Schistosoma Japonicum Peptide SJMHE1 Inhibits Acute And Chronic Colitis Induced By Dextran Sulphate Sodium In Mice

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Research

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Abstract

Background: Harnessing helminth-based immunoregulation is a novel therapeutic strategy for many immune dysfunction disorders, including inflammatory bowel diseases. We previously identified a small-molecule peptide from *Schistosoma japonicum* and named it SJMHE1. SJMHE1 can suppress delayed-type hypersensitivity, collagen-induced arthritis, and asthma in mice. In this study, we assessed the effects of SJMHE1 on dextran sulfate sodium (DSS)-induced acute and chronic colitis.

Methods: C57BL/6 mice were induced acute and chronic colitis by DSS, and were injected by emulsifier SJMHE1 or PBS. Then the mice were examined body weight loss, disease activity index, colon lengths, histopathology change, cytokine expression and helper T (Th) cell subset distribution.

Results: We found that SJMHE1 treatment significantly suppressed DSS-induced acute and chronic colitis, improved disease activity and colon pathological damage. SJMHE1 treatment modulated the expression of pro-inflammatory and anti-inflammatory cytokines in splenocytes and the colon. Furthermore, SJMHE1 treatment reduced the percentage of Th1 and Th17 cells and increased the percentage of Th2 and regulatory T (Treg) cells in the splenocytes and mesenteric lymph nodes (MLNs) from mice with acute colitis. Similarly, SJMHE1 treatment upregulated the expression of IL-10 mRNA, and downregulated the expression of IL-17 mRNA, and modulated the Th cell balance in mice with chronic colitis.

Conclusions: Our data show that SJMHE1 provided protection against acute and chronic colitis by restoring immune balance. As a small molecule, SJMHE1 might be a novel agent for the treatment of IBD without immunogenicity concerns.

Background

Inflammatory bowel diseases (IBDs), including ulcerative colitis (UC) and Crohn's disease (CD), are chronic and inflammatory diseases without a real cure, and IBD incidence has increased rapidly worldwide, including China[1]. They are mainly treated with conventional substances, including corticosteroids (e.g., budesonide and prednisone) and immunomodulators (e.g., azathioprine and methotrexate). Cytokines, monoclonal antibodies, cytokine antagonists, and soluble receptors are used to treat patients with IBD and poor or no response to conventional drugs[2, 3]. However, almost all available drugs for the treatment of IBD have adverse side effects. Biologics such as cytokines and antibodies mentioned above have high preparation costs and pose the main challenge in IBD management[1, 4, 5]. Thus, novel therapeutics for IBD with a safe and minimal cost should be developed.

The hygiene hypothesis has been confirmed to be associated with the growing incidence of IBD. Studies have shown that the exposure to infections is reduced, especially the reduction of helminth infection, due to improved sanitation, vaccination, and the use of antibiotics. The reduced infection then leads to the rapid increase in the incidence of IBD worldwide [6, 7]. Helminths have co-evolved with human hosts and developed various mechanisms to modulate the immune network of hosts[8]. Harnessing helminth-driven

immunoregulation has been applied to treat patients with IBD[9]. *Schistosoma mansoni* (*S. mansoni*) infection fails to induce colitis in mice through the intrarectal administration of trinitrobenzenesulfonic acid (TNBS) [10]. Furthermore, TNBS-induced colitis in mice can be decreased by oral eggs of *Schistosoma japonicum* (*S.japonicum*) [11]. Twenty-nine patients with active CD are administered with *Trichuris suis* ova at an interval of 3 weeks for 24 weeks. On week 24, the activity index of CD in 23 patients decreased, and 21 of the 29 patients remitted. Furthermore, no adverse events occur[12]. Furthermore, helminths secrete immunomodulatory molecules, including glycans, proteins, lipids, and nucleic acids, have been confirmed to protect mice against colitis. The excretory/secretory (ES) from the hookworm *Ancyclostoma caninum* (*A.caninum*) can suppress TNBS-induced colitis in mice by inhibiting inflammatory cytokine production[13]. SJMHE1, an anti-inflammatory peptide from the HSP60 protein of S. *japonicum*, suppresses delayed-type hypersensitivity[14], collagen-induced arthritis[15], and asthma[16] in mice. Helminth molecules, such as SJMHE1, should be safer and cheaper for the treatment of IBD than biologics and have the potential to be next-generation drugs.

In the present study, using the DSS model of colitis, we demonstrated that SJMHE1 protected against inducible acute and chronic colitis in mice. SJMHE1 treatment regulated the cytokine expression in the colon and splenocytes. It also modulated Th cell balance in splenocytes and MLNs in mice with acute and chronic colitis. SJMHE1 might be considered a small-molecule peptide as a novel treatment for IBD.

Results

SJMHE1 treatment alleviates DSS-induced acute colitis in mice

Acute colitis was induced by 2.5% DSS. The treatment regimen is illustrated in Fig. 1A. Treatment with SJMHE1 alleviated colitis in mice, as indicated by a decrease in DAI and weight loss and shortening of the colon (Figs. 1B–1E). Histological analysis of the colons of mice showed that SJMHE1 treatment significantly reduced the infiltration of inflammatory cells, mucosal injury, and edema (Fig. 1F). The histological score of the colons was lower in SJMHE1-treated mice than in DSS and DSS/PBS groups (Fig. 1G). These results indicated that SJMHE1 treatment attenuated the disease activity in mice with DSS-induced acute colitis.

SJMHE1 treatment regulates cytokine expression in the splenocytes and colon in DSS- induced acute colitis of mice

Proinflammatory cytokines play a key role in the pathogenesis of IBD[17, 18]. Helminth infection can evoke the production of regulatory cytokines to protect against inflammatory diseases, including IBD[7, 9, 19]. Thus, we investigated the expression of cytokines in the splenocytes and colon of mice from normal, DSS, DSS/PBS, and DSS/SJMHE1 groups. In Figs. 2A–2F, the mRNA expression level of IL-17 in the splenocytes of the DSS and DSS/PBS groups was higher than that of the normal control group. The mRNA expression levels of IL-4, IL-10, and TGF-β of the DSS and DSS/PBS groups were less than that of the normal control group. However, the mRNA expression levels of IL-4 and TGF-β in the splenocytes of the DSS/SJMHE1 group were upregulated, and the mRNA expression levels IFN-γ and IL-17 were

downregulated compared with those in the DSS and DSS/PBS groups. The mRNA expression level of IL-35 in the DSS/SJMHE1 group was higher than that in the DSS/PBS group. The mRNA expression levels of IFN-γ and IL-17 in the colon of DSS and DSS/PBS groups were higher than those of the normal control group. The mRNA expression levels of IL-4, TGF-β, and IL-35 of of DSS and DSS/PBS groups were less than those of the normal control group (Figs. 2G–2L). However, the mRNA expression levels of IFN-γ and IL-17 in the colon of the mice in the DSS/SJMHE1 group were less than those in the DSS and/or DSS/PBS group. The mRNA expression levels of IL-4, IL-10, TGF-β, and IL-35 in the DSS/SJMHE1 group were higher than those in the DSS and/or DSS/PBS group (Figs. 2G–2L). These results suggested that SJMHE1 treatment could regulate the expression of cytokines in the splenocytes and colons of mice with acute colitis.

SJMHE1 treatment modulates Th cell balance in the splenocytes and MLNs in mice with DDS-induced acute colitis

Dysregulated $CD4^+$ T cells have also been demonstrated to play a crucial role in the pathogenesis of IBD[20, 21]. The Th subsets were detected in the splenocytes and MLNs of the mice from normal, DSS, DSS/PBS, and DSS/SJMHE1 groups. In Fig. 3, the proportions of $CD4^+IFN\text{-}\gamma^+$ Th1 and $CD4^+IL\text{-}17^+$ Th17 cells increased in the splenocytes of the DSS groups compared with those of the normal control group. However, SJMHE1 treatment significantly reduced the proportions of Th1 and Th17 cells and increased the percentage of $CD4^+IL\text{-}4^+$ Th2 and $CD4^+CD25^+Foxp3^+$ Treg cells in the splenocytes compared with those in the DSS and/or DSS/PBS groups (Fig. 3). The proportions of Th1 and Th17 cells decreased and the proportions of Th2 and Treg cells increased in the MLNs of the mice in the DSS/SJMHE1 group (Fig. 4). These results indicated that SJMHE1 treatment downregulated Th1 and Th17 cells and upregulated Th2 and Treg cells. Therefore, it might provide protection to mice against DSS-induced acute colitis.

Improvement of DSS-induced chronic colitis in SJMHE1-treated mice

The mice were induced by 2% DSS on days 0–5, 10–15, and 20–25 to detect the effects of SJMHE1 treatment on chronic colitis[22]. The treatment regimen is displayed in Fig. 5A. Similar to the effect of SJMHE1 on acute colitis, decrease in DAI and weight loss and shortening of the colon were induced by SJMHE1 treatment (Figs. 5B–5E). The crypt damage, ulceration, and infiltration of inflammatory cells were presented in DSS- and DSS/PBS-treated mice through histological analysis. However, SJMHE1 treatment decreased the degree of mucosal injury, the number of infiltrating cells, and the histological scores compared with those in the DSS and DSS/PBS groups (Figs. 5F and 5G). These results suggested that SJMHE1 could suppress DSS-induced chronic colitis in mice.

