Sishen Pill Regulates Memory T Cells in Mice with Colitis via JAK/STAT 5 Signaling Pathway

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Research

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Abstract

Background: Memory T cell (T_m) has a pivotal role as host protection in autoimmune inflammatory diseases via a JAK/STAT signaling pathway. Sishen Pill (SSP) is a classic prescription used to treat chronic ulcerative colitis (UC). However, it is still unclear whether SSP can regulate immune memory function. In this study, we examined the effect of SSP and whether it can regulate immune memory function through a JAK/STAT5 signaling pathway.

Methods: Mice were randomly divided into four groups (10 mice per group): Normal group (Normal), health mice without DSS administration; DSS group (DSS), mice with DSS administration; Sishen pill treated (DSS+SSP) group, colitis mice treated with SSP for 7 days; Mesalazine controlled (DSS+5-ASA) group, colitis mice treated with mesalazine for 7 days. The therapeutic effect of SSP was evaluated by macroscopic and microscopic observation; T_m and their subsets were analyzed by flow cytometry; Activation of the JAK/STAT signaling pathway was analyzed using a Western blot.

Results: SSP significantly reversed weight loss and colonic injury (colon weight increase and colonic length shortening) caused by 3% dextran sodium sulfate (DSS). Flow cytometry showed that the percentages of CD4⁺ and CD8⁺ expressions on central memory T cells were enhanced after SSP treatment, while the CD4⁺ T_{cm}, CD4⁺ mT_{fh} (memory T follicular helper) cells and their subpopulations were also significantly increased. Moreover, SSP inhibited the expression of STAT5 signaling pathway proteins JAK1, PIAS3, STAT5, p-STAT5, BIM, BAX, Caspase-3, and β -casein and promoted the expression of JAK3, PISA1, Bcl-2, and Caveolin-1.

Conclusions: SSP can be used to effectively treat DSS-induced colitis by improving the status of immune memory via the JAK/STAT signaling pathway.

Background

Inflammatory bowel diseases (IBD) are a group of chronic recurrent intestinal diseases involving the ileum, colon, and rectum, including ulcerative colitis (UC) and Crohn's disease (CD). The clinical symptoms include loss of body weight, abdominal pain, diarrhea, and bloody stools. The etiology of IBD is complex and involves the co-regulation of factors such as immunity, infection, genetics, and environment, among which immune dysfunction is the main factor. In the initial stage, T cells are activated and differentiated into effector T cells. After binding with antigens and removing them, most of the effector T cells undergo apoptosis, while some become memory T cells. Memory T cells can quickly activate and defend against secondary antigen invasion. Once memory T cells function is imbalanced, secondary antigen stimulation may promote the differentiation of the memory T cells into the abnormal effective T cell and further induce autoimmune diseases, such as UC and CD. It is believed that abnormal or dysfunctional memory CD4⁺T cell can lead to a variety of chronic or recurrent inflammatory and immune-mediated diseases as well as the progression of tumor diseases [1; 20].

UC is a complex non-specific intestinal inflammatory disease characterized by ulcers. At present, no cure for UC has been developed. Compared to western medicine, which has a higher cost and is associated with side effects and poor patient tolerance, Chinese medicine is widely accepted as an alternative treatment.

As a classic prescription of traditional Chinese medicine-treated UC, Sishen Pill (SSP) has anti-inflammatory and immunomodulatory effects, and has been treating chronic enteritis in China for thousands of years. For example, a clinical study of 204 patients with IBD found that the total effective rate of SSP was 75.98%, and the recurrence rate within 6 months was only 8.1%, while the recurrence rate with sulfasalazine was 23.3% [02]. In the previous study, we had performed the pharmacodynamic evaluation of the different doses (5, 2.5 and 1.25 g·kg⁻¹) of SSP treated colitis induced by TNBS in rats. The above results were reported in the J Ethnopharmacol that the 2.5 g·kg⁻¹ SSP effectively treated TNBS-induced colitis, which was the best dose to regulate the expression of IL-10 and IL-4 mRNA [2]. And these results were repeatedly proved in our previous other studies. So, in the present study, we selected the 2.5 g·kg⁻¹ SSP to explore the mechanism of SSP treated colitis. While our research in 2020 has shown that SSP regulates the interaction between inflammatory DCs and gut microbiota and the differentiation and function of follicular T helper cell (T_{fh}) cells to treat IBD. However, it is still unclear whether SSP can regulate immune memory function. In this study, we examined the effect of SSP, and whether it can regulate immune memory function through a JAK/STAT5 signaling pathway.

