Brief Report

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Involvement of adaptive immune responses in a model of subacute colitis induced with dextran sulfate sodium in C57BL/6 mice

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SUMMARY Inflammatory bowel disease (IBD) is a non-specific chronic intestinal inflammatory disorder. Pharmacotherapy serves as the main treatment strategy for IBD; however, the current medications have certain limitations, such as inefficacy and a tendency to induce tolerance, thereby requiring the development of innovative drugs to fulfill therapeutic requirements. A model of acute colitis induced with a solution of approx. 3% dextran sulfate sodium (DSS) has been widely used in preclinical drug development. Nevertheless, this model has some drawbacks, including rapid disease progression leading to mortality in some mice and disparities between the inflammatory characteristics of mice and the pathological features of human IBD. The current study found that mice freely consuming a lower concentration of a DSS solution (1-1.5%) for 10-15 days exhibited milder colitis symptoms. Continued consumption of the DSS solution for 15-20 days resulted in chronic inflammation in colon tissue, accompanied by a significant increase in the proportion of Th1 cells, indicating the involvement of adaptive immune responses. Subsequently, mice were treated with mesalazine or Centella triterpenes while concurrently consuming the DSS solution for 10 days. The treated mice had significant improvements in body weight and colon length compared to the control group. The advantages of this subacute model include minimal mortality among experimental mice and the fact that intestinal mucosal inflammation in mice resembles the pathological features of human IBD, enabling the assessment of drug efficacy against IBD.

Keywords inflammatory bowel disease (IBD), colitis, dextran sulfate sodium (DSS), Th1 cells, adaptive immune response

1. Introduction

Inflammatory bowel disease (IBD) is a non-specific chronic intestinal inflammatory disorder that mainly includes ulcerative colitis and Crohn's disease (*1*). The former mainly damages the colon and rectum, while the latter can damage any part of the gastrointestinal tract from the mouth to the anus, with the terminal ileum and colon often affected (*2,3*). Patients often present with symptoms such as abdominal pain, diarrhea, bloody stools, and weight loss (*2,3*). IBD is difficult to cure, prone to recur, and has a potential risk of cancer, seriously affecting the quality of life of patients (*4*). The etiology of IBD remains unclear, but it is associated with abnormal immune responses in the gastrointestinal tract (*5*). Drug therapy is the main treatment strategy for IBD, with commonly used drugs including mesalazine, corticosteroids, and biologics targeting inflammatory mediators (*6,7*). However, existing drugs have drawbacks such as inefficacy and the potential to induce tolerance, necessitating the development of new drugs to meet treatment needs (*8*).

A model of acute colitis induced with dextran sulfate sodium (DSS) is widely used in preclinical drug development (*9,10*). In this model, DSS is typically used at a concentration of approx. 3%, and mice exhibit obvious colitis symptoms such as diarrhea, rectal bleeding, and weight loss within 3-7 days (*11*). The advantages of this model lie in its convenience and rapidity, and it can respond to some commonly used anti-IBD drugs (*11*). However, this model also has some drawbacks, evident in the large individual differences among mice, with some showing mild or no obvious colitis symptoms while others exhibit severe symptoms such as significant rectal bleeding and weight loss. This can easily lead to death and result in missing data. Second, DSS-induced acute inflammation progresses rapidly, and it can heal on its own after DSS is discontinued. Therefore, preventive administration is generally used to evaluate the efficacy of compounds,

while it is seldom used to evaluate the efficacy of drugs in the disease model. Finally and most importantly, human IBD is a chronic inflammation that involves adaptive immune response that plays an important role in disease pathology (*5*). However, studies have shown that acute intestinal inflammation can still be induced in mice with defective adaptive immune responses, indicating that the pathological features of DSS-induced acute inflammation in mice differ from those of human IBD (*12*). Therefore, the mouse model of DSS-induced acute colitis needs to be improved to better evaluate the efficacy of compounds.

Reported here is a mouse model of subacute DSS-induced colitis in which mice was allowed free access to a low concentration of a DSS solution (1%-1.5%) for 40-45 days. This model is characterized by diarrhea in mice, adaptive immune response involvement, and generally no mortality during the experiment. It can be used to evaluate the therapeutic and/or preventive effects of drugs.