SJMHE1 treatment decreased the mRNA expression of IL-17 and increased the mRNA expression of IL-10 in the splenocytes and colon of mice with DSS-induced chronic colitis

The expression of cytokines in the splenocytes and colon was examined to evaluate the cytokine production of SJMHE1 treatment in mice with DSS-induced chronic colitis. In Fig. 6, the mRNA expression

levels of IFN-γ and IL-35 in the splenocytes of the DSS group were less than those of the normal control group. The mRNA expression level of IL-17 of the DSS group was higher than that of the normal control group. SJMHE1 treatment reduced the mRNA expression level of IL-17 and increased the mRNA expression level of IL-10 compared with those of the DSS and/or DSS/PBS group. The IL-4 level slightly increased in the DSS and DSS/PBS groups compared with that in the normal group. SJMHE1 treatment reduced the mRNA expression of IL-4, but this decrease was not statistically significant (Figs. 6A–6F). Similarly, the mRNA expression of IFN-γ in the colon of DSS- and DSS/PBS-treated mice was less than that of the normal mice (Figs. 6G–6L). SJMHE1 treatment downregulated the mRNA expression of IL-17 and upregulated the mRNA expression of IL-10 in the colon of mice compared with that of the DSS/PBS or DSS group (Fig. 6B). These results suggested that the inhibitory effect of SJMHE1 on DSS-induced chronic colitis might be associated with the modulation of the mRNA expression levels of IL-17 and IL-10.

SJMHE1 treatment regulated the distribution of Th cells in the splenocytes and MLNs of mice with DSS-induced chronic colitis

The Th cells in the splenocytes and MLNs were examined through flow cytometry to test the effect of SJMHE1 treatment on the Th subsets of mice with DSS-induced chronic colitis. In Fig. 7, the percentages of $CD4^+IL\text{-}4^+$ Th2, and $CD4^+IL\text{-}17^+$ Th17 cells in the splenocytes of the DSS-treated mice increased compared with those of the normal mice. However, SJMHE1 treatment significantly reduced the proportions of Th17 cells and increased the proportions of Th1 and Treg cells in the splenocytes compared with those in the DSS group. Consistent with the decrease in the mRNA expression of IL-4 (Fig. 6B), decreased proportions of Th2 cells were induced by SJMHE1 treatment in the splenocytes, but this decrease was not significant. Furthermore, the DSS-treated mice had increased percentages of Th2 cells and decreased percentages of Treg cells in MLNs compared with those of the normal mice (Fig. 8). SJMHE1 treatment also increased the percentage of Th1 and Treg cells in MLNs compared with that of the DSS and DSS/PBS groups. However, SJMHE1 treatment slightly decreased the proportion of Th17 cells in MLNs compared with that in the DSS or DSS/PBS group (Fig. 8). These results suggested that SJMHE1 suppressed DSS-induced chronic colitis partly mediated by regulating the balance of Th cells.

Discussion

IBD is a chronic relapsing inflammation characterized by intestinal epithelial injury and immune homeostasis disruption, including CD and UC. The pathogenesis of IBD has not yet been identified, and the available treatment for IBD is still limited. Helminth therapy for IBD has been displayed to inhibit colitis in different animal models. The pig whipworm *Trichuris suis* ova (TSO) has been assessed in patients with CD and UC in phase 1 trials. TSO significantly improves the symptoms of colitis in patients compared with those who received placebo after 12 weeks of therapy [23]. Patients who have CD and percutaneous infection of low doses of *Necator americanus*, and patients remained in the trial for 1 year until disease remission[24]. Further research found that ES products (ESPs) from helminths also have therapeutic properties. Soluble proteins from *S. mansoni* and *A. caninum* can ameliorate TNBS-induced colitis in mice [13]. Crude and ES products from *Ancylostoma ceylanicum* can also inhibit DSS-induced

colitis in mice [25]. Although ESPs instead of helminth infection can avoid many drawbacks via currently available helminth therapies, crude helminth products as a drug are limited[8]. A recombinant of anti-inflammatory protein from *A. caninum* (AIP-1) can suppress TNBS-induced colitis in mice [26]. Furthermore, an increasing number of immunomodulatory proteins from helminths were identified. Notably, ES-62 from *Acanthocheilonema vitae* (*A. vitae*) contains phosphorylcholine (PC), and PC is the bioactive moiety of ES-62. Small drug-like analogs of PC have therapeutic potential for arthritis and lung fibrosis, but they can avoid immunogenicity concerns with the whole ES-62 protein [27, 28]. Thus, helminth-derived anti-inflammatory small molecules for the treatment of colitis should be further explored.

In this study, SJMHE1, a small-molecule peptide from *S. japonicum*, was used to treat mice with DSS-induced acute and chronic colitis. The results revealed that SJMHE1 treatment significantly reduced the DAI and weight loss and shortened the colon of the mice with acute and chronic colitis. This treatment also improved the survival rate of the mice with acute colitis (data not shown). The mice with colitis showed cellular infiltration, epithelial erosion, and villus atrophy in colon tissues, but SJMHE1 treatment significantly protected the mice against DSS-induced gut pathology. Thus, SJMHE1 alleviated disease activity and inflammatory response in mice with acute colitis induced by DSS.

Many proinflammatory cytokines play a crucial role in the pathogenesis of IBD. The pathogenesis of CD and UC is usually considered different. CD is dominated by IFN-γ and IL-17A from Th1 and Th17 cells, respectively, whereas UC is mediated by IL-4 and IL-13 from Th2 cells [1]. Consistent with the findings in mice with DSS-induced acute colitis [29, 30, 31], the mRNA expression of IL-17 in the splenocytes and the mRNA expression of IFN-γ and IL-17 in the colon increased in the DSS and DSS/PBS groups. However, SJMHE1 treatment reduced the mRNA expression levels of IFN-γ and IL-17, increased the mRNA expression levels of IL-4 and TGF-β in the splenocytes, and increased the mRNA expression levels of IL-4, IL-10, TGF-β, and IL-35 in the colon of mice. SJMHE1 administration provided protection against DSS-induced acute colitis in mice. This observation might be related to the regulation of cytokine expression by SJMHE1. Similar results have been reported that SEA from *S. mansoni* and cystatin and Sj16 from *S. japonicum* [31, 32, 33].

Dysregulated $CD4^+$ T cells have also been reported to play key role in the pathogenesis of IBD. Th1 and Th17 responses dominate in patients with CD, whereas Th2 response is mediated in patients with UC [1]. DSS-induced colitis is originally considered a T cell-independent model [34]. However, further studies suggested that DSS colitis is a Th1- or Th17-mediated inflammation [35, 36]. Consistent with these studies, the acute model demonstrated that the proportions of Th1 and Th17 cells in the splenocytes of the DSS and DSS/PBS groups were higher than those of the normal mice (Fig. 3). The percentage of Th17 cells in the MLNs of the DSS/PBS group was higher than that of the normal mice (Fig. 4). However, SJMHE1 treatment reduced the proportions of Th1 and Th17 cells and increased the percentage of Th2 and Treg cells in the splenocytes and MLNs (Fig. 4). Helminth infection or their products can skew the immune response toward Th2 and Treg responses, which were generally considered to suppress Th1- and

Th17-mediated inflammation, including IBD [7, 37]. As a small-molecule peptide from helminths, SJMHE1 inhibits the development of DSS-induced acute colitis through the induction of a Th2/Treg profile.

In the present study, SJMHE1 had the protective effect on DSS-induced chronic colitis in mice. SJMHE1 administration attenuated the clinical disease activity and pathological response of mice with chronic colitis (Fig. 5). In contrast to mice with acute colitis, the mRNA expression of IFN-γ in the splenocytes and colon of DSS-treated mice with chronic colitis decreased, whereas the mRNA expression of IL-17A in the splenocytes increased. SJMHE1 treatment decreased the mRNA expression of IL-17A and increased the mRNA expression of IL-10 in the splenocytes and colon compared with those of the DSS-treated mice with chronic colitis (Fig. 6). The proportion of Th1 cells in the splenocytes of the mice with DSS-induced chronic colitis decreased compared with those of mice with acute colitis. By contrast, the percentages of Th2 and Th17 cells in mice from the DSS-induced chronic colitis increased compared with that in the normal mice (Fig. 7). However, SJMHE1 treatment reduced the percentage of Th17 cells and increased the proportion of Th1 and Treg cells in the splenocytes. SJMHE1 also increased the percentage of Th1 and Treg cells but slightly reduced the percentage of Th17 cells in MLNs (Fig. 8). These results suggested that SJMHE1 could inhibit Th2 and Th17-mediated chronic colitis in mice through the upregulation of Th1 and Treg cells. DSS-induced colitis is originally considered Th1- or Th17-mediated inflammation [7, 37]. Most of these findings are based on an acute colitis model, i.e., oral administration of DSS for 7 days [38]. However, chronic colitis was induced by multiple cycles of DSS, its histopathology is similar to UC, and UC is a Th2-dominated inflammation [39]. Dieleman and colleagues [40] demonstrated that DSS-induced chronic colitis is characterized by Th1 and Th2 cytokines. Th17 cells have been reported to participate in the development of DSS-induced chronic colitis in mice [41, 42]. Interestingly, SJMHE1 inhibits not only Th1- and Th17-mediated acute colitis but also Th2- and Th17-mediated chronic colitis in mice. A similar helminth molecule is ES-62 from *A. vitae*. ES-62 inhibits inflammation by restoring immune balance regardless of inflammatory phenotype [42, 43, 44]. As a small-molecule peptide from helminths that coevolved with humans, SJMHE1 might be a drug development perspective for the treatment of various inflammatory diseases, such as asthma, arthritis, and colitis.

Conclusions

In conclusion, SJMHE1 from *S. japonicum* alleviates DDS-induced acute and chronic colitis in mice. SJMHE1 treatment regulates cytokine expression and Th balance in the splenocytes and inflammatory site (colon or MLNs) from mice with acute and chronic colitis. SJMHE1 may be a novel therapeutic agent that can be exploited for the treatment of IBD without immunogenicity concerns.