Materials And Methods

Animals

Forty Male BALB/c mice, weighing 22 ± 2 g, were purchased from Hunan Shrek Jingda Experimental Animal Co., Ltd (Changsha, China) (Permission No: SCXK (Xiang) 2016-0002). All the animals were housed in an environment with a temperature of 22 ± 1 °C, relative humidity of $50 \pm 1\%$, and a light/dark cycle of 12/12 hr and were given food and water *ad libitum*. All animal studies (including the mice euthanasia procedure) were done in compliance with the regulations and guidelines of Jiangxi University of Chinese Medicine (JXUCM) institutional animal care and conducted according to the AAALAC and the IACUC guidelines (No: JZ2018-139).

Drugs

DSS (No. 160110, molecular weight 36,000–50,000KDa) was obtained from MP Biomedicals (California CA, USA). SSP (batch number 17080051, Tongrentang Natural Medicine Co. Ltd. (Beijing, China), was composed of *Euodia rutaecarpa* (Juss.) Benth., *Schisandra chinensis* (Turcz.) Baill, *Psoralea corylifolia* L., *Myristica fragrans* Houtt., *Ziziphus jujuba* Mill. and *Zingiber officinale* Rosc. (Pharmacopoeia of China (2015)), which were prepared into pills according to the dose ratio (100g, 200g, 400g, 200g, 200g, and 200g, ratio: 1:2:4:2:2:2, respectively). The main active ingredients were measured by High-Performance Liquid Chromatography (HPLC) according to a previously described approach [9]. ASA (Mesalazine

enteric-coated tablets) (batch number 181003) was obtained from Sunflower Pharmaceutical Group Jiamusi Luling Pharmaceutical Co., Ltd. (Heilongjiang, China).

Experimental design and Therapeutic protocols

Mice were randomly divided into four groups (10 mice per group): Normal group (Normal), health mice without DSS administration; DSS group (DSS), mice with DSS administration; Sishen pill treated (DSS + SSP) group, colitis mice treated with SSP; Mesalazine controlled (DSS + 5-ASA) group, colitis mice treated with mesalazine. According to the previous literature [6], the mice in the Normal group had free access to drinking water, while other mice were treated with 3% (w/v) DSS solution prepared with the drinking water for 7 days to induce in ulcerative colitis. On the 8th day, the mice in the DSS + SSP group were treated with 2.5 g/kg Sishen pill suspension, while the mice in the DSS + 5-ASA group were treated with 300 mg/kg mesalazine suspension. The mice in the Normal group and DSS group were fed with an equivalent volume of physiological saline solution for 7 days. Body hair, drinking water and food intake, fecal traits, hematochezia, and body weight were monitored and analyzed once per day.

Macroscopical observation

On day 15, all animals were sacrificed under sodium pentobarbital (50 mg/kg i.p.) anesthesia. After measuring the body weight, peripheral blood was collected with disposable vacuum blood collection (including heparin anticoagulant) and stored at 4°C. The colon tissue was then quickly isolated, placed on ice cake; the colonic length and weight (n = 10) were measured, and the colonic weight index (n = 10) was calculated as follows: $colonic\ weight\ index = colonic\ weight/body\ weight \cdot 100\%$. The separated colon was longitudinally opened and rinsed with cold phosphate-buffered saline (PBS).

Evaluation of colitis

The distal colon of each mouse was fixed in 4% formaldehyde solution for one week, and the colon tissue was dehydrated, embedded in paraffin, sectioned at 4 μm, and then stained with hematoxylin and eosin. Next, a histological score of the colon was evaluated as double-blind analysis by two pathologists, and the degree of inflammatory infiltration and injury of the colon were evaluated according to the criteria described by Dieleman *et al.* The histological grading of colitis was performed as follows [3]: inflammation, 0: none; 1: slight; 2: moderate; 3: severe; extent, 0: none; 1: mucosa; 2: mucosa and submucosa; 3: transmural; regeneration, 4: no tissue repair; 3: surface epithelium not intact; 2: regeneration with crypt depletion; 1: almost complete regeneration; 0: complete regeneration or normal tissue; crypt damage, 0: none; 1: 1/3 damaged basal ganglia; 2: 2/3 damaged basal ganglia; 3: only surface epithelium would be intact; 4: entire crypt and epithelium lost; percentage of involvement, 1: 1–25%; 2: 26–50%; 3: 51–75%; 4: 76–100%.

Flow cytometry analysis

The obtained lymphocytes (n = 10) were incubated with fluorescein isothiocyanate (FITC)-conjugated monoclonal antibodies. Flow cytometry analysis was performed on a FACSCalibur system (BD Biosciences, Franklin Lakes, NJ, USA).

