gene sequencing.

Results

Rg1 and ADSC showed significantly ameliorated colon inflammation, such as body weight loss, shortening of colon length, histology score. Rg1 and ADSC treatment downregulated the level of proinflammatory cytokines, including IL-1β, TNF-α, IL-6, IL-4 and IL-17A and upregulated the immunosuppressive cytokine IL-10 in serum. We observed that the structure of the microbial community in Rg1 + ADSC group were significantly changed compared to that of ADSC and Rg1 groups, respectively. Additionally, Rg1 and ADSC treated selectively upregulated the percentage of spleen regulatory T (Treg) cells as well as downregulated the frequency of T helper type 17 (Th17) cells, ameliorating the Treg/Th17 balance to maintain intestinal homeostasis. Furthermore, we found the combination of ADSC + Rg1 groups showed more efficiently than that of ADSC and Rg1 alone, respectively, which indicates that the regulation effect of Rg1 on gut microbiome may enhance the effects of ADSCs in restoring immune balance.

Conclusions

Our study indicated that the combination of Rg1 and ADSC can alleviate dextran sulfate sodium-induced colitis more efficiently than that of ADSC alone, Rg1 can enhance the effect of ADSC on DSS-induced
Background

The two major clinically defined forms of inflammatory bowel disease (IBD), Crohn's disease (CD) and ulcerative colitis (UC), are chronic remittent or progressive inflammatory conditions that can affect the entire gastrointestinal tract and the colonic mucosa [2]. Recently, increasing studies indicated that the interactions of gut microbiome, mucosal immune system and the manner in which environmental factors modify these relationships appear particularly relevant for the development of IBD[3]. IBD is associated with substantial morbidity, decreased quality of life, and colitis-correlated colorectal cancer (CAC) development, it has increasingly emerged as a public health challenge worldwide[4]. However, the drugs treated IBD are far from optimal, and patients have to face lifelong treatment and debility. So, it is urgent to develop treatment which could reduce side effects and improve long-term effect of IBD.

As an emerging therapy for patients with IBD, mesenchymal stem cells (MSCs) have promising future in restoring epithelial barrier integrity, homing to the damaged tissue, inhibiting inflammatory response, and regulating immunity[5–7] [8–10]. Moreover, a lot of studies demonstrated that systemic administration of MSC by the intravenous or intraperitoneal injection could alleviate colitis of mouse model [11–14]. As a category of MSCs, We and other groups had reported that the ADSC was a feasible and effective treatment for Crohn’s fistula-in-ano, compared with traditional incision and thread-drawing, ADSC therapy could protect anal function of patients, relieve pain, allow quick recovery, be well-tolerated, and improve the quality of life during perioperative period[15].

Ginsenoside Rg1 is a traditional stem extract and is one of the main active ingredients of ginseng [16, 17]. It has been reported that ginsenoside Rg1 can promote stem cell orientation transformation and induce stem cell proliferation [18, 19]. For example, Rg1 could enhance the proliferation, differentiation, and soft tissue regeneration of human breast adipose ADSCs in collagen type I sponge scaffolds in vitro and in vivo, and a broad new organizational network was also formed[20]. Moreover, Zhu et al., reported that Rg1 markedly reduces proinflammatory cytokines that released from dendritic cells in a mouse DSS-induced colitis model [1]. To help to develop novel therapeutic procedures for IBD patients, in the current study, we investigate the role of the combination therapy of Rg1 and ADSC administration in a mouse DSS-induced colitis and explore the specific mechanisms involved in this process.