Materials And Methods

Mice

C57BL/6 male mice aged 6–8 weeks (each weighing 19–22 g) were purchased from Cavins Experimental Animals Co., Ltd. (Changzhou, China; No. 201911131). They were fed at a specific-free level in the

Experimental Animal Center at Jiangsu University. Animal experiments were conducted in accordance with the Guidelines for the Care and Use of Laboratory Animals and approved by the Animal Research Ethics Committee of Jiangsu University (UJS-LEAR-AP-2018030601).

Peptides

S. japonicum peptide SJMHE1 was synthesized and purified by ChinaPeptides (Shanghai, China) as described previously [14, 15, 16]. The peptide was detected through high-performance liquid chromatography with a purity of more than 98% and stored at −80 °C.

Experimental colitis induction via DSS and SJMHE1 treatment

Male C57BL/6 mice were randomly divided into four groups: normal, DSS, DSS/PBS, and DSS/SJMHE1 groups. For acute colitis, the mice were drunk with 2.5% DSS (molecular weight of 36–50 kDa; MP Biomedicals, USA) solution continuously for 10 days in accordance with a previously described method [22]. The mice in the DSS/SJMHE1 and DSS/PBS groups were subcutaneously injected with 0.1ml emulsifier SJMHE1 (10 μg) or PBS with incomplete Freund's adjuvant (IFA, Sigma, Poole, UK) on days 0 and 7, respectively. For chronic colitis, the mice were drunk with 2% DSS solution on days 0–5, 10–15, and 20–25 as described previously[22]. In the DSS/SJMHE1 and DSS/PBS groups, the mice were treated with 0.1ml emulsifier SJMHE1 (10 μg) or PBS with IFA on days 0, 14, and 28, respectively. During treatment, the mice were monitored daily for fecal traits, blood in stool, and weight change. The disease activity index (DAI) was calculated in accordance with the scoring criteria of Holger Sann [22]. Then, the mice were anesthetized and killed to evaluate the length and inflammation of the colon.

Histopathologic analysis

Colon tissue segments (about 0.5 cm) were collected and fixed with a formalin solution at room temperature for 2 days. Then, these segments were dehydrated, embedded in paraffin, sectioned, and stained with hematoxylin and eosin (H&E). The pathological changes and inflammation of colon tissues were assessed using a standard histopathological score as previously described [45].

Flow cytometry analysis

The spleen and mesenteric lymph nodes (MLNs) were aseptically separated, and single cell suspension was prepared as described previously [14, 15, 16]. Single-cell suspensions from the spleen and MLNs were stimulated with a brefeldin/monensin mixture (Brefeldin A, Multisciences) and a phorbol-12-myristate-13-acetate/ionomycin mixture (Multisciences) for 5 h. FITC-anti-mouse-CD4 (eBioscience) was used for surface staining. APC-anti-mouse-IFN-γ (eBioscience), APC-anti-mouse-IL-4 (Biolegend), and PE-anti-mouse-IL-17A (Biolegend) were utilized for intracellular staining after the membrane was ruptured with fixation/permeabilization (BD Biosciences) to analyze Th1, Th2, and Th17 cells as described previously[16, 46]. Mononuclear cells were stained with FITC-anti-mouse-CD4, APC-anti-mouse-CD25, and PE-anti-mouse Foxp3 by using a mouse regulatory T cell staining kit (eBioscience) to evaluate Treg cells.

All the samples were detected with a BD FACSCcanto flow cytometer (BD Biosciences), and data were analyzed with Flowjo v10.0.7 (Tree Star, Ashland, OR, USA).

RNA extraction and quantitative RT-PCR (qRT-PCR)

Total RNA was extracted from the spleen cells by using a Trizol reagent (Invitrogen, CA, USA). First-strand cDNA was synthesized with a reverse transcription kit (GeneCopoeia, Germantown, MD, USA). RNA was isolated from the colon tissue by using a tissue RNA purification kit Plus (Yishan Biological Technology, Shanghai, China). The cDNA of mRNA was synthesized with a reverse transcription kit (TakaRa, Tokyo, Japan). All-in-one^{TM} qPCR primer sets for IFN-γ (Cat. No. MQP027401), IL-4 (Cat. No. MQP032451), IL-17A (Cat. No. MQP029457), IL-10 (Cat. No. MQP029453), TGF-β (Cat. No. MQP030343), IL-35 (Cat. No. MQP027412), and GAPDH (Cat. No. MQP027158) were obtained from GeneCopoeia. The PCR amplification and calculation of the relative mRNA expression were based on previously described methods [16, 46].

Statistical analysis

Data were expressed as mean ± SEM, and statistical significance was analyzed through one-way ANOVA followed by Dunn's multiple comparison with GraphPad Prism 8.0.0 (GraphPad, USA). Data with $P < 0.05$ were considered statistically significant.

Declarations

Author Contributions

Conceived and designed the experiments: WQS WZZ XFW. Performed the experiments: WQS WZZ FX YBM. Analysed the data: LYD TW YZ. Contributed reagents/materials/ analysis tools: DQF MC GYY. Wrote the paper: WQS WZZ XFW. All authors read and approved the final manuscript.

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Availability of data and materials

The dataset supporting the conclusions of this article is included within the article.

Ethics approval and consent to participate

As no human material was used, no ethical approval from human patients could be obtained. Animal experiments were conducted in accordance with the Guidelines for the Care and Use of Laboratory Animals and approved by the Animal Research Ethics Committee of Jiangsu University (UJS-LEAR-AP-2018030601).

Consent for publication

Not applicable

Competing interests

The authors declare that they have no conflicts interests.

References

1. Yeshi K, Ruscher R, Hunter L, Daly NL, Loukas A, Wangchuk P. Revisiting Inflammatory Bowel Disease: Pathology, Treatments, Challenges and Emerging Therapeutics Including Drug Leads from Natural Products. J Clin Med. 2020;9 5; doi:10.3390/jcm9051273. https://www.ncbi.nlm.nih.gov/pubmed/32354192.
2. Yamamoto-Furusho JK. Inflammatory bowel disease therapy: blockade of cytokines and cytokine signaling pathways. Curr Opin Gastroenterol. 2018;34 4:187–93; doi: 10.1097/MOG.0000000000000444. https://www.ncbi.nlm.nih.gov/pubmed/29846261.
3. Fernandez-Clotet A, Castro-Poceiro J, Panes J. Tofacitinib for the treatment of ulcerative colitis. Expert Rev Clin Immunol. 2018;14 11:881–92; doi: 10.1080/1744666X.2018.1532291. https://www.ncbi.nlm.nih.gov/pubmed/30285500.
4. Kim JW, Lee CK, Lee JK, Jeong SJ, Oh SJ, Moon JR, et al. Long-term evolution of direct healthcare costs for inflammatory bowel diseases: a population-based study (2006–2015). Scand J Gastroenterol. 2019;54 4:419–26; doi:10.1080/00365521.2019.1591498. https://www.ncbi.nlm.nih.gov/pubmed/30905222.
5. Mak JW, Sung JJ. The Use of Biologics and Biosimilar in Asian patients with IBD: Are we ready? J Gastroenterol Hepatol. 2019;34 8:1269–70; doi: 10.1111/jgh.14817. https://www.ncbi.nlm.nih.gov/pubmed/31456235.
6. Leong RW, Mitrev N, Ko Y. Hygiene Hypothesis: Is the Evidence the Same All Over the World? Dig Dis. 2016;341–2:35–42; doi: 10.1159/000442922. https://www.ncbi.nlm.nih.gov/pubmed/26982573.
7. Heylen M, Ruyssers NE, Gielis EM, Vanhomwegen E, Pelckmans PA, Moreels TG, et al. Of worms, mice and man: an overview of experimental and clinical helminth-based therapy for inflammatory bowel disease. Pharmacol Ther 2014;143 2:153 – 67; doi: 10.1016/j.pharmthera.2014.02.011. https://www.ncbi.nlm.nih.gov/pubmed/24603369.
8. Ryan SM, Eichenberger RM, Ruscher R, Giacomin PR, Loukas A. Harnessing helminth-driven immunoregulation in the search for novel therapeutic modalities. PLoS Pathog 2020;16 5:e1008508;

doi:10.1371/journal.ppat.1008508. https://www.ncbi.nlm.nih.gov/pubmed/32407385.