2. Materials and Methods

2.1. Agents and animals

DSS (M.W. 40,000) and mesalazine were purchased from Shanghai Aladdin Biochemical Technology Co., Ltd. (Shanghai, China). Centella triterpene tablets were manufactured by Shanghai Shyndec Pharmaceutical Co., Ltd. (Shanghai, China). Female C57BL/6 mice (8-10 weeks old, 18-22 g) were purchased from Beijing Vital River Laboratory Animal Technology Co., Ltd. (Beijing, China). The animals were housed under pathogen-free conditions. The research protocol was in accordance with the institutional guidelines of the Animal Care and Use Committee.

2.2. Histological processing and hematoxylin and eosin (H&E) staining

After mice were sacrificed, the entire colon and rectal segment were removed, rinsed thoroughly with pre-cooled normal saline, blot dried with filter paper, and visually inspected for macroscopic changes. The length of the colorectum was measured. One-cm sections of the colon were cut, fixed in 4% paraformaldehyde, and embedded in paraffin. Six-μm thick sections then prepared for H&E staining. Digital micrographs were taken with a Nikon N*i* microscope.

2.3. Extraction of mouse colorectal mucosal lymphocytes

After the mouse colon was washed with pre-cooled normal saline, the tissue was cut into approximately 0.5-1-cm fragments. An appropriate amount of separation solution was added (20 mL of a separation solution containing 18.52 mL of sterile calcium- and magnesium-free Hank's balanced salt solution, 1 mL of fetal calf serum, 200 μL of 100 mM dithiothreitol, 200 μL of 1 M HEPES, and 80 μL of 0.5 M EDTA). The tissue fragments were shaken (250 r/min) at 37°C for 15 min and then filtered through a 100-μm nylon mesh to obtain a filtrate containing intraepithelial lymphocytes and intestinal epithelial cells. The intestinal tissue fragments were then added to an appropriate amount of a digestion solution (10.5 mL of a digestion solution containing 500 μL of fetal calf serum, 15 mg of collagenase VIII, 10 mg of DNase I, and 10 mL of calcium- and magnesium-free PBS). The mixture was shaken (250 r/min) at 37°C for 45 min and then filtered through a 100-μm nylon mesh to obtain a filtrate containing lamina propria lymphocytes. The filtrate was centrifuged (860× *g*) at 4°C for 10 min, and the supernatant was discarded. The cell pellet was resuspended in 8 mL of 40% isotonic Percoll solution and slowly layered over 4 mL of 80% isotonic Percoll solution along the tube wall. After being left to stand for 10 min, the mixed liquid was subjected to density gradient centrifugation (1000× *g*) at 20°C for 20 min. The opaque cell layer between the two layers of the interface was aspirated and placed into a centrifuge tube, and PBS was added. After mixing, it was centrifuged (800× *g*) at 4°C for 8 min. The cell pellet was resuspended in 1 mL of RPMI 1640 medium containing 10% fetal calf serum, and then an intestinal mucosal lymphocyte suspension was obtained.

2.4. Determination of Th1/Th2/Th17 $CD4^+$ T cells

After resuspending the extracted intestinal mucosal lymphocytes in PBS, Fixable Viability Stain 780 (BD Biosciences, NJ, USA) was added to the suspension and incubated in the dark for 10-15 min. After the mixture was washed and centrifuged (350× *g*, 5 min), mouse BD Fc Block (BD Biosciences, NJ, USA) was added and incubated at 4°C for 5 min to block the Fc receptors of the cells. Then appropriate amounts of antibodies against CD3 (FITC anti-mouse CD3, BioLegend, CA, USA), CD4 (BB700 rat anti-mouse CD4, BioLegend, CA, USA), and CD25 (APC anti-mouse CD25, BioLegend, CA, USA) were added and incubated at 4°C in the dark for 30 min. After the mixture was washed and centrifuged (350× *g*, 5 min), 1 mL of 1× FIX/Perm was added to each tube, and the mixture was incubated at 4°C in the dark for 40 min. After the mixture was washed and centrifuged (450× *g*, 5 min), appropriate amounts of antibodies against IFN-γ (Brilliant Violet 650™ anti-mouse IFN-γ, BioLegend, CA, USA), IL-4 (PE/Cyanine7 anti-mouse IL-4, BioLegend, CA, USA), and IL-17A (Brilliant Violet 421™ anti-mouse IL-17A, BioLegend, CA, USA) were added to each tube. The mixture was incubated at 4°C in the dark for 45 min and then washed and centrifuged (450× *g*, 5 min). The cells were resuspended in 500 μL of staining buffer, filtered through a 300-mesh filter, and analyzed using flow

cytometry. Prior to flow cytometry, compensation beads (BD Biosciences, NJ, USA) were used to adjust the flow cytometer.