A lymphocyte suspension was prepared as follows: 100 μ L of anticoagulant, 100 μ L of RPMI 1640 medium, and 1 mL of hemolysin were added to fresh blood and incubated for 15 min. The mixture was centrifuged at 300 g for 5 min, and the supernatant was removed. Then, 1 mL of stain buffer was added, and the cells were rinsed and centrifuged at 300g for 5 min, and re-suspended with 100 μ L staining buffer and 1 μ g FC blocking sealant at 4 °C for 8 min. Samples were then incubated with primary antibodies for 15 min at room temperature, washed with 1 mL staining buffer, and mixed with 100 μ L staining buffer. The following monoclonal antibodies (mAbs) were used: PE anti-mouse CD45RA, PE-CY7 anti-mouse CD62L, APC-H7 anti-mouse CD4, FITC anti-mouse CD8, Alexa Fluor 647 anti-mouse CCR7; second group: PE anti-mouse GL7, PE-CY7 anti-mouse CD62L, Alexa Fluor 647 anti-mouse CCR7, FITC anti-mouse CXCR5, APC-H7 anti-mouse CD4) (BD Biosciences, San Jose, CA) were added, incubated for 30 mins in the dark. and then it was added 1ml stain buffer (BD Biosciences, 554656), centrifuged to the supernatant (500 $\times$ g, 5 mins, 4°C), and discarded the supernatant. The 500 ul stain buffer was added into the stained cells which were detected using FACS Calibur (BD Biosciences), and the detection results were analyzed by FlowJo VX10 (Treestar, Ashland, OR).

Western blot

Colon tissues (n = 6) were quickly shredded and mixed with protease inhibitor cocktails. The mixture was homogenized by ultrasound on crushed ice and centrifuged at 13000 g at 4°C for 10 min, the supernatant was extracted, and the protein concentration was determined by BCA protein assay. Equal amounts of total protein (60 μ g) were separated by SDS-PAGE and transferred to polyvinylidene fluoride (PVDF) membrane using a semi-dry transfer slot (BIO-RAD, USA). After blocking with 5% nonfat dried milk for 1 h, the membrane was incubated with proportionally diluted antibodies at 4°C overnight. The antibodies included rabbit anti-GAPDH (1:2000), anti-BCL-2 (1:2000), anti-BIM-1 (1:1000), anti-Caspase-3 (1:500), anti-BAX (1:1000), anti-PP2A (1:1000), anti- β -casein (1:2500), anti-Caveolin-1 (1:1000), anti-Pim-1 (1:1000), anti-JAK1 (1:1000), anti-JAK3 (1:1000), anti-PIAS1 (1:2000), anti-PIAS3 (1:2000), anti-Socs-1 (1:1000), anti-P-STAT5 (1:1000), and anti-STAT5 (1:1000)(Abcam). Then, samples were incubated with a corresponding secondary antibody (Abcam) for 1 h. After washing three times with TBST, the blots were visualized by the Proteogel imaging system (FluorChem M, ProteinSimple, USA) and quantified with the Quantity One System (Bio-Rad).

Statistical analysis

Statistical analysis was performed using GraphPad Prism 8.0 (La Jolla, CA, USA), results are presented as mean $\pm$ average standard error (SEM). Differences between groups were examined using unpaired Student's t-tests. P values < 0.05 were considered to be statistically significant.

Results

SSP ameliorated DSS-induced colitis in mice

DSS-induced mice colitis is an alternative and classic model widely applied to study IBD treatment. In this study, mice were treated with 3% (w/v) DSS solution for 7 days to induce ulcerative colitis. As expected, two days after administration of 3% DSS, the weight of mice in the Model group began to gradually decrease and mice began showing poor spirits, hunched posture, reduced mobility, and reduced diet. From the 5th to 7th day, the above reported signs became more obvious and loose and bloody stools were observed. On the 8th day, mice were treated with SSP and 5-ASA for 7 consecutive days. After treatment, shortened colon length was rapidly restored in DSS + SSP and DSS + 5-ASA groups compared with the DSS group (Fig. 1A and B). At the same time, the colon weight (Fig. 1E) and colon weight index (Fig. 1G) were significantly reduced. The body weight of mice also showed a recovery trend in the DSS + SSP group; it was higher in the DSS + 5-ASA group compared to the DSS group (Fig. 1F).