9. Abdoli A. Therapeutic Potential of Helminths and Helminth-Derived Antigens for Resolution of Inflammation in Inflammatory Bowel Disease. Arch Med Res. 2019;50 1:58–9; doi:10.1016/j.arcmed.2019.03.001. https://www.ncbi.nlm.nih.gov/pubmed/30879759.
10. Elliott DE, Urban JJ, Argo CK, Weinstock JV. Does the failure to acquire helminthic parasites predispose to Crohn's disease? FASEB J. 2000;14 12:1848–55; doi:10.1096/fj.99-0885hyp. https://www.ncbi.nlm.nih.gov/pubmed/10973934.
11. Xia CM, Zhao Y, Jiang L, Jiang J, Zhang SC. Schistosoma japonicum ova maintains epithelial barrier function during experimental colitis. World J Gastroenterol. 2011;17 43:4810–6; doi:10.3748/wjg.v17.i43.4810. https://www.ncbi.nlm.nih.gov/pubmed/22147983.
12. Summers RW, Elliott DE, Urban JF Jr, Thompson R, Weinstock JV. Trichuris suis therapy in Crohn's disease. Gut. 2005;54 1:87–90; doi:10.1136/gut.2004.041749. https://www.ncbi.nlm.nih.gov/pubmed/15591509.
13. Ruyssers NE, De Winter BY, De Man JG, Loukas A, Pearson MS, Weinstock JV, et al. Therapeutic potential of helminth soluble proteins in TNBS-induced colitis in mice. Inflamm Bowel Dis 2009;15 4:491–500; doi:10.1002/ibd.20787. https://www.ncbi.nlm.nih.gov/pubmed/19023900.
14. Wang X, Wang J, Liang Y, Ni H, Shi L, Xu C, et al. Schistosoma japonicum HSP60-derived peptide SJMHE1 suppresses delayed-type hypersensitivity in a murine model. Parasit Vectors 2016;9:147; doi:10.1186/s13071-016-1434-4. https://www.ncbi.nlm.nih.gov/pubmed/26971312.
15. Wang X, Li L, Wang J, Dong L, Shu Y, Liang Y, et al. Inhibition of cytokine response to TLR stimulation and alleviation of collagen-induced arthritis in mice by Schistosoma japonicum peptide SJMHE1. J Cell Mol Med. 2017;21 3:475–86; doi:10.1111/jcmm.12991. https://www.ncbi.nlm.nih.gov/pubmed/27677654.
16. Zhang W, Li L, Zheng Y, Xue F, Yu M, Ma Y, et al. Schistosoma japonicum peptide SJMHE1 suppresses airway inflammation of allergic asthma in mice. J Cell Mol Med. 2019;23 11:7819–29; doi:10.1111/jcmm.14661. https://www.ncbi.nlm.nih.gov/pubmed/31496071.
17. Neurath MF. Cytokines in inflammatory bowel disease. Nat Rev Immunol 2014;14 5:329–42; doi:10.1038/nri3661. https://www.ncbi.nlm.nih.gov/pubmed/24751956.
18. Salas A, Hernandez-Rocha C, Duijvestein M, Faubion W, McGovern D, Vermeire S, et al. JAK-STAT pathway targeting for the treatment of inflammatory bowel disease. Nat Rev Gastroenterol Hepatol. 2020;17 6:323 – 37; doi: 10.1038/s41575-020-0273-0. https://www.ncbi.nlm.nih.gov/pubmed/32203403.
19. Maruszewska-Cheruiyot M, Donskow-Lysoniewska K, Doligalska M. Helminth Therapy: Advances in the use of Parasitic Worms Against Inflammatory Bowel Diseases and its Challenges. Helminthologia 2018;55 1:1–11; doi:10.1515/helm-2017-0048. https://www.ncbi.nlm.nih.gov/pubmed/31662622.
20. Boden EK, Lord JD. CD4 T Cells in IBD: Crossing the Line? Dig Dis Sci. 2017;62 9:2208–10; doi: 10.1007/s10620-017-4655-2. https://www.ncbi.nlm.nih.gov/pubmed/28646283.

21. Tindemans I, Joosse ME, Samsom JN. Dissecting the Heterogeneity in T-Cell Mediated Inflammation in IBD. Cells. 2020;9 1; doi: 10.3390/cells9010110. https://www.ncbi.nlm.nih.gov/pubmed/31906479.
22. Yoshihara K, Yajima T, Kubo C, Yoshikai Y. Role of interleukin 15 in colitis induced by dextran sulphate sodium in mice. Gut. 2006;55 3:334–41; doi:10.1136/gut.2005.076000. https://www.ncbi.nlm.nih.gov/pubmed/16162679.
23. Summers RW, Elliott DE, Urban JF Jr, Thompson RA, Weinstock JV. Trichuris suis therapy for active ulcerative colitis: a randomized controlled trial. Gastroenterology 2005;128 4:825–32; doi:10.1053/j.gastro.2005.01.005. https://www.ncbi.nlm.nih.gov/pubmed/15825065.
24. Croese J, O'Neil J, Masson J, Cooke S, Melrose W, Pritchard D, et al. A proof of concept study establishing Necator americanus in Crohn's patients and reservoir donors. Gut. 2006;55 1:136–7; doi:10.1136/gut.2005.079129. https://www.ncbi.nlm.nih.gov/pubmed/16344586.
25. Cancado GG, Fiuza JA, de Paiva NC, Lemos Lde C, Ricci ND, Gazzinelli-Guimaraes PH, et al. Hookworm products ameliorate dextran sodium sulfate-induced colitis in BALB/c mice. Inflamm Bowel Dis. 2011;17 11:2275–86; doi:10.1002/ibd.21629. https://www.ncbi.nlm.nih.gov/pubmed/21290484.
26. Ferreira IB, Pickering DA, Troy S, Croese J, Loukas A, Navarro S. Suppression of inflammation and tissue damage by a hookworm recombinant protein in experimental colitis. Clin Transl Immunology. 2017;6 10:e157; doi:10.1038/cti.2017.42. https://www.ncbi.nlm.nih.gov/pubmed/29114386.
27. Doonan J, Lumb FE, Pineda MA, Tarafdar A, Crowe J, Khan AM, et al. Protection Against Arthritis by the Parasitic Worm Product ES-62, and Its Drug-Like Small Molecule Analogues, Is Associated With Inhibition of Osteoclastogenesis. Front Immunol. 2018;9:1016; doi:10.3389/fimmu.2018.01016. https://www.ncbi.nlm.nih.gov/pubmed/29867986.
28. Suckling CJ, Mukherjee S, Khalaf AI, Narayan A, Scott FJ, Khare S, et al. Synthetic analogues of the parasitic worm product ES-62 reduce disease development in in vivo models of lung fibrosis. Acta Trop 2018;185:212–8; doi:10.1016/j.actatropica.2018.05.015. https://www.ncbi.nlm.nih.gov/pubmed/29802846.
29. Wang J, Goepfert C, Mueller N, Piersigilli A, Lin R, Wen H, et al. Larval Echinococcus multilocularis infection reduces dextran sulphate sodium-induced colitis in mice by attenuating T helper type 1/type 17-mediated immune reactions. Immunology. 2018;154 1:76–88; doi:10.1111/imm.12860. https://www.ncbi.nlm.nih.gov/pubmed/29121394.
30. Kim JH, Won YS, Cho HD, Hong SM, Moon KD, Seo KI. Protective Effect of Prunus mume Fermented with Mixed Lactic Acid Bacteria in Dextran Sodium Sulfate-Induced Colitis. Foods. 2020;10 1; doi: 10.3390/foods10010058. https://www.ncbi.nlm.nih.gov/pubmed/33383792.
31. Wang L, Xie H, Xu L, Liao Q, Wan S, Yu Z, et al. rSj16 Protects against DSS-Induced Colitis by Inhibiting the PPAR-alpha Signaling Pathway. Theranostics 2017;7 14:3446–60; doi:10.7150/thno.20359. https://www.ncbi.nlm.nih.gov/pubmed/28912887.

32. Elliott DE, Li J, Blum A, Metwali A, Qadir K, Urban JF Jr, et al. Exposure to schistosome eggs protects mice from TNBS-induced colitis. Am J Physiol Gastrointest Liver Physiol. 2003;284 3:G385-91; doi:10.1152/ajpgi.00049.2002. https://www.ncbi.nlm.nih.gov/pubmed/12431903.
33. Wang S, Xie Y, Yang X, Wang X, Yan K, Zhong Z, et al. Therapeutic potential of recombinant cystatin from Schistosoma japonicum in TNBS-induced experimental colitis of mice. Parasit Vectors 2016;9:6; doi:10.1186/s13071-015-1288-1. https://www.ncbi.nlm.nih.gov/pubmed/26728323.
34. Dieleman LA, Ridwan BU, Tennyson GS, Beagley KW, Bucy RP, Elson CO. Dextran sulfate sodium-induced colitis occurs in severe combined immunodeficient mice. Gastroenterology. 1994;107 6:1643–52; doi:10.1016/0016-5085(94)90803-6. https://www.ncbi.nlm.nih.gov/pubmed/7958674.
35. Brown JB, Cheresh P, Zhang Z, Ryu H, Managlia E, Barrett TA. P-selectin glycoprotein ligand-1 is needed for sequential recruitment of T-helper 1 (Th1) and local generation of Th17 T cells in dextran sodium sulfate (DSS) colitis. Inflamm Bowel Dis. 2012;18 2:323–32; doi:10.1002/ibd.21779. https://www.ncbi.nlm.nih.gov/pubmed/22009715.
36. Ito R, Kita M, Shin-Ya M, Kishida T, Urano A, Takada R, et al. Involvement of IL-17A in the pathogenesis of DSS-induced colitis in mice. Biochem Biophys Res Commun. 2008;377 1:12 – 6; doi: 10.1016/j.bbrc.2008.09.019. https://www.ncbi.nlm.nih.gov/pubmed/18796297.
37. Khan AR, Fallon PG. Helminth therapies: translating the unknown unknowns to known knowns. Int J Parasitol. 2013;43 3–4:293–9; doi:10.1016/j.ijpara.2012.12.002. https://www.ncbi.nlm.nih.gov/pubmed/23291459.
38. Egger B, Bajaj-Elliott M, MacDonald TT, Inglin R, Eysselein VE, Buchler MW. Characterisation of acute murine dextran sodium sulphate colitis: cytokine profile and dose dependency. Digestion. 2000;62 4:240–8; doi:10.1159/000007822. https://www.ncbi.nlm.nih.gov/pubmed/11070407.
39. Cooper HS, Murthy SN, Shah RS, Sedergran DJ. Clinicopathologic study of dextran sulfate sodium experimental murine colitis. Lab Invest. 1993;69(2):238–49. https://www.ncbi.nlm.nih.gov/pubmed/8350599.
40. Dieleman LA, Palmen MJ, Akol H, Bloemena E, Pena AS, Meuwissen SG, et al. Chronic experimental colitis induced by dextran sulphate sodium (DSS) is characterized by Th1 and Th2 cytokines. Clin Exp Immunol. 1998;114 3:385–91; doi:10.1046/j.1365-2249.1998.00728.x. https://www.ncbi.nlm.nih.gov/pubmed/9844047.
41. Dong YL, Duan XY, Liu YJ, Fan H, Xu M, Chen QY, et al. Autotaxin-Lysophosphatidic Acid Axis Blockade Improves Inflammation by Regulating Th17 Cell Differentiation in DSS-Induced Chronic Colitis Mice. Inflammation. 2019;42 5:1530–41; doi:10.1007/s10753-019-01015-z. https://www.ncbi.nlm.nih.gov/pubmed/31102124.
42. Zhang H, Dai Y, Liu Y, Wu T, Li J, Wang X, et al. Helicobacter pylori Colonization Protects Against Chronic Experimental Colitis by Regulating Th17/Treg Balance. Inflamm Bowel Dis. 2018;24 7:1481–92; doi:10.1093/ibd/izy107. https://www.ncbi.nlm.nih.gov/pubmed/29788098.
43. Harnett W. Secretory products of helminth parasites as immunomodulators. Mol Biochem Parasitol 2014;195 2:130–6; doi:10.1016/j.molbiopara.2014.03.007.

https://www.ncbi.nlm.nih.gov/pubmed/24704440.