Material And Method

Adipose Derived Stem Cell Derivation

Human abdomen or buttock adipose tissues were collected with informed consent from patients receiving regenerative medicine using ADSC at Nanjing Hospital of Chinese Medicine Affiliated to Nanjing University of Chinese Medicine (Nanjing, China). Adipose tissues were collected from healthy adult male patient who provided informed consent at Nanjing Hospital of Chinese Medicine Affiliated to Nanjing
University of Chinese Medicine (Nanjing, China). ADSC were isolated from samples, and stromal vascular fraction were cultured with serum-free culture medium, at 37°C in an atmosphere containing 5% CO2. After reaching confluence, adherent cells were trypsinized and replated. Cells were passaged using serum-free culture medium three times, and the characteristics of ADSC were verified by analysis of the differentiation, proliferation, and immunologic phenotypes. The cells were frozen and delivered to Nanjing Medical University. After thawing, the cells were immediately washed, counted, suspended in phosphate-buffered saline (PBS), For flow cytometry analysis, ADSCs were harvested, washed, and incubated with specific MSC marker antibodies CD90-FITC, CD44-PE, CD105-FITC, CD73-PE, CD34-PE, and CD45-FITC.

Animal

Mice six-to eight-week-old C57BL/6 male mice were purchased from experiment center of Nanjing Medical University (Nanjing, China) and housed under controlled temperature, humidity, and light cycle conditions. All animal experiments were conducted in compliance with regulations and approved by the Institutional Animal Care and committee the Nanjing Medical University.

Induction of experimental colitis and study design

Colitis was induced by providing drinking water containing 3% DSS (molecular weight 36,000–50,000; MP Biomedicals, Santa Ana, CA) for 7 days followed by regular water for 7 days. Mice were divided into five groups, ADSC and ADSC + Rg1 groups were injected intravenously with 1*10^6 ADSC[21] on day 4th and 7th, whereas Control, Rg1 and DSS groups were injected with PBS. Moreover, Rg1 and ADSC + Rg1 groups were administered with Rg1 (at a dose of 20 mg/kg[24]) by gavage once daily from the rst day to the 14th day (Start with DSS treatment), whereas others were administered with PBS by gavage once daily. All were sacrificed on the 15th day after the start of the experiment.

Body Weight and Assessment of Colon Length

To evaluate the therapeutic effects of ADSC and Rg1, the body weight, and colon length were analyzed. Body weight was recorded daily, colon lengths were measured from the anus to the cecum soon after harvesting the colon. Samples were measured as an indirect assessment of inflammation.

Histopathological analysis

Histological score was calculated as follows. The colon was excised, fixed in 10% formalin, embedded in paraffin wax, and sliced into 4-µm-thick sections. After hematoxylin and eosin (H&E) staining, histological evaluation was performed in a blinded manner according to a previously published scoring system. Histology was scored as described previously [22]. Simply, score 1: mild mucosal inflammatory cell infiltrates with intact epithelium; Score 2: inflammatory cell infiltrates into mucosa and submucosa with undamaged epithelium; Score 3: mucosal infiltrates with focal ulceration; Score 4: inflammatory cell infiltrates in mucosa and submucosa and focal ulceration; Score 5: moderate inflammatory cell infiltration into mucosa and submucosa with extensive ulcerations; Score 6: transmural inflammation and extensive ulceration.

Serum Parameter Analysis
We collected all the mice' blood serum and tested 6 immune cytokines, including IL-10, IL-6, IL-17A, IL-33, IL-1β, and TNF-α following the company’s kit procedures. All of assay kits are purchased from R&D System. All immune cytokines were statistically analyzed by T-test included in GraphPad Prism software 8.0.

**Flow Cytometry**

Spleen were removed and mechanically dissociated into single cell suspensions. Splenocytes were incubated with Fc block (CD16/32) for 10 minutes to block non-specific binding before stained with the conjugated antibodies. For surface staining, cells were incubated with specific antibodies for 30 minutes on 4°C followed by washing twice. For intracellular cytokine staining, cells were stimulated with phorbol myristate acetate and ionomycin and in the presence of monensin (eBioscience) for 5 h prior to staining with the BD Fixation/Permeabilization Solution kit. Treg cells were fixed and permeabilized using the eBioscience Foxp3/transcription factor staining buffer kit (Invitrogen) according to manufacturer’s instruction. Flow cytometry data were acquired on a BD FACSVerse flow cytometer (BD Bioscience) and analyzed using FlowJo version 10 software.