2.5. Statistical analysis

Data are expressed as the mean ± SEM and were analyzed using a one-way analysis of variance (ANOVA) followed by Dunnett's multiple range tests. The limit of statistical significance was $p < 0.05$. Statistical analysis was performed with the software SPSS/Win 16.0 (SPSS, Chicago, IL, USA).

3. Results and Discussion

3.1. Subacute colitis induced with DSS

Mice were allowed free access to a 1-1.5% DSS solution for about 40-45 days (Figure 1A). Their feces were examined daily for any changes and their weight was recorded every three days. On days 10-15, the mice exhibited diarrhea and were allowed to continue drinking the DSS solution until day 30. During day 15 to 30, the mice showed obvious symptoms of diarrhea, indicating the presence of colitis. During the first 30 days of the study, the mice maintained a relatively stable weight (Figure 1B). Starting on day 30, the mice were given therapeutic agents while still having access to the DSS solution for about 10-15 days.

3.2. Pathology of subacute colitis in C57BL/6 mice

Colon tissues of mice were collected at the beginning and on day 15, day 30, and day 40. After H&E staining, the pathological changes in the colon were examined (Figure 2). At the beginning, the colonic mucosa of mice had abundant and regularly arranged crypts (Figure 2A). Few white blood cells were observed in the mucosal layer. On day 15, mice developed diarrhea, and the quantity and quality of crypts decreased (Figure 2B). The number of goblet cells decreased, and the submucosa became thicker with leukocyte infiltration. On day 30, the number of crypts decreased further, and large amounts of leukocytes were observed in the mucosal and submucosal layers (Figure 2C). Isolated lymphoid follicles were also observed, indicating an adaptive immune response. On day 40, the number of crypts decreased significantly, and the mucosal and submucosal layers were infiltrated by a large number of plasma cells and lymphocytes (Figure 2D).

To further clarify the type of adaptive immune response in the colonic mucosa, on day 30 colonic mucosal lymphocytes were extracted from mice and analyzed for the Th subgroups of $CD4^+$ T cells using flow cytometry (Figure 3). The results indicated that the proportion of Th1 cells in the colonic mucosa of

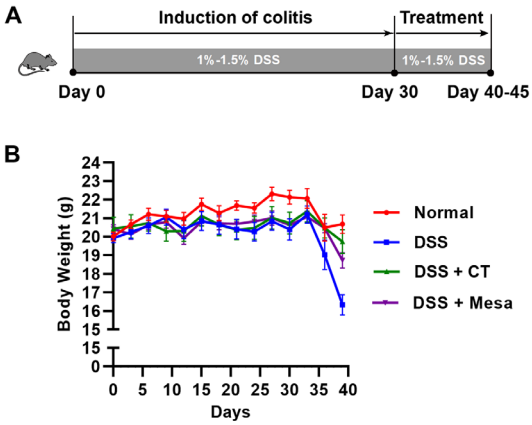

Figure 1. DSS (1%-1.5%)-induced subacute colitis in C57BL/6 mice. (**A**) The whole experiment could be divided into two phases. In phase 1, colitis was induced and maintained until day 30, when the colon mucosa exhibited chronic inflammatory characteristics. In phase 2, mice were treated with agents that have potential anti-colitis action. (**B**) The body weight of mice was monitored throughout the whole study. Body weight was stable during the first phase but tended to decrease during the second phase. CT, Centella triterpenes; Mesa, mesalazine.