44. Crowe J, Lumb FE, Doonan J, Broussard M, Tarafdar A, Pineda MA, et al. The parasitic worm product ES-62 promotes health- and life-span in a high calorie diet-accelerated mouse model of ageing. PLoS Pathog. 2020;16 3:e1008391; doi:10.1371/journal.ppat.1008391. https://www.ncbi.nlm.nih.gov/pubmed/32163524.
45. Sun X, Somada S, Shibata K, Muta H, Yamada H, Yoshihara H, et al. A critical role of CD30 ligand/CD30 in controlling inflammatory bowel diseases in mice. Gastroenterology. 2008;134 2:447 – 58; doi: 10.1053/j.gastro.2007.11.004. https://www.ncbi.nlm.nih.gov/pubmed/18242212.
46. Xue F, Yu M, Li L, Zhang W, Ma Y, Dong L, et al. Elevated granulocytic myeloid-derived suppressor cells are closely related with elevation of Th17 cells in mice with experimental asthma. Int J Biol Sci. 2020;16 12:2072–83; doi:10.7150/ijbs.43596. https://www.ncbi.nlm.nih.gov/pubmed/32549755.

Figures

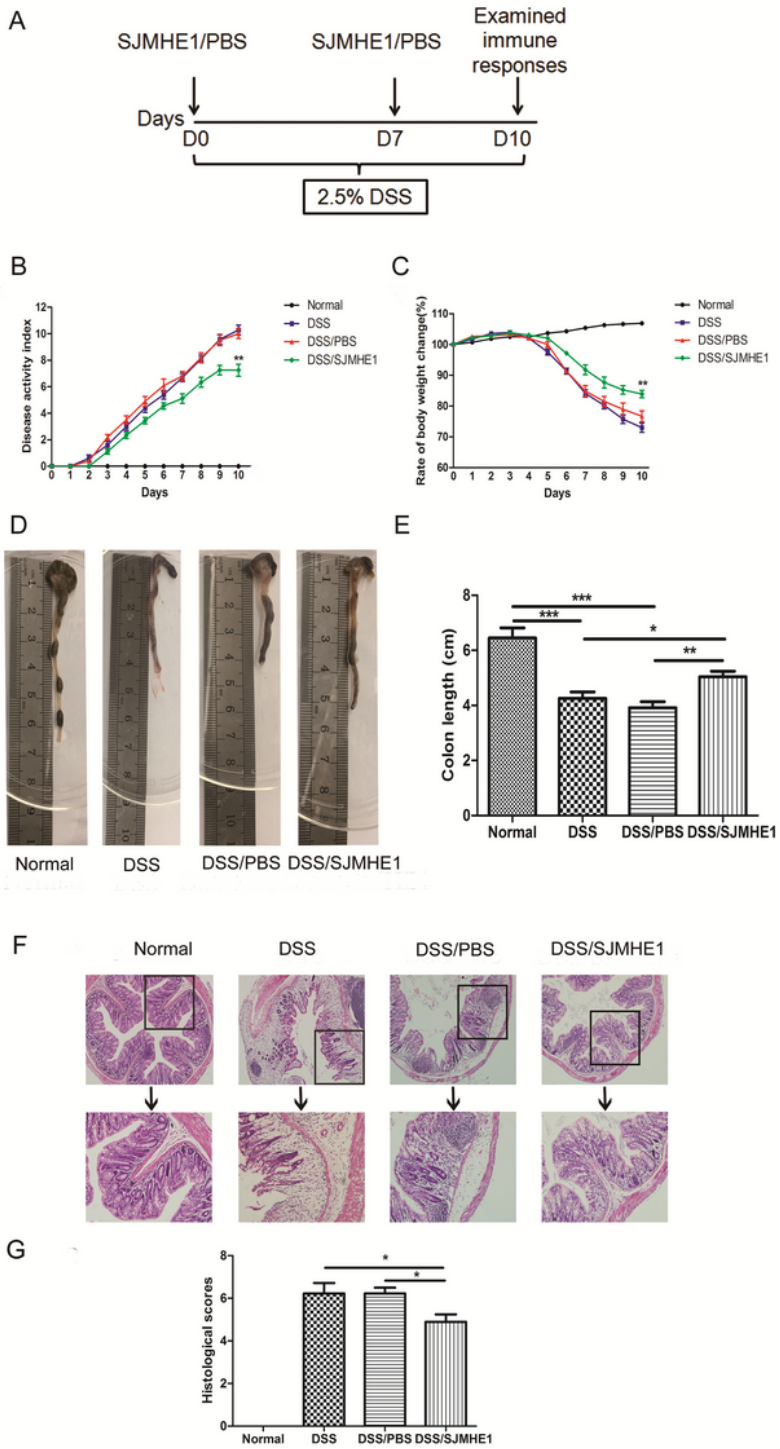

Figure 1

SJMHE1 treatment alleviates colon inflammation in mice with DDS-induced acute colitis. (A) Experimental scheme. C57BL/6 mice were drunk with 2.5% DSS for 10 days. They were injected with SJMHE1 or PBS emulsified in IFA on days 0 and 7. The mice were killed on day 10. (B) Disease activity index (DAI) and (C) rate of body weight change in each group. (D) Macroscopic appearance of colon. (E) Colon length in each group. (F) Histological analysis of the colon from mice via H&E staining (20×

magnification). Images were the representative of three independent experiments (n = 6 mice per group). (G) Histological scores were assessed from each mouse. Data were presented as mean ± SEM (n = 18) from three independent experiments. * P < 0.05, ** P < 0.01, *** P < 0.001.

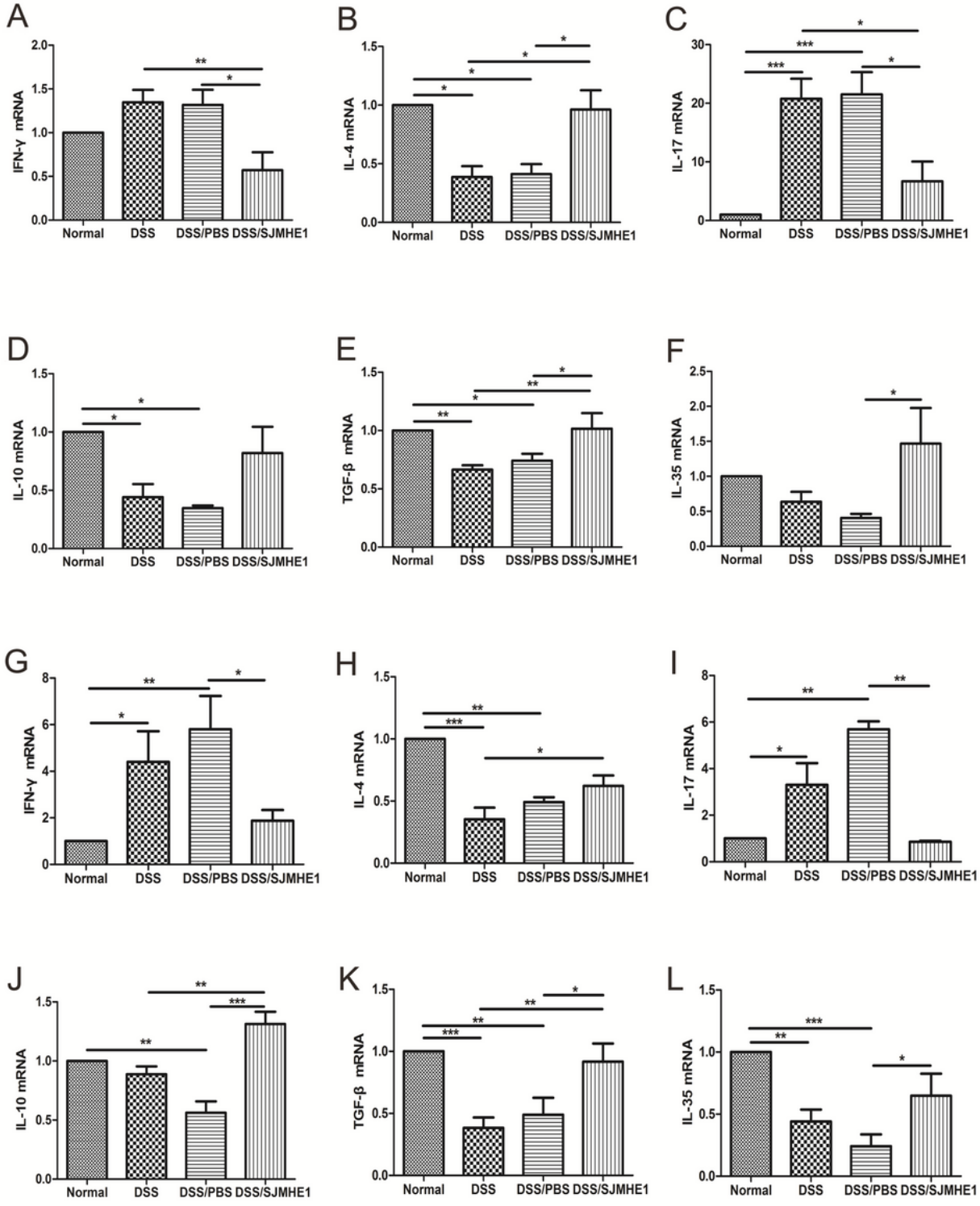

Figure 2

SJMHE1 treatment regulates the expression of cytokines from the splenocytes and colon of mice with DSS-induced acute colitis. On day 10, the mice were killed, and the splenocytes (A) and (B) colon from

each mouse were tested for the mRNA expression levels of IFN-γ, IL-4, IL-17, IL-10, TGF-β, and IL-35 through qRT-PCR. Data were presented as mean ± SEM of 18 mice from three independent experiments. * P < 0.05, ** P < 0.01, *** P < 0.001.