Microscopic evaluation showed that DSS-induced colitis was characterized by decreased glands, loss of mucus layer, extensive inflammatory cell infiltration into the lamina propria, especially neutrophils, intestinal epithelial erosion, and even ulceration. In contrast, SSP treatment obviously ameliorated the pathological symptoms (Fig. 1C). Moreover, the histological scores in the colon of mice from the DSS group were significantly higher than that in the normal group, and the histological scores in DSS + SSP and DSS + 5-ASA groups were significantly lower than that in the DSS group (Fig. 1D). These results demonstrated that SSP has a therapeutic role for DSS-induced colitis in mice.

SSP promotes CD4⁺ and CD8⁺ expressions on Tcm cells in colitis mice

Memory T cells have considerable functional and phenotypic heterogeneity. The expression of chemokine CCR7 and homing receptor CD62L is the most commonly used surface marker to distinguish memory T cells and their subsets. In this study, we used CD62L and CCR7 to characterize the activation states of the cells and reflect the level of Tcm. The percentage of CD45RA⁺CD62L⁺CCR7⁺ Tcm cells (Fig. 2A, B and C) was significantly reduced in the DSS group compared to the Normal group and SSP and/or 5-ASA group. Furthermore, when stimulated by antigen, CD4⁺ (Fig. 2A, B and D) and CD8⁺ (Fig. 2A, B and E) expression on Tcm cells were decreased in the DSS group compared with the these in the Normal group, DSS + SSP group, and DSS + 5-ASA group. These results indicate that SSP promotes the expressions of CD4⁺ and CD8⁺ on the surface of CD45RA⁺CD62L⁺CCR7⁺ Tcm cells in colitis mice.

SSP regulates CD4⁺ Tcm cells and their subpopulations in colitis mice

Next, we analyzed the numbers of different subpopulations of CD4⁺Tcm cells in peripheral blood of mice by flow cytometry. In the present study, the results showed that the proportions of CD4⁺CCR7⁺GL7⁺T (Fig. 3A, B, and C) and CD4⁺CCR7⁺CD62⁺ T (Fig. 3A, B and D) cells in the DSS group were significantly down-regulated when they were compared with the Normal group, DSS + SSP group, and DSS + 5-ASA group. In addition, the proportion of CD4⁺CCR7⁺CD62⁺GL7⁺T cells (Fig. 3A, B and E) in colitis mice after

SSP and 5-ASA treatment was significantly higher than that in those colitis mice before treatment. The results suggested that SSP increased the number of CD4⁺Tcm cells and their subsets in DSS-induced colitis.

SSP regulates CD4⁺ mTfh cells and their subpopulations in colitis mice

Tfh cells exist in lymphoid organs, such as germinal centers, and memory Tfh cells (mTfh) can be found in peripheral blood or local tissues. mTfh cells are also an important part of T cell memory in both mice and humans [4]. In this study, we evaluated whether cell percentages of CD4⁺CCR7⁺CXCR5⁺ (CD4⁺mTfh) cells changed in experimental colitis. As shown in Fig. 4, the percentages of CD4⁺mTfh cells in untreated mice with colitis were reduced when they were compared to those in the Normal group, DSS + SSP group, and DSS + 5-ASA group (Fig. 4A, B and E). Furthermore, the change in the numbers of different subpopulations of CD4⁺mTfh cells in colitis mice is associated with SSP treatment. As shown in Fig. 4, the levels of CD4⁺CCR7⁺CXCR5⁺GL7⁺ T cells (Fig. 4A, B and C) and CD4⁺CCR7⁺CXCR5⁺ CD62L⁺ T cells (Fig. 4A, B and D) from the DSS group were significantly lower than those in the Normal group, but they were significantly higher than the DSS + SSP and DSS + 5-ASA groups. These data indicated that SSP administration regulates the number of CD4⁺mTfh cells and their subpopulations in DSS-induced colitis.

SSP modulates protein levels of JAK/STAT5 signal molecule in colonic tissues

The activation of members of the JAK/STAT5 signal transduction pathway is considered to be the necessary pathway for intestinal inflammation. To further explore molecular mechanisms, the related protein expression levels of the JAK/STAT5 signal were determined using Western blot. The present results showed that JAK1 (Fig. 5A1 and A2), p-STAT5 (Fig. 5B1 and B5), and STAT5 (Fig. 5B1 and B6) were up-regulated, and JAK3 (Fig. 5A1 and A3) was down-regulated in the DSS group when compared with them in the Normal group, SS + SSP, and DSS + 5-ASA group, thus suggesting that the JAK/STAT5 signaling pathway was activated, and SSP deactivated JAK/STAT5 signal. In addition, the ratio of p-STAT5/STAT5 (Fig. 5B7) was increased after mice with experimental colitis were treated after SSP. The results indicated that SSP could restrain the activation of the JAK/STAT5 pathway in experimental colitis induced by DSS.