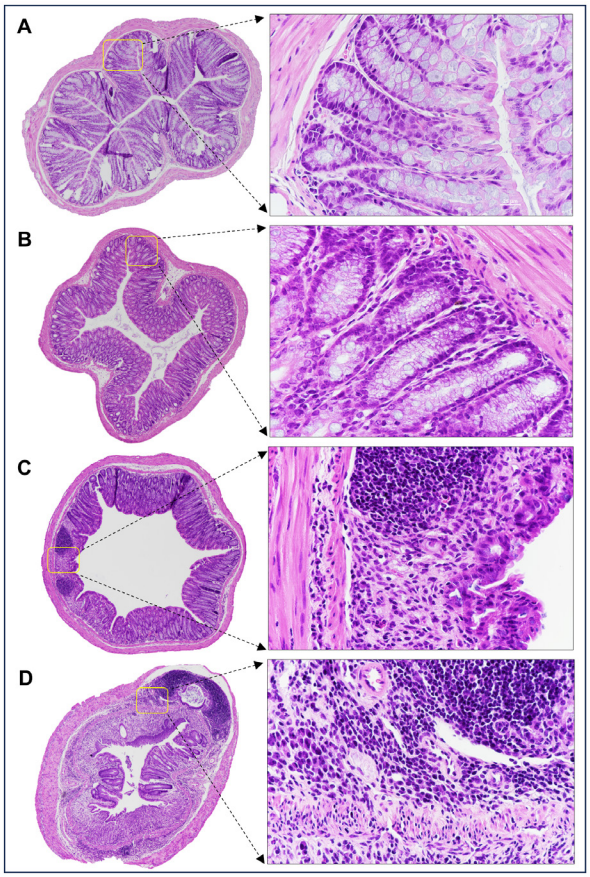

Figure 2. H&E staining of colon tissues removed on day 0, 15, 30, and 40 of the experiment. (**A**) Normal colon tissue on day 0. (**B**) Mild colitis on day 15, when the mice developed diarrhea. (**C**) The colon mucosa exhibited chronic inflammatory characteristics on day 30. (**D**) Colitis worsened on day 40, when a large number of leucocytes such as plasma cells and lymphocytes were observed in the mucosa and submucosa of colon tissue. Original magnification: 40× (left), 400× (right).