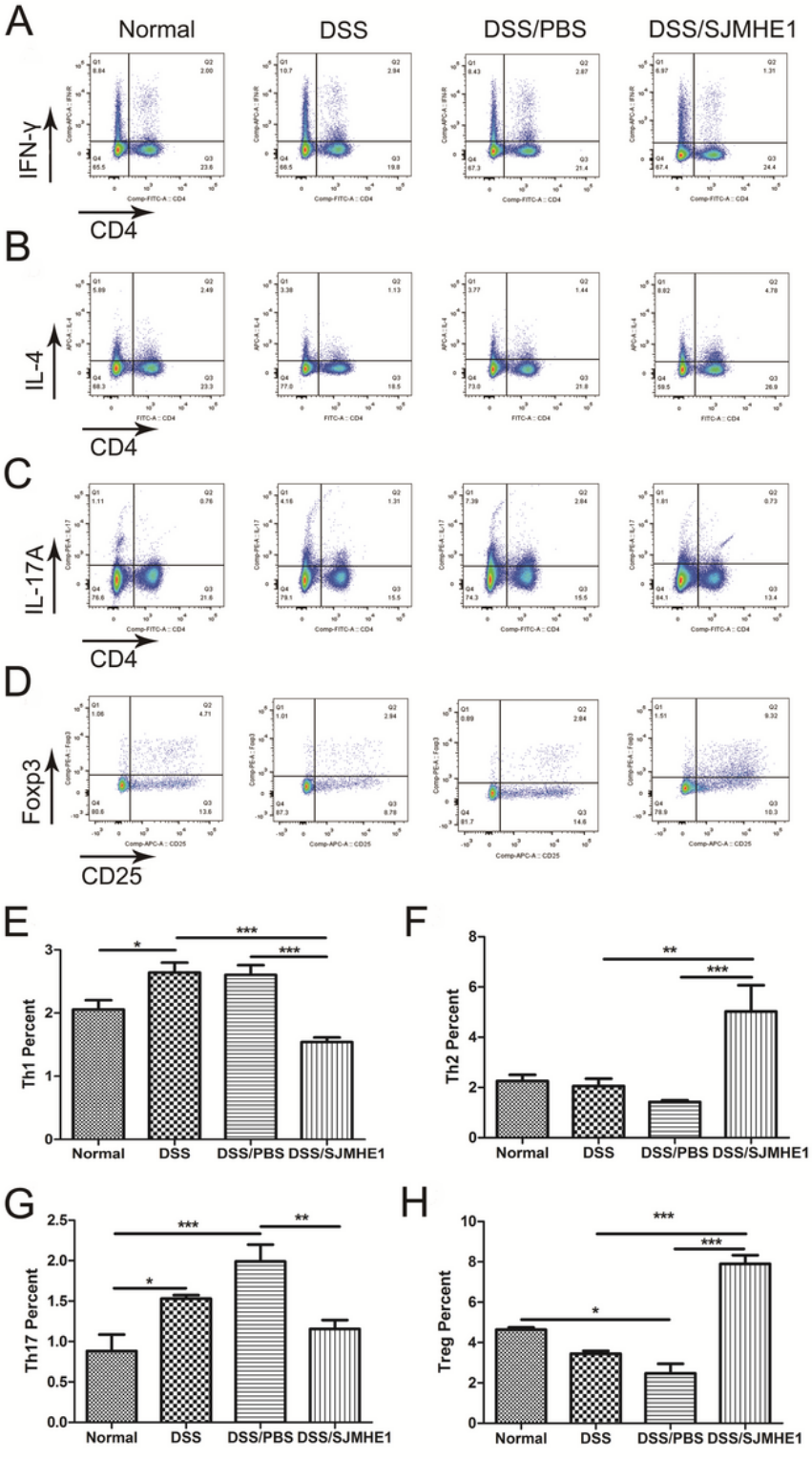

Figure 3

SJMHE1 treatment regulates Th1/Th2/Th17/Treg cell distribution from the splenocytes of mice with DSS-induced acute colitis. On day 10, the mice were killed, and splenocytes from each mouse were tested

for Th1/Th2/Th17/Treg subsets through flow cytometry. (A) CD4+IFN-γ+ Th1 cells, (B) CD4+IL4+ Th2 cells, (C) CD4+IL17+ Th17 cells, and (D) CD4+CD25+Foxp3+ Tregs in each group. Data were representative of the experiments. The percentages of (E) CD4+IFN-γ+ Th1 cells, (F) CD4+IL4+ Th2 cells, (G) CD4+IL17+ Th17 cells, and (H) CD4+CD25+Foxp3+ Tregs in each group. Results were presented as mean ± SEM of 18 mice from three independent experiments. * P < 0.05，** P < 0.01, *** P < 0.001.

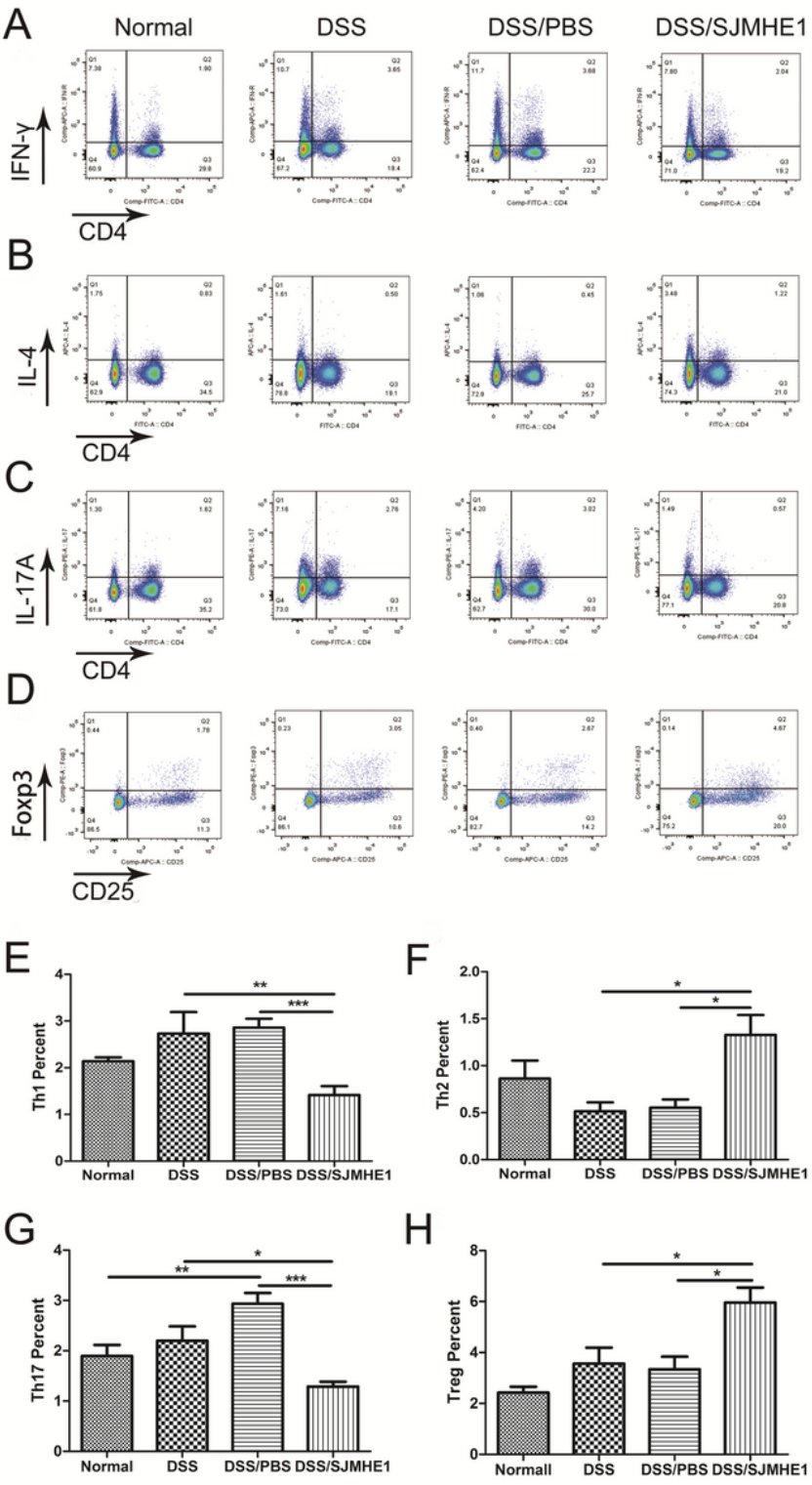

Figure 4

SJMHE1 treatment modulates the Th1/Th2/Th17/Treg cell distribution in the MLNs of mice with DSS-induced acute colitis. On day 10, the mice were killed, and the MLNs from each mouse were tested for Th1/Th2/Th17/Treg subsets through flow cytometry. (A) CD4+IFN-γ+ Th1 cells, (B) CD4+IL4+ Th2 cells, (C) CD4+IL17+ Th17 cells, and (D) CD4+CD25+Foxp3+ Tregs in each group. Data were representative of the experiments. The percentages of (E) CD4+IFN-γ+ Th1 cells, (F) CD4+IL4+ Th2 cells, (G) CD4+IL17+ Th17 cells, and (H) CD4+CD25+Foxp3+ Tregs in each group. Results were presented as mean ± SEM of 18 mice from three independent experiments. * P < 0.05，** P < 0.01, *** P < 0.001.