Furthermore, we found that regulators of the JAK/STAT5 pathway, including PIAS1 (Fig. 5B1 and B2) and Socs-1 (Fig. 5B1 and B4) were decreased, and the expression of PIAS3 (Fig. 5B1 and B3) was increased in the DSS group when they were compared with these in the Normal group, DSS + SSP and DSS + 5-ASA group.

SSP regulates the expression of apoptotic genes in the mitochondrial pathway of colonic tissues

JAK/STAT5 signaling pathway has a significant role in the process of the differentiation and activation of memory T cells, which included its downstream proteins as BIM-1, BCL-2, Caspase-3, BAX, PP2A and so on. As shown in Fig. 6, the expression of BCL-2 (Fig. 6A and B) and Caveolin-1 (Fig. 6A and H) were lower, while the expression of BIM-1 (Fig. 6A and C), Caspase-3 (Fig. 6A and D), BAX (Fig. 6A and E), and β -casein (Fig. 6A and G) were higher in the DSS group than in the Normal group, DSS + SSP group, and DSS + 5-ASA group. Although it is not discrepant when the PP2A (Fig. 6A and F) and Pim-1 (Fig. 6A and I) expressions in the DSS group were contrast to them in the Normal group, their expressions were dramatically decreased in the DSS + SSP and DSS + 5-ASA groups when they were compared with them in the DSS group. These data suggest that SSP effectively regulates the activation of the downstream proteins of the JAK/STAT5 pathway.

Discussion

The exact etiology and pathogenesis of UC are still unclear. It is believed that the abnormal immunological status induced by immunological memory disorder or unbalance of memory immune cells subsets is one of the main pathogenic factors leading to UC. Previous studies have indicated that abnormal immune memory is an important characteristic of inflammatory bowel disease, including UC [1; 20]. In the present study, mice colitis was successfully induced by DSS administration. The mice developed diarrhea, bloody stool, ulcer formation, crypt disappear and abundant inflammatory cells infiltration, and so on, which is consistent with the animal model of ulcerative colitis previously reported [4]. DSS mice developed significant changes in memory T cells compared to normal control mice. The total of memory T cells (as $CD45RA^+CD62L^+CCR7^+$ and $CD4^+CCR7^+CD62L^+$ T cells) and memory T follicular helper cell (mTfh) (as $CD4^+CCR7^+CXCR5^+$ T cells) were markedly decreased, while the expression of $CD4^+$, $CD8^+$, $GL7^+$, $CD62L^+$ molecules on the surface of memory T cells and mTfh cells were distinctly reduced in the DSS mice, which suggested that low-level immune memory has an important role in DSS-induced colitis.

SSP is a classic and frequently-used traditional Chinese medicine used to treat chronic ulcerative colitis (UC). In this study, we examined the effect of SSP on immune memory function in DSS mice. SSP effectively alleviated the pathological colonic mucosa damage induced by DSS and improved the total of the memory T cells (as $CD45RA^+CD62L^+CCR7^+$ and $CD4^+CCR7^+CD62L^+$ T cells) and memory T follicular helper cell (mTfh) (as $CD4^+CCR7^+CXCR5^+$ T cells). In addition, SSP increased the expression of $CD4^+$, $CD8^+$, $GL7^+$, $CD62L^+$ molecules on their surface. These results revealed that SSP-treated UC is closely related to the restored memory T cells differentiation and function.

In the early stage, T cells are activated and differentiated into effector T cells. After binding with antigens, most of the effector T cells undergo apoptosis, while some became memory T cells. In the present study, as a special type of memory T cells, $CD45RA^+CCR7^+CD62L^+$ T cell can be divided into $CD4^+$ and $CD8^+$ two subtypes [6]. Many studies have shown that the immune memory dysfunction represented by memory T cells is involved in the onset of UC and has become one of the main reasons for the prolonged and

recurrent onset of UC [2]. As shown in Fig. 2–4, the total of CD45RA⁺CCR7⁺CD62L⁺T cell, CD4⁺CD45RA⁺CCR7⁺CD62L⁺T cell, and CD8⁺CD45RA⁺CCR7⁺CD62L⁺T cell was decreased in DSS mice, while memory Tfh cells showed similar change, which suggests that the low-level immune memory function is one of the main pathogenetic characters of UC.