**DNA isolation and 16S rRNA gene sequencing**

In total, 100-mg stool samples were used to extract total bacteria genome DNA following the protocol of the DNA extraction kit (#DP328, Tiangen Company, Beijing, China). The concentration and purity of the extracted bacterial DNA were detected using the Qubit 2.0 Fluorometer (Thermo Scientific, USA). The V3 and V4 regions of 16S rRNA genes were amplified using composite specific primer. The 16S rRNA V3 and V4 specific primers are 338F (5'-ACTCCTACGGGAGGCAGCAG-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3'). The 16S rDNA data were analyzed using QIIME software package 1.9, all the analysis and calculation methods used in-house Perl scripts, with special reference to these two articles[23, 24].

**Statistical analysis**

Student's t-test (unpaired, two-tailed) was used to figure out levels of significance for comparisons between two groups by using Graphpad Prism 8.0 software. Results are shown as mean ± SD, P value less than 0.05 was considered significant.

**Results**

**Characterization of Human ADSC**

ADSC expressed all specific MSC markers CD73, CD105, CD90, CD44 and lacked expression of the hematopoietic markers CD34 and CD45. Differentiation to adipocytes, chondrocytes, and osteocytes was observed in ADSC (Fig. s1).
ADSC and Rg1 administration ameliorated DSS-induced colitis

As Fig. 1b shown, the body weights of the mice in the DSS groups were consistently reduced since the fifth day, while the mice in the ADSC + Rg1 groups and Rg1 groups mice showed a significant improvement in weight loss, although there was an apparent decrease in the ADSC groups (P < 0.05). In the colitis model, disease severity is typically associated with colon length shortening due to intestinal inflammation [25]. The length of colon in the DSS groups were shorter than in the ADSC groups, Rg1 groups, and ADSC + Rg1 groups (p < 0.05).

Consistent with previous studies [11], H&E colon analysis (Fig. 1e) showed that DSS-induced colitis resulted in extended ulcerations, destroyed crypts and transmural inflammatory infiltration, with a barely complete mucosal structure. However, in mice treated with ADSC and Rg1, histological damage was ameliorated, as evidenced by a preserved mucosal architecture showing focal erosions and mild/moderate mucosa inflammatory infiltration. To evaluate the intestinal mucosal architecture and inflammatory infiltration, we used the histopathology score system[22] to quantify inflammatory severity degree, and the mice treated with ADSC and Rg1 had a significantly lower score than the mice treated with DSS only (P < 0.05) (Fig. 1f).

**ADSC and Rg1 treatment could alleviate colitis by regulating pro/anti-inflammatory cytokines**

Pro-inflammatory cytokines play a crucial role in the progression of DSS-induced colitis [26]. To explore whether ADSC and Rg1 treatment could alleviate colitis by regulating inflammation, we detected the cytokine expression in blood serum. As shown in Figure .2a-h, the levels of inflammatory cytokines IL-6, IL-33, TNF-α, IL-1β and IL-17A in Rg1, ADSC, and ADSC + Rg1 groups decreased significantly and IL-10 increased compared with those in the DSS groups. Moreover, we found that the combined of ADSC + Rg1 groups can significantly inhibit the expression of inflammatory cytokines expression compared to Rg1 and ADSC alone groups (Fig. 2a, b, c, d, e, f, g, h). Therefore, Rg1 can enhance the effect of ADSC on DSS-induced colitis in a mouse model.

**ADSC and Rg1 regulated Treg/Th17 balance to maintain intestinal homeostasis**

Previous study has demonstrated that ADSC could inhibit Th17 response in T-cell-mediated autoimmune diseases[27]. It has also been reported that ADSC could inhibit Treg/Th17 differentiation in DSS-induced colitis model mice[15]. So we further compared the number of Th17 cells between groups. We found the number of Th17 cells were much higher in the DSS group than in the Rg1, ADSC, and ADSC + Rg1 groups (Fig. 3b, c, e). But rather, the percentage of Treg cells was remarkably higher in Rg1, ADSC, and ADSC + Rg1 groups compared with DSS groups. In summary, it indicated that Rg1 and ADSC administration
selectively upregulated the frequency of Treg cells as well as downregulated the ratio of Th17 cells against DSS-induced colitis, improving the Treg/Th17 balance to maintain intestinal homeostasis. (Fig. 3a, d, f). Besides, the ADSC and Rg1 treated simultaneously showed better trends of recovering Treg/Th17 balance than ADSC and Rg1 groups alone.