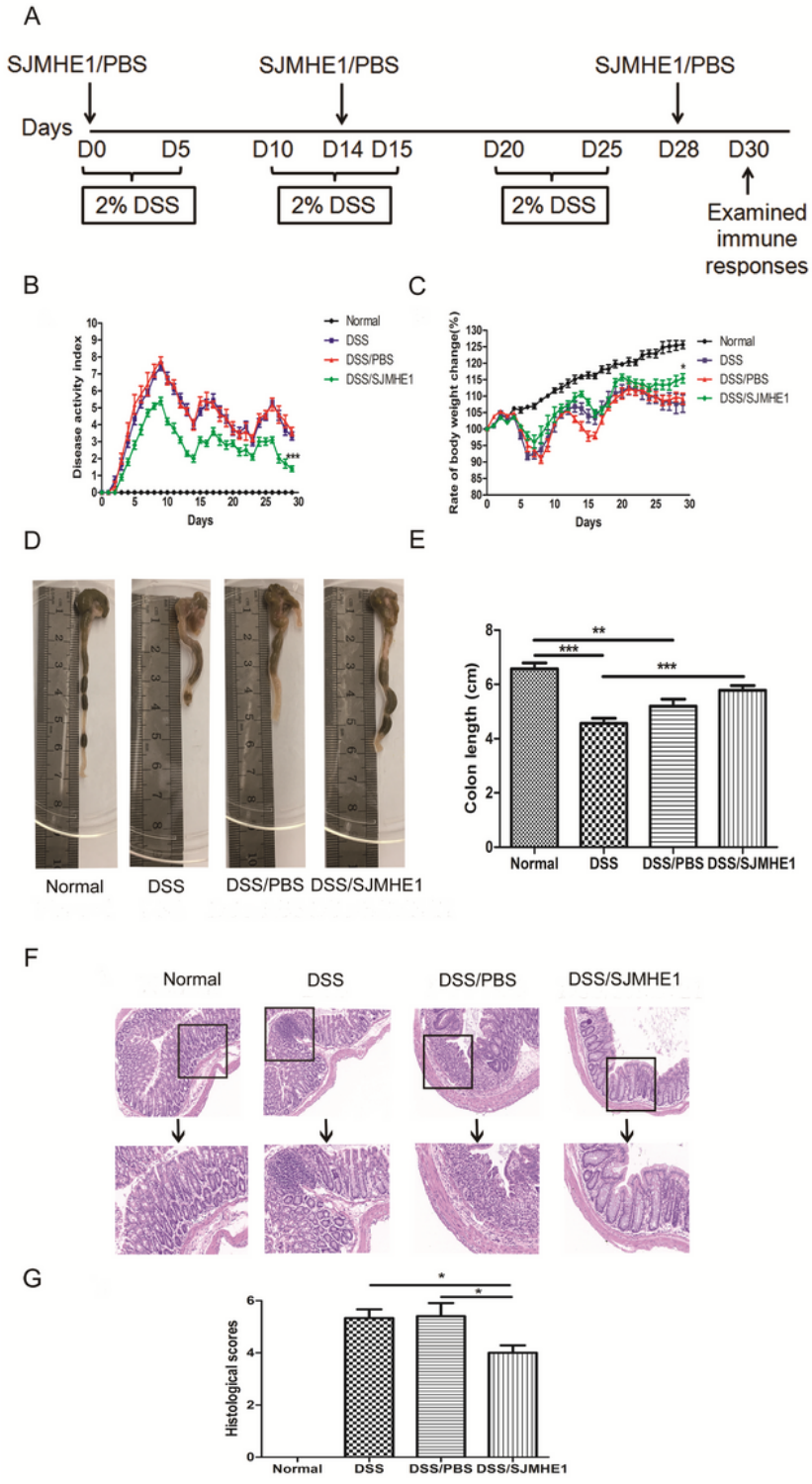

Figure 5

SJMHE1 treatment protects against the induction of chronic colitis by DSS in mice. (A) Experimental scheme. C57BL/6 mice were drunk with 2% DSS on days 0–5, 10–15, and 20–25. Mice were injected with SJMHE1 or PBS emulsified in IFA on days 0, 14, and 28. The mice were killed on day 30. (B) Disease activity index (DAI) and (C) rate of body weight change in each group. (D) Macroscopic appearance of the colon. (E) Colon length in each group. (F) Histological analysis of the colon from mice via H&E staining (20× magnification). Images were representative of three independent experiments (n = 6 mice per group). (G) Histological scores were assessed from each mouse. Data were presented as mean ± SEM (n = 18) from three independent experiments. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

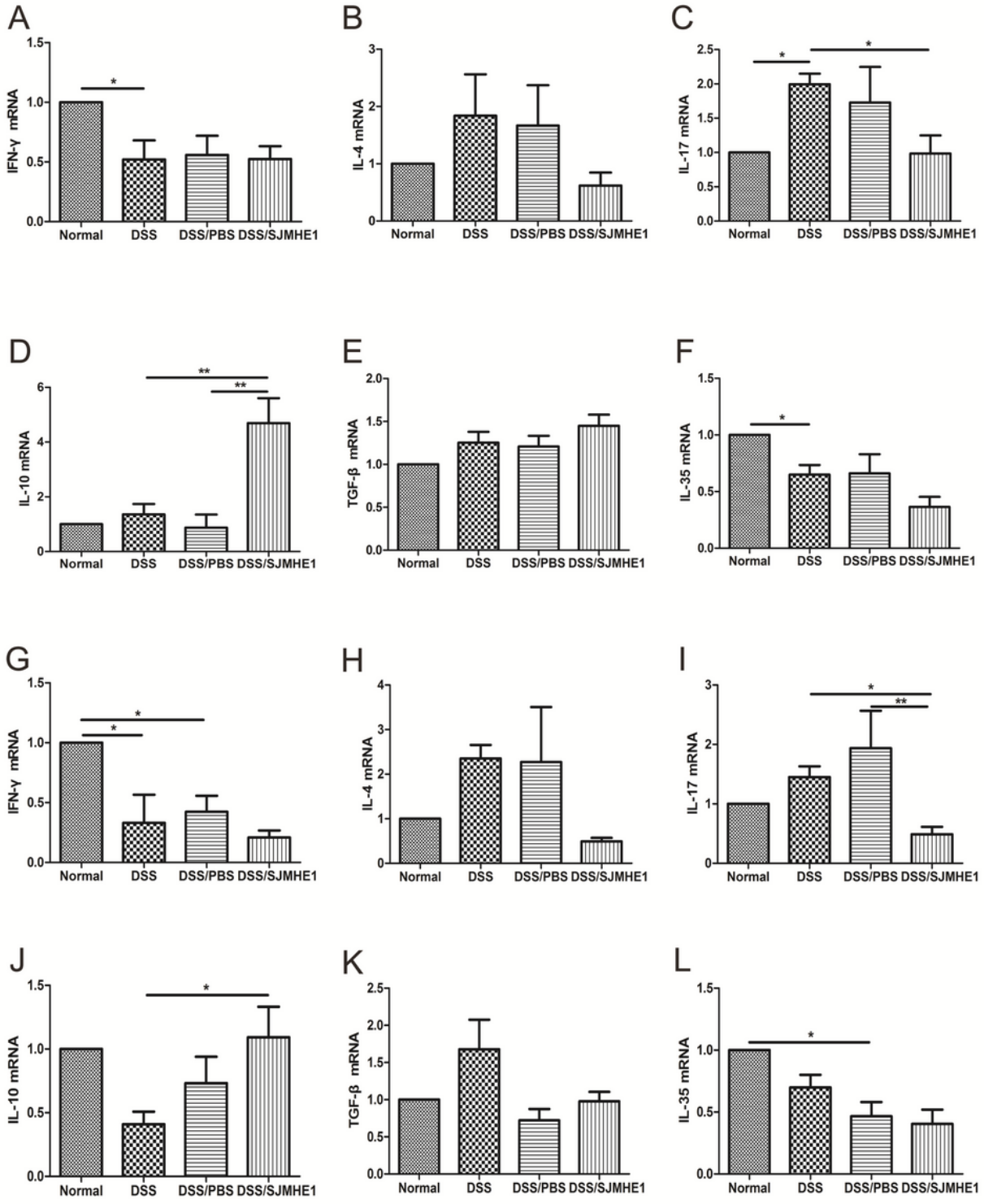

Figure 6

SJMHE1 treatment regulates the expression of cytokines from the splenocytes and MLNs of mice with DSS-induced chronic colitis. On day 30, the mice were killed, and the splenocytes (A) and (B) colon from each mouse were tested for the mRNA expression levels of IFN-γ, IL-4, IL-17, IL-10, TGF-β, and IL-35 through qRT-PCR. Data were presented as mean ± SEM of 18 mice from three independent experiments. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