After acute infection or antigen clearance, the differentiation of memory T cells no longer relies on the classical antigen-recognition MHC- α pathway but mainly on intracellular signals (such as Jak/STAT and PI3K/Akt signaling pathways) and inflammatory mediators. Previous studies have shown that the overactivation of the JAK/STAT signaling pathway is closely related to the pathogenesis of colitis, which seems to be involved in the regulation of physiological processes such as proliferation, apoptosis, and differentiation of various immune cells. The JAK/STAT signaling pathway has an important role in the cellular immune response. When the receptor on the surface of T lymphocytes receives the stimulus signal, the receptor configuration changes, and this change will cause the activation of intracellular protein tyrosine kinase. This activation signal is then transmitted to the nucleus through STAT, causing the occurrence of the immune response. STAT5 activation occurs primarily through the activation of JAK1 and JAK3. Yet, the SOCS, PTPs, and PIAS are the main negative regulators of JAK-STAT signaling. Overexpression of SOCS1 leads to a decrease in p-JAKs and p-STATs; PIAS1 and PIAS3 can inhibit the binding of STATS to target gene promoters by coupling with STATS molecules and inhibit the transcriptional activity of STATS by modifying the phosphorylation sites of STAT. In this study, the expressions of SOCS-1, PIAS3, and JAK3 in the colon tissues were significantly decreased in mice with colitis, while the expressions of PIAS1 and JAK1 were significantly increased, as well as the activities of p-STAT5 and STAT5 were also significantly increased compared with the normal group. This indicates that the JAK/STAT5 signaling pathway is activated during UC.

Activation of the JAK/STAT5 signaling pathway led to low expression of the downstream target gene Bcl-2, which inhibited the anti-apoptotic effect, thereby promoting the apoptosis of memory T cells and aggravating intestinal inflammation. Interestingly, we found that SSP treatment inhibited the JAK/STAT5 signaling pathway, which is consistent with the levels of T cells that regulate immune memory.

Survival and maintenance of memory T cells depend on the expression of Bcl-2 and Bim-1. Bcl-2 promotes the survival of T cells, and Bim-1 drives the death of activated T cells [6; 20]. Bim-1, which is not bound to Bcl-2, can promote apoptosis by activating Bax and/or Bak. The balance between Bcl-2 and Bim-1 has a key role in maintaining the survival and homeostasis of memory T cells [7]. Bcl-2 and Bax can jointly activate pro-apoptotic factor Caspase-3. Compared with the normal group, the expression of Bcl-2 was decreased, while the expression of Bim-1, Bax, and Caspase-3 were significantly increased in the DSS group, appeared and accompanied with memory T cells dysfunction. However, after experimental colitis was treated with SSP for 7 days, the protein expression of Bax and Caspase-3 in colon tissue of experimental colitis mice was significantly down-regulated, while the protein expression of Bcl-2 was up-regulated. The SSP stimulated and restored the balance of memory T cells.

The expression of Pim-1 and PP2A decreased after SSP treatment. Pim-1 and PP2A are also closely related to cell differentiation. Pim-1 is a target gene of the STAT5 signaling pathway and a key kinase that regulates cell differentiation [3]. PP2A is involved in a variety of life activities such as cell cycle, metabolism, and migration. Recent studies have found that PP2A is widely involved in immune dysregulation diseases and is closely related to T cells and B cells.

We found that SSP treatment significantly reduced the expression of β -Casein and increased the expression of Caveolin-1. β -Casein is a pro-inflammatory cytokine regulated by STAT5 that induces a T-cell-mediated inflammatory response [11]. Caveolin-1 is a multifunctional scaffold protein that acts as a platform for cell signal transduction and has an important role in the inflammatory response. Caveolin-1 inhibits cytokine signal transduction by inhibiting the kinase activity of JAK family members [4], induces T cell proliferation and secretion of cytokines [13], and has a protective role in ulcerative colitis [2].

Previous studies have reported that SSP and its effective components can inactivate the JAK/STAT signaling pathway in other diseases. SSP inhibits the activation of p-STAT5, increases the expression of STAT5, and regulates the differentiation and function of Tfh cells in treatment of IBD. It has also been reported that the main effective components of SSP include evodiamine, rutaecarpine, schisandrin B, and psoralen. Evodiamine inhibits the migration and invasion of colorectal cancer by down-regulating the JAK/STAT signaling pathway [11]. As shown in the above analysis, SSP can be used to treat experimental colitis by JAK/STAT signaling pathway.

Conclusion

SSP can effectively treat colitis induced by DSS, thus potentially improving the status of immune memory via the JAK/STAT signaling pathway. However, this study has a few limitations. A direct correlation between the JAK/STAT5 signaling pathway and memory T cells has not been confirmed. In our next study, we plan to use JAK^{-/-} or STAT5^{-/-} mice and effective components of SSP and JAK inhibitor to confirm the close relationship between memory T cells and UC. We also plan to use genomics, bioinformatics, and other technologies to define the mechanism SSP regulated memory T cell to treat UC.