**ADSC and Rg1 treatment significantly altered the gut microbiota diversity and composition**

Gut microbiota is an important factor in regulate intestinal inflammation [28]. Therefore, we further compared the microbiota composition of five groups. The alpha diversity of bacterial communities was evaluated according to Shannon's diversity index (Fig. 4a). The Shannon's diversity index of ADSC and Rg1 showed no statistical difference with DSS groups (Fig. 4a, P > 0.05). The PCoA scatterplot revealed the clear clustering of gut bacterial communities between five groups (Fig. 4c). To compare the gut microbiota composition of the five groups, we then conducted a statistical analysis of gut microbiota at the genus level using the Kruskal–Wallis test. At the genera level, compared to DSS groups, the gut microbiota of ADSC, Rg1, and ADSC + Rg1 groups was characterized by an increased Rikenellaceae RC9 and Ruminococcaceae UCG-013 level (Fig. 4d) and a lower ratio of Escherichia-shigella (Fig. 4d). Interestingly, the ratio of Rikenellaceae RC9, Ruminococcaceae UCG-013, Esripelatoclostridium and Escherichia-shigella lever in ADSC + Rg1 groups were more similar to control groups. In addition, we also found that the effect of ADSC on gut microbiota disturbance induced by DSS was not significantly improved, there was even a worsening trend. However, Rg1 can restore the disturbed gut microbiota induced by DSS treated by ADSC.

We also identified significant changes in the multiple biological pathways of five groups (Fig. 4e). As shown in Fig. 4e, the fifteen modules in the five groups were involved in L-arginine degradation, superpathway of L-arginine, putrescine, and 4-aminobutanoate degradation, superpathway of methylglyoxal degradation, glyoxylate cycle, Citrate cycle (TCA cycle), L-methionine biosynthesis, and heme biosynthesis, and methanogenesis from acetate. The overrepresentation of the L-arginine degradation pathway in DSS groups may be related to the high abundance of Escherichia-shigella, which has been shown to have a wide capacity for degrading polysaccharides in those samples[29]. In all, the predication analysis indicated that gut dysbiosis may induce a disease-linked state through the interference of physiological metabolic functions.

**Discussion**

In our study, we found that ADSC and Rg1 administrated could substantially ameliorate the colitis compared with ADSC and Rg1 treated alone, as indicated by lower body weight loss, colon length shortening, and better histological scores. Additionally, some of the potential mechanisms were presented, including (i) amelioration of colon mucosal barrier damage; (ii) modulation of the inflammatory response; (iii) reshaping the gut microbiota; and (iv) regulation of Treg/Th17 differentiation.
The excessive response of Th17 cells and insufficient function of Treg cells correlate with the onset of IBD. Previous study has demonstrated that ADSC could inhibit Th17 response in T-cell-mediated autoimmune diseases [27] and inhibit Treg/Th17 differentiation DSS-induced colitis model mice [30]. Consistent with previous study, we found ADSC treatment improved the Treg/Th17 balance. Th17 cells produce pro-inflammatory cytokines IL-17A, which contribute to the progression of IBD [31]. Tregs are derived from the thymus with the function of suppressing the innate immune response [32]. Previous study indicated that improving Treg/Th17 balance contributed to the re-establishment of intestinal immune homeostasis in IBD [33]. In this study, we also found the Rg1 can enhance the effect of ADSC of improving the Treg/Th17 balance.