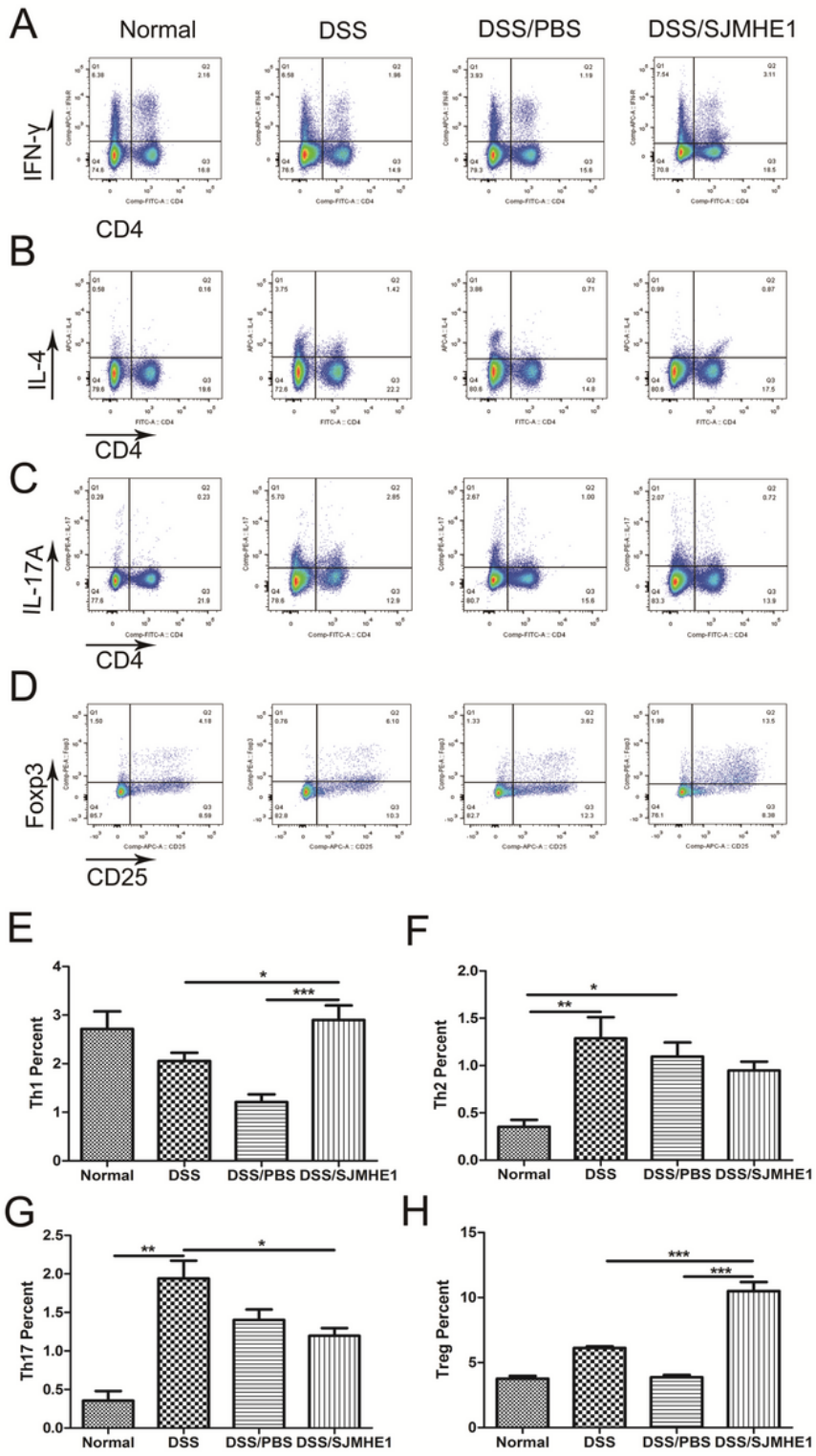

Figure 7

SJMHE1 treatment regulates Th1/Th2/Th17/Treg cell distribution from the splenocytes of mice with DSS-induced chronic colitis. On day 30, the mice were killed, and splenocytes from each mouse were tested for Th1/Th2/Th17/Treg subsets through flow cytometry. (A) CD4+IFN-γ+ Th1 cells, (B) CD4+IL4+ Th2 cells, (C) CD4+IL17+ Th17 cells, and (D) CD4+CD25+Foxp3+ Tregs in each group. Data were representative of the experiments. The percentages of (E) CD4+IFN-γ+ Th1 cells, (F) CD4+IL4+ Th2 cells,

(G) CD4+IL17+ Th17 cells, and (H) CD4+CD25+Foxp3+ Tregs in each group. Results were presented as mean ± SEM of 18 mice from three independent experiments. * P < 0.05，** P < 0.01, *** P < 0.001.

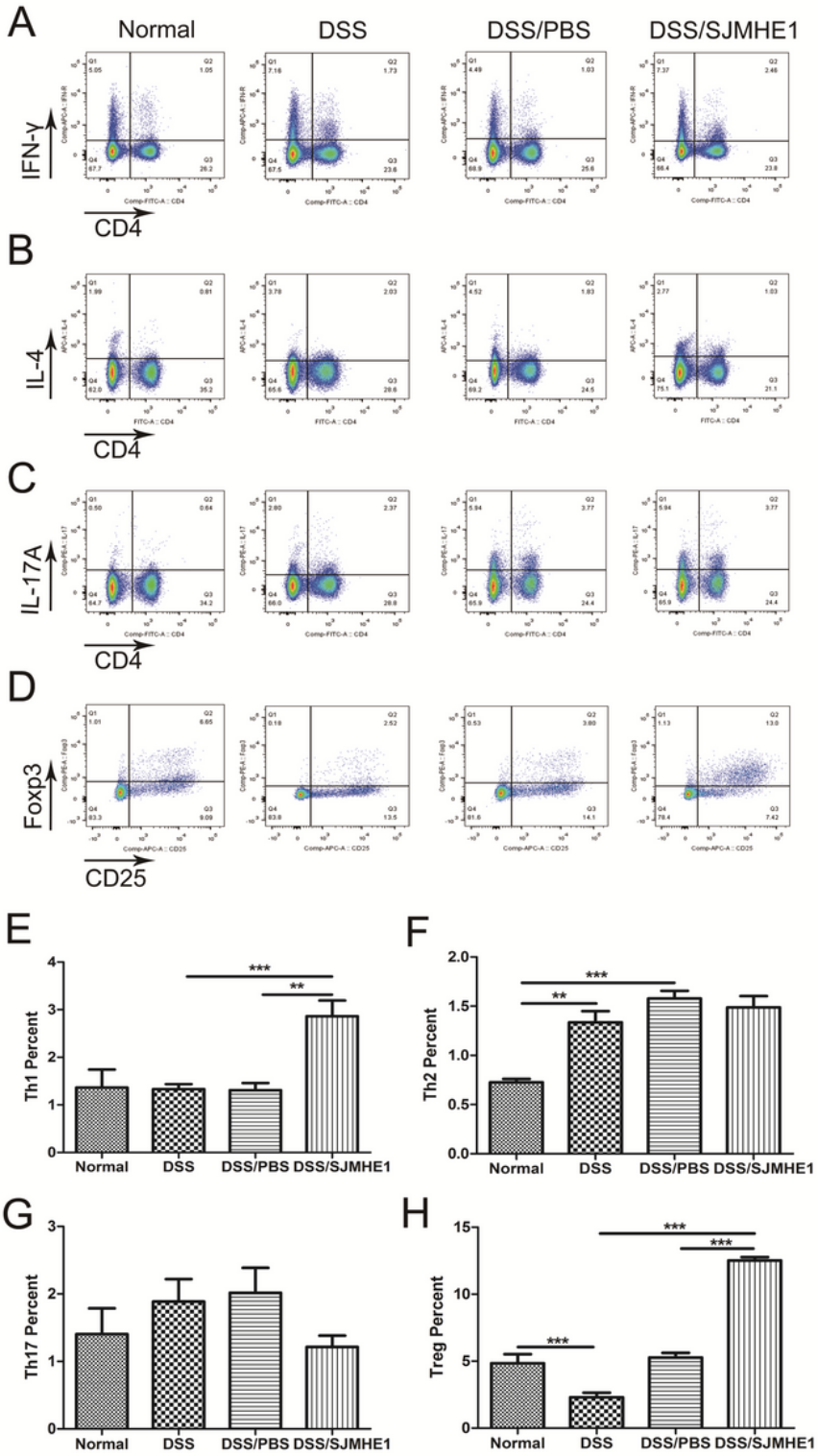

Figure 8

SJMHE1 treatment regulates Th1/Th2/Th17/Treg cell distribution from the MLNs of mice with DSS-induced acute colitis. On day 30, the mice were killed, and splenocytes from each mouse were tested for Th1/Th2/Th17/Treg subsets through flow cytometry. (A) CD4+IFN-γ+ Th1 cells, (B) CD4+IL4+ Th2 cells,

(C) CD4+IL17+ Th17 cells, and (D) CD4+CD25+Foxp3+ Tregs in each group. Data were representative of the experiments. The percentages of (E) CD4+IFN-γ+ Th1 cells, (F) CD4+IL4+ Th2 cells, (G) CD4+IL17+ Th17 cells, and (H) CD4+CD25+Foxp3+ Tregs in each group. Results were presented as mean ± SEM of 18 mice from three independent experiments. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

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