Abbreviations

UC, Ulcerative colitis; Tm, Memory T cell; SSP, Sishen Pill; JAK, Janus kinase; SAT, Signal transducer and activator of transcription; DSS, Dextran sulfate sodium; mTfh, memory T follicular helper; PIAS, Protein inhibitors of activated STATs; BIM, Bcl-2-like protein 11; BAX, BCL-2 associated X; Caspase, Cysteine Aspartyl Proteases; IBD, Inflammatory bowel disease; CD, Crohn's disease; DC, Dendritic cell; HPLC, High Performance Liquid Chromatography; Tcm, Central Memory T cell; SOCS, Suppressors of cytokine signaling; BCL, B-cell lymphoma; PTPs, Protein Tyrosine Phosphatases; PP2A, Protein phosphatase 2A.

Declarations

Acknowledgements

Not applicable.

Authors' Contributions

Mengxue Wand and Youbao Zhong contributed equally to this work as co-first authors. Conceived and designed the experiments: D-Y LIU and X-Y HUANG. Performed the experiments: M-X WANG, Z-P, KANG, Y-B, ZHONG, S-Y WEI and J-Q HUAGN, Contributed reagents/ materials/ analytical tools: D-Y LIU and H-M ZHAO. Analyzed the data: Y-B, ZHONG, M-X WANG and D-Y LIU. Wrote the paper: M-X WANG and D-Y LIU.

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Availability of data and materials

The data used to support the findings of this study are available from the [cor\[1\]](#) responding author upon request.

Declarations

Ethics approval and consent to participate

All procedures were performed in accordance with the guidelines of the Jiangxi University of Chinese Medicine ethics committee.

Consent for publication

Not applicable.

Competing Interests

All authors declare that they have no competing interests.

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Figures

Figure 1

SSP ameliorates DSS-induced colitis in mice. (A) Changes in colon length observed by the naked eye. (B) Colon length. (C) Representational tissues were stained with hematoxylin and eosin (H&E), (scale bar: 40um and 100um). (D) Histological scores. (E) Colon weight. (F) Body weight of mice. (G) Colon weight index. Data are presented as mean $\pm$ SEM (n = 10). #p < 0.05 and ##p < 0.01 versus Normal group; *p < 0.05 and **p < 0.01 versus DSS group.

Figure 2

SSP promotes CD4+ and CD8+ expressions on Tcm cells in colitis mice. Peripheral blood cells were isolated from mice and stained with different fluorescent antibodies; the cells were gated sequentially on living lymphocytes, CCR7+, and then on CD62L+ and CD45RA+ cells. The proportion of CD45RA+CD62L+CCR7+ Tcm cells was determined. Then, Tcm cells were gated on CD4 and CD8, the proportion of CD4+Tcm cells and CD8+Tcm cells in lymphocytes was analyzed. (A) Total lymphocytes in peripheral blood. (B) Relative percentages of CCR7+ labeled lymphocytes. (C) Relative percentages of CD45RA+CD62L+CCR7+ T cells in UC mice pre- and post-treatment. C1: Normal group; C2: DSS; C3: DSS+SSP; C4: DSS+5-ASA; C5: CD45RA+CD62L+CCR7+ T cells level. (D) Relative percentages of CD4+ Tcm cells in UC mice pre- and post-treatment. D1: Normal group; D2: DSS; D3: DSS+SSP; D4: DSS+5-ASA; D5: CD4+CD45RA+CD62L+CCR7+ T cells level. (E) Relative percentages of CD8+ Tcm cells in UC mice pre- and post-treatment. E1: Normal group; E2: DSS; E3: DSS+SSP; E4: DSS+5-ASA; E5: CD8+CD45RA+CD62L+CCR7+ T cells level. Data are presented as mean $\pm$ SEM (n = 10). #p < 0.05 and ##p < 0.01 versus Normal group; *p < 0.05 and **p < 0.01 versus DSS group.

