Journal of Inflammation Research

Dovepress
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ORIGINAL RESEARCH

Saccharomyces boulardii Alleviates Colitis by Regulating FXR-NLRP3 Mediated Macrophage Pyroptosis

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Background: Ulcerative colitis (UC), a typical inflammatory bowel disease (IBD), is pathologically defined by mucosal inflammation confined to the colonic mucosa. *Saccharomyces boulardii* (Sb), a commonly utilized probiotic yeast for managing digestive disorders like UC, has not been thoroughly investigated regarding its precise mechanisms for alleviating colitis. Increasing evidence indicates the involvement of FXR in UC. Meanwhile, the regulatory role of FXR on NLRP3 has garnered increasing attention. This study investigated the therapeutic effects of Sb supernatant (SbS) on colitis and elucidated the role of the FXR-NLRP3 signaling pathway in this process.

Methods: A murine model of colitis was established through administration of dextran sulfate sodium (DSS), followed by oral gavage with either SbS or the control Sabouraud dextrose broth (SDB) culture medium. The FXR activation, NLRP3 inflammasome inhibition, and macrophage pyroptosis were evaluated both in vivo and in vitro. The effects of SbS in activating FXR, suppressing NLRP3 inflammasome and alleviating the colitis were assessed.

Results: SbS ameliorated symptoms of DSS-induced colitis. Our data demonstrated that SbS elicited activation of FXR and concomitantly suppressed NLRP3 expression within the colonic tissue samples. Additionally, SbS was further observed to suppress the expression of cleaved caspase-1, a key effector protein in pyroptosis process, within the $F4/80^+$ macrophage population. Moreover, SbS modulated the expression of genes and proteins associated with pyroptosis, collectively suggesting its potential to ameliorate intestinal pyroptosis, potentially via its direct impact on macrophage. Consistently, SbS ameliorated pyroptosis of macrophages in vitro through activating FXR and inhibiting NLRP3 inflammasome. However, the therapeutic effect of SbS was reversed by the FXR inhibitor, guggulsterone, resulting in increased levels of pyroptosis-related proteins.

Conclusion: SbS demonstrated a beneficial effect in alleviating UC by modulating the FXR-NLRP3 signaling pathway, thereby mitigating macrophage pyroptosis. This discovery presents new insights into effects of Sb on alleviating UC.