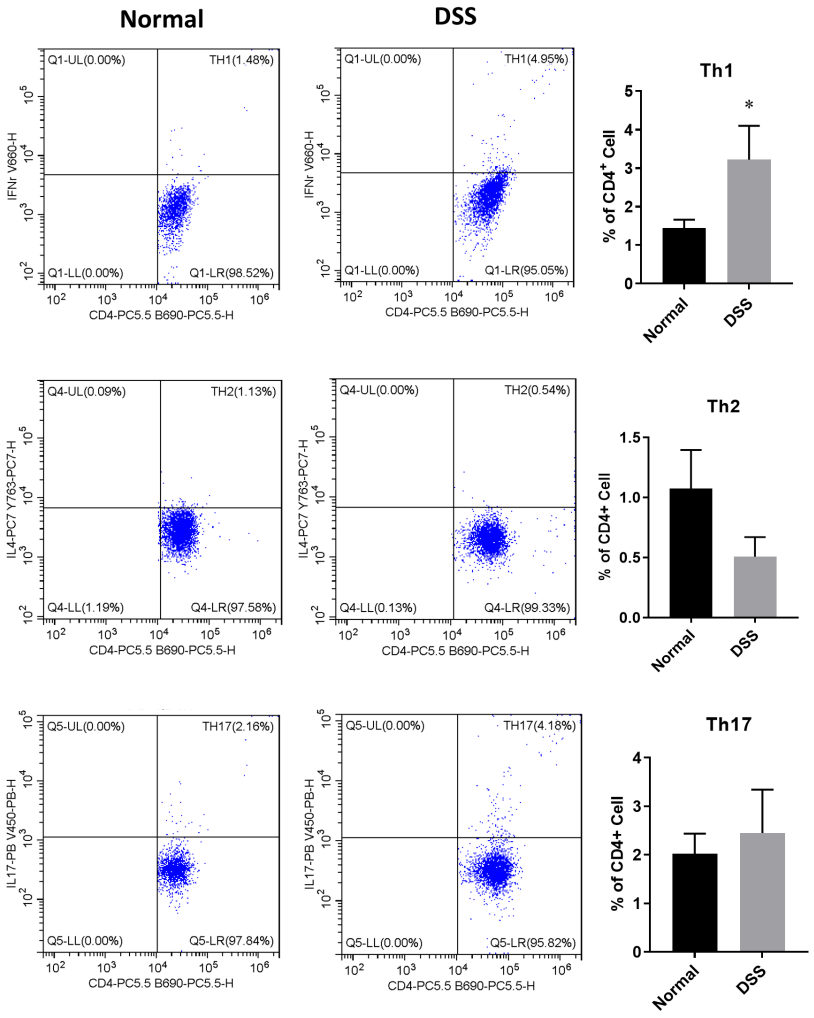

Figure 3. Alteration of Th cells in the mucosa of inflamed colon tissue. The mice were sacrificed on day 30 and the colon tissues were removed. Lymphocytes were extracted and subjected to flow cytometry. Cells that express both CD4 and IFN-γ were designated as Th1 cells, those that express both CD4 and IL-4 were designated as Th2 cells, and those that express both CD4 and IL-17A were designated as Th17 cells. The proportion of Th1 cells increased significantly in mice with DSS-induced colitis compared to that in normal mice. * $p < 0.05$.

mice with colitis increased significantly compared to that in normal mice, and the proportion of Th17 cells also tended to increase. Th1 and Th2 cells are generally believed to mediate the immune response in Crohn's disease and ulcerative colitis, respectively, while Th17 cells play an important role in both diseases (*13*). Therefore, this model of subacute colitis has certain prospects for use in the study of IBD.

3.3. Drug response in the model of subacute colitis

Mesalazine is an effective drug for treating IBD. The efficacy of mesalazine was noted in this model of subacute colitis. Starting on day 30, mice were orally administered mesalazine at a dose of 200 mg/kg twice daily for 10 days. At the end of the experiment, mice in the mesalazine treatment group had a significantly greater body weight and significantly longer colon length than those in the model group (Figure 4), indicating that mesalazine is effective at alleviating colitis in this model.

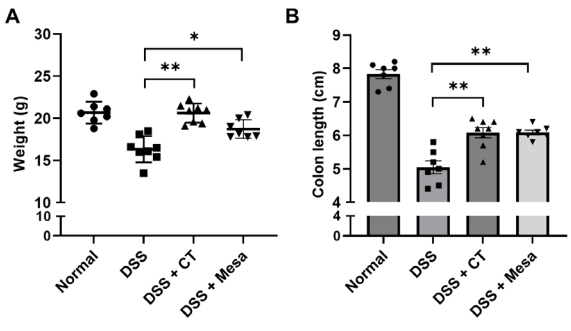

Figure 4. The body weight (A) and colon length (B) of mice at the end of the experiment. Starting on day 30, the mice were treated with Centella triterpenes or mesalazine for 10 days. Centella triterpenes and mesalazine markedly increased the body weight and colon length of mice compared to the vehicle. CT, Centella triterpenes; Mesa, mesalazine. * $p < 0.05$; ** $p < 0.01$.

Centella triterpenes mainly contain asiaticoside and madecassoside, which have anti-inflammatory

and wound healing action (*14*). Clinically, they are used to treat trauma, surgical wounds, burns, scars, and scleroderma in China. Studies have found that asiaticoside and madecassoside can alleviate the symptoms of acute colitis in a model at a dose of 50 mg/kg (*15*). In the current model, Centella triterpenes were orally administered at a dose of 1 mg/kg twice daily, significantly increasing the body weight and colon length of mice with colitis (Figure 4). Considering the conversion factor between mice and humans, this dose is close to the clinical dose of the drug, indicating that the mouse model of subacute colitis has advantages in evaluating the efficacy of drugs.

4. Conclusion

The use of a high-concentration DSS solution (approx. 3%) to induce acute colitis in mice in order to evaluate the efficacy of drugs has certain limitations. The current study found that mice, when freely consuming a lower concentration of a DSS solution (1-1.5%) for a duration of 10-15 days, exhibited milder symptoms of colitis. When intake of the DSS solution was continued for another 15-20 days, the mucosa of colon tissues exhibited characteristics of chronic inflammation, accompanied by a significant increase in the proportion of Th1 cells, indicating the involvement of an adaptive immune response. Subsequently, the mice were administered drug treatment for 10-15 days while consuming the DSS solution, allowing for the evaluation of the drug's efficacy against colitis. The advantages of this subacute model include minimal mortality of mice during the experimental process, the fact that the characteristics of murine intestinal mucosal inflammation resemble the pathological features of IBD in humans, and evaluating the efficacy of drugs to treat colitis in mice.

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Conflict of Interest: The authors have no conflicts of interest to disclose.

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