Figure 3

SSP regulates CD4⁺ Tcm cells and their subsets in colitis mice. After being stained with different fluorescent antibodies, cells were sequentially gated on living lymphocytes, CD4⁺, CCR7⁺, GL7⁺, and CD62L⁺. CD4⁺CCR7⁺GL7⁺, CD4⁺CCR7⁺CD62L⁺, and CD4⁺CCR7⁺CD62L⁺GL7⁺ Tcm cells were analyzed by flow cytometry. (A) Total lymphocytes in peripheral blood. (B) The proportion of CCR7⁺ labeled CD4⁺ T cells. (C) Relative percentages of CD4⁺CCR7⁺GL7⁺ cells in UC mice pre- and post-treatment, C1: Normal group; C2: DSS; C3: DSS+SSP; C4: DSS+5-ASA; C5: CD4⁺CCR7⁺GL7⁺ cells level. (D) Relative percentages of CD4⁺CCR7⁺CD62L⁺ cells in UC mice pre- and post-treatment. D1: Normal group; D2: DSS; D3: DSS+SSP; D4: DSS+5-ASA; D5: CD4⁺CCR7⁺CD62L⁺ cells level. (E) Relative percentages of CD4⁺CCR7⁺CD62L⁺GL7⁺ cells in UC mice pre- and post-treatment. E1: Normal group; E2: DSS; E3: DSS+SSP; E4: DSS+5-ASA; E5: CD4⁺CCR7⁺CD62L⁺GL7⁺ cells level. Data are presented as mean $\pm$ SEM (n = 10). #p < 0.05 and ##p < 0.01 versus Normal group; *p < 0.05 and **p < 0.01 versus DSS group.

Figure 4

SSP regulates CD4⁺ mTfh cells and their subsets in colitis mice. After being stained with different fluorescent antibodies, the cells were sequentially gated on living lymphocytes, CD4⁺, CCR7⁺, and CXCR5⁺. The proportion of CD4⁺CCR7⁺CXCR5⁺ mTfh cells was determined. Subsequently, CD4⁺mTfh cells were gated on GL7 and CD62L, the proportion of GL7⁺CD4⁺mTfh cells and CD62⁺CD4⁺mTfh cells in lymphocytes was analyzed. (A) Total lymphocytes in peripheral blood. (B) The proportion of CCR7⁺ labeled CD4⁺ T cells. (C) Relative percentages of GL7⁺CD4⁺mTfh cells in UC mice pre- and post-treatment. C1: Normal group; C2: DSS; C3: DSS+SSP; C4: DSS+5-ASA; C5: GL7⁺CD4⁺cells level. (D) Relative percentages of CD62⁺CD4⁺mTfh cells in UC. D1: Normal group; D2: DSS; D3: DSS+SSP; D4: DSS+5-ASA; D5: CD62⁺CD4⁺cells level. (E) Relative percentages of total CD4⁺mTfh cells in UC. E1: Normal group; E2: DSS; E3: DSS+SSP; E4: DSS+5-ASA; E5: CD4⁺CCR7⁺CXCR5⁺cells level. Data are presented as mean $\pm$ SEM (n = 10). #p < 0.05 and ##p < 0.01 versus Normal group; *p < 0.05 and **p < 0.01 versus DSS group.

Figure 5

SSP modulates protein levels of JAK/STAT5 signal molecule in colonic tissues. Colon tissue homogenates were collected after SSP treatment, after which the expression levels of JAK/STAT5 signal molecule were analyzed by Western blot analysis. (A) Expression of JAK1 and JAK3. (A1) Quantitative analysis of JAK1. (A2) Quantitative analysis of JAK3. (B) Expression of PIAS1, PIAS3, Socs-1, P-STAT5, and STAT5. (B1) Quantitative analysis of PIAS1. (B2) Quantitative analysis of PIAS3. (B3) Quantitative analysis of Socs-1. (B4) Quantitative analysis of P-STAT5. (B5) Quantitative analysis of STAT5. Data are presented as mean $\pm$ SEM (n = 6). #p < 0.05 and ##p < 0.01 versus Normal group; *p < 0.05 and **p < 0.01 versus DSS group.

Figure 6

SSP modulates protein levels of JAK/STAT5 signal molecule in colonic tissues. Colon tissue homogenates were collected after SSP treatment, after which the expression levels of JAK/STAT5 signal molecule were analyzed by Western blot analysis. (A) Expression of JAK1 and JAK3. (A1) Quantitative analysis of JAK1. (A2) Quantitative analysis of JAK3. (B) Expression of PIAS1, PIAS3, Socs-1, P-STAT5, and STAT5. (B1) Quantitative analysis of PIAS1. (B2) Quantitative analysis of PIAS3. (B3) Quantitative analysis of Socs-1. (B4) Quantitative analysis of P-STAT5. (B5) Quantitative analysis of STAT5. Data are presented as mean $\pm$ SEM (n = 6). #p < 0.05 and ##p < 0.01 versus Normal group; *p < 0.05 and **p < 0.01 versus DSS group.