We observed that the microbial community diversity and structure in ADSC, Rg1, and ADSC + Rg1 groups were significantly changed compared to those of DSS groups. For example, in DSS groups, we found that reduction of *Rikenellaceae RC9* and *Ruminococcaceae UCG-013* and increase of *Erysipelatoclostridium* and *Escherichia-shigella* compared with other groups. *Rikenellaceae_RC9_gut_group* is a dominant group of *Bacteroidetes*, which might affect intestinal permeability, oxidative stress and energy metabolism, and might contribute to the pathogenesis of acute myocardial ischemia [34] and inflammation [35]. The *Ruminococcaceae* family is a member of the Firmicutes phylum and comprises a broad spectrum of species with different functional properties. An underrepresentation of species belonging to the family has previously been reported in IBD [36]. *Erysipelatoclostridium* and *Escherichia-shigella* have been reported that play an important role in inflammatory response [37, 38]. Besides, our results also showed that ADSC transplantation after combined with the ginsenoside Rg1 administration could significantly improve the ratio of *Rikenellaceae RC9* and *Ruminococcaceae UCG-013* and reduces the level of *Erysipelatoclostridium* and *Escherichia-shigella*, which was superior to the ADSC and Rg1 administration alone.

The complex interactions between biological pathways and gut microbiota are intense associated with host-microbe. Notably, gut microbiota plays an essential role in IBD through the pathways such as glyoxylate cycle, Citrate cycle (TCA cycle), and L-arginine degradation [39–41], consistent with this, the KEGG pathways in the ADSC, Rg1 and ADSC + Rg1 group were different from DSS groups, such as glyoxylate cycle, Citrate cycle (TCA cycle), and L-arginine pathway reduced significantly, which implicated ADSC and Rg1 may modulate these pathway though restoring composition of gut microbiota.

Taken together, the study suggested the combination of ADSC and Rg1 administration may enhance clinical efficacy of IBD through restore the Treg/Th17 balance and gut microecological structure.

**Conclusion**

Overall, our results showed that Rg1 and ADSC treatment restore balance of pro/anti-inflammatory cytokines, Treg/Th17 balance, gut microecology. We confirmed that the combination of Rg1 and ADSC administration could alleviate DSS-induced colitis more efficient than that of Rg1 or ADSC treatment alone.
Abbreviations

IBD: Inflammatory bowel diseases; MSCs: Mesenchymal stem cells; ADSC: Adipose Derived Stem Cell.

Declarations

Authors’ contributions

BJ, MN and XL conceived and designed the study project. RZ, QQ, YN, BZ, CG, LW, QZ and YQ did experiments and performed analysis. RZ wrote the manuscript. BJ, MN and XY finalized the manuscript. All authors contributed at all stages and critically reviewed the content. All authors read and approved the final manuscript.

Acknowledgements

We thank the doctors who helped with clinical experience at Anorectal Surgery of Zhongda Hospital Southeast University.

Competing interests

The authors declare that they have no competing interests.

Availability of data and materials

Our study was approved by the Ethics Committee of Nanjing Medical University. All methods were carried out in accordance with the relevant guidelines.

Consent for publication

Not applicable.

Ethics approval and consent to participate

Our study was approved by the Ethics Committee of Nanjing Medical University. All participants provided a written informed consent upon enrolment. The methods were carried out in accordance with the relevant guidelines.

Funding

This work was supported by the National Natural Science Foundation of China (No. 81803926); Science and Technology Development Project of Nanjing of China (No. ZKKX17034) and Key Scientific Research Projects of Jiangsu Commission of Health (No. ZDB2020002).

References
1. Zhu G, Wang H, Wang T, Shi F. Ginsenoside Rg1 attenuates the inflammatory response in DSS-induced mice colitis. Int Immunopharmacol. 2017;50:1–5.

2. Kaser A, Zeissig S, Blumberg RS. Inflammatory bowel disease. Annu Rev Immunol. 2010;28:573–621.

3. Lightner AL. Stem Cell Therapy for Inflammatory Bowel Disease. Clin Transl Gastroenterol. 2017;8(3):e82.

4. Kaplan GG, Ng SC. Understanding and Preventing the Global Increase of Inflammatory Bowel Disease. Gastroenterology. 2017;152(2):313–21. e312.

5. Xing Y, Chen X, Cao Y, Huang J, Xie X, Wei Y. Expression of Wnt and Notch signaling pathways in inflammatory bowel disease treated with mesenchymal stem cell transplantation: evaluation in a rat model. Stem Cell Res Ther. 2015;6:101.

6. Wang X, Lazorchak AS, Song L, Li E, Zhang Z, Jiang B, Xu RH. Immune modulatory mesenchymal stem cells derived from human embryonic stem cells through a trophoblast-like stage. Stem Cells. 2016;34(2):380–91.

7. Song WJ, Li Q, Ryu MO, Ahn JO, Bhang DH, Jung YC, Youn HY. TSG-6 released from intraperitoneally injected canine adipose tissue-derived mesenchymal stem cells ameliorate inflammatory bowel disease by inducing M2 macrophage switch in mice. Stem Cell Res Ther. 2018;9(1):91.

8. Li X, Wang Q, Ding L, Wang YX, Zhao ZD, Mao N, Wu CT, Wang H, Zhu H, Ning SB. Intercellular adhesion molecule-1 enhances the therapeutic effects of MSCs in a dextran sulfate sodium-induced colitis models by promoting MSCs homing to murine colons and spleens. Stem Cell Res Ther. 2019;10(1):267.

9. Gong W, Guo M, Han Z, Wang Y, Yang P, Xu C, Wang Q, Du L, Li Q, Zhao H, et al. Mesenchymal stem cells stimulate intestinal stem cells to repair radiation-induced intestinal injury. Cell Death Dis. 2016;7(9):e2387.

10. Udalamaththa VL, Jayasinghe CD, Udagama PV. Potential role of herbal remedies in stem cell therapy: proliferation and differentiation of human mesenchymal stromal cells. Stem Cell Res Ther. 2016;7(1):110.

11. Goncalves Fda C, Schneider N, Pinto FO, Meyer FS, Visioli F, Pfaffenseller B, Lopez PL, Passos EP, Cime-Lima EO, Meurer L, et al. Intravenous vs intraperitoneal mesenchymal stem cells administration: what is the best route for treating experimental colitis? World J Gastroenterol. 2014;20(48):18228–39.

12. Hu J, Zhao G, Zhang L, Qiao C, Di A, Gao H, Xu H. Safety and therapeutic effect of mesenchymal stem cell infusion on moderate to severe ulcerative colitis. Exp Ther Med. 2016;12(5):2983–9.

13. Yan Y, Zhao N, He X, Guo H, Zhang Z, Liu T. Mesenchymal stem cell expression of interleukin-35 protects against ulcerative colitis by suppressing mucosal immune responses. Cytoteraphy. 2018;20(7):911–8.

14. Zhou C, Li M, Zhang Y, Ni M, Wang Y, Xu D, Shi Y, Zhang B, Chen Y, Huang Y, et al. Autologous adipose-derived stem cells for the treatment of Crohn's fistula-in-ano: an open-label, controlled trial.
Stem Cell Res Ther. 2020;11(1):124.

15. Heidari M, Pouya S, Baghaei K, Aghdaei HA, Namaki S, Zali MR, Hashemi SM. The immunomodulatory effects of adipose-derived mesenchymal stem cells and mesenchymal stem cells-conditioned medium in chronic colitis. J Cell Physiol. 2018;233(11):8754–66.

16. Zhang Q, Zhao YH. Therapeutic angiogenesis after ischemic stroke: Chinese medicines, bone marrow stromal cells (BMSCs) and their combinational treatment. Am J Chin Med. 2014;42(1):61–77.

17. Chu SF, Zhang Z, Zhou X, He WB, Chen C, Luo P, Liu DD, Ai QD, Gong HF, Wang ZZ, et al. Ginsenoside Rg1 protects against ischemic/reperfusion-induced neuronal injury through miR-144/Nrf2/ARE pathway. Acta Pharmacol Sin. 2019; 40(1):13–25.

18. Zhang JT. [Nootropic mechanisms of ginsenoside Rg1—influence on neuronal plasticity and neurogenesis]. Yao Xue Xue Bao. 2005;40(5):385–8.

19. Guo YH, Zhao S, Du YX, Xing QJ, Chen BL, Yu CQ. Effects of ginsenoside Rg1-loaded alginate-chitosan microspheres on human bone marrow stromal cells. Biosci Rep. 2017;37(3).

20. Dong J, Zhu G, Wang TC, Shi FS. Ginsenoside Rg1 promotes neural differentiation of mouse adipose-derived stem cells via the miRNA-124 signaling pathway. J Zhejiang Univ Sci B. 2017;18(5):445–8.

21. Bao C, Wang Y, Min H, Zhang M, Du X, Han R, Liu X. Combination of ginsenoside Rg1 and bone marrow mesenchymal stem cell transplantation in the treatment of cerebral ischemia reperfusion injury in rats. Cell Physiol Biochem. 2015;37(3):901–10.

22. Llewellyn SR, Britton GJ, Contijoch EJ, Vennaro OH, Mortha A, Colombel JF, Grinspan A, Clemente JC, Merad M, Faith JJ. Interactions Between Diet and the Intestinal Microbiota Alter Intestinal Permeability and Colitis Severity in Mice. Gastroenterology. 2018;154(4):1037–46 e1032.

23. Guo M, Wang H, Xu S, Zhuang Y, An J, Su C, Xia Y, Chen J, Xu ZZ, Liu Q, et al. Alteration in gut microbiota is associated with dysregulation of cytokines and glucocorticoid therapy in systemic lupus erythematosus. Gut Microbes. 2020;11(6):1758–73.

24. Chen K, Luan X, Liu Q, Wang J, Chang X, Snijders AM, Mao JH, Secombe J, Dan Z, Chen JH, et al. Drosophila Histone Demethylase KDM5 Regulates Social Behavior through Immune Control and Gut Microbiota Maintenance. Cell Host Microbe. 2019;25(4):537–52 e538.

25. Wirtz S, Popp V, Kindermann M, Gerlach K, Weigmann B, Fichtner-Feigl S, Neurath MF. Chemically induced mouse models of acute and chronic intestinal inflammation. Nat Protoc. 2017;12(7):1295–309.

26. Andujar I, Recio MC, Giner RM, Cienfuegos-Jovellanos E, Laghi S, Muguera B, Rios JL. Inhibition of ulcerative colitis in mice after oral administration of a polyphenol-enriched cocoa extract is mediated by the inhibition of STAT1 and STAT3 phosphorylation in colon cells. J Agric Food Chem. 2011;59(12):6474–83.

27. Withers DR, Hepworth MR, Wang X, Mackley EC, Halford EE, Dutton EE, Marriott CL, Brucklacher-Waldert V, Veldhoen M, Kelsen J, et al. Transient inhibition of ROR-gammat therapeutically limits...
intestinal inflammation by reducing TH17 cells and preserving group 3 innate lymphoid cells. Nat Med. 2016;22(3):319–23.

28. Sartor RB, Wu GD. Roles for Intestinal Bacteria, Viruses, and Fungi in Pathogenesis of Inflammatory Bowel Diseases and Therapeutic Approaches. Gastroenterology. 2017;152(2):327–39. e324.

29. Miscevic D, Mao JY, Mozell B, Srirangan K, Abedi D, Moo-Young M, Chou CP. Bio-based production of poly(3-hydroxybutyrate-co-3-hydroxyvalerate) with modulated monomeric fraction in Escherichia coli. Appl Microbiol Biotechnol. 2021;105(4):1435–46.

30. Karlsson C, Emanuelsson K, Wessberg F, Kajic K, Axell MZ, Eriksson PS, Lindahl A, Hyllner J, Strehl R. Human embryonic stem cell-derived mesenchymal progenitors—potential in regenerative medicine. Stem Cell Res. 2009;3(1):39–50.

31. van Wijk F, Cheroutre H. Mucosal T cells in gut homeostasis and inflammation. Expert Rev Clin Immunol. 2010;6(4):559–66.

32. Fontenot JD, Gavin MA, Rudensky AY. Foxp3 programs the development and function of CD4 + CD25 + regulatory T cells. Nat Immunol. 2003;4(4):330–6.

33. Bian X, Wu W, Yang L, Lv L, Wang Q, Li Y, Ye J, Fang D, Wu J, Jiang X, et al. Administration of Akkermansia muciniphila Ameliorates Dextran Sulfate Sodium-Induced Ulcerative Colitis in Mice. Front Microbiol. 2019;10:2259.

34. Sun L, Jia H, Li J, Yu M, Yang Y, Tian D, Zhang H, Zou Z. Cecal Gut Microbiota and Metabolites Might Contribute to the Severity of Acute Myocardial Ischemia by Impacting the Intestinal Permeability, Oxidative Stress, and Energy Metabolism. Front Microbiol. 2019;10:1745.

35. Gao X, Chang S, Liu S, Peng L, Xie J, Dong W, Tian Y, Sheng J. Correlations between alpha-Linolenic Acid-Improved Multitissue Homeostasis and Gut Microbiota in Mice Fed a High-Fat Diet. mSystems. 2020; 5(6).

36. Hedin CR, McCarthy NE, Louis P, Farquharson FM, McCartney S, Taylor K, Prescott NJ, Murrells T, Stagg AJ, Whelan K, et al. Altered intestinal microbiota and blood T cell phenotype are shared by patients with Crohn's disease and their unaffected siblings. Gut. 2014;63(10):1578–86.

37. Xie J, Liu Y, Chen B, Zhang G, Ou S, Luo J, Peng X. Ganoderma lucidum polysaccharide improves rat DSS-induced colitis by altering cecal microbiota and gene expression of colonic epithelial cells. Food Nutr Res. 2019; 63.

38. Robinson AM, Rahman AA, Miller S, Stavely R, Sakkal S, Nurgali K. The neuroprotective effects of human bone marrow mesenchymal stem cells are dose-dependent in TNBS colitis. Stem Cell Res Ther. 2017;8(1):87.

39. Bernstein CN. The Brain-Gut Axis and Stress in Inflammatory Bowel Disease. Gastroenterol Clin North Am. 2017;46(4):839–46.

40. Klinsoda J, Votterl J, Koger S, Metzler-Zebeli BU. Dietary Phytase- and Lactic Acid-Treated Cereals Caused Greater Taxonomic Adaptations than Functional Adaptations in the Cecal Metagenome of Growing Pigs. Appl Environ Microbiol. 2020; 87(1).
41. Kim J, Choi JH, Ko G, Jo H, Oh T, Ahn B, Unno T. Anti-Inflammatory Properties and Gut Microbiota Modulation of Porphyra tenera Extracts in Dextran Sodium Sulfate-Induced Colitis in Mice. *Antioxidants (Basel).* 2020; 9(10).

**Figures**

**Figure 2**

ADSCs and Rg1 treated alter expression of cytokines associated with inflammation in DSS-induced colitis mice. Serum cytokines were detected in each group. (a) IL-33 (pg/ml). (b) TNF-α (pg/ml). (c) IL-1β (pg/ml). (d) IL-6 (pg/ml). (e) IL-10 (pg/ml). (f) IL-17A (pg/ml) n= 8, **P < 0.01, ***P < 0.001.
Figure 3

The ADSC and Rg1 improves Treg/Th17 balance in the spleen in DSS-induced colitis mice. Flow cytometry analysis spleen cells. Cells were surface stained with anti-CD3, anti-CD4 and anti-CD25, and intracellularly stained with anti-IL-17A, anti-FoxP3. (a,b) Representative dot plots from spleen. (c,e) The percentage of Th17 cells CD3+CD4+ IL-17A+ and Treg (CD4+CD25+Foxp3+). (d,f) The MFI of Th17 cells CD3+CD4+ IL-17A+ and Treg (CD4+CD25+Foxp3+). * P < 0.05, **P < 0.01, ***P < 0.001.
Figure 4

ADSC and Rg1 changed the structure of gut microbiota in DSS-induced colitis mice. (a) Alpha diversity boxplot of the shannon index in each groups. (b) Phylogenetic distances between samples from five groups were figured out by Unweighted UniFrac PCoA (Principal co-ordinates analysis) of the overall gut microbiota. (c) Heatmap of normalized relative abundance levels of OTUs in each group. (d) The average
abundance of 15 KEGG modules differentially enriched in every groups. (e). Correlation analysis of these differential genera and cytokines between any two groups. n=8.

**Supplementary Files**

This is a list of supplementary files associated with this preprint. Click to download.

- figs1.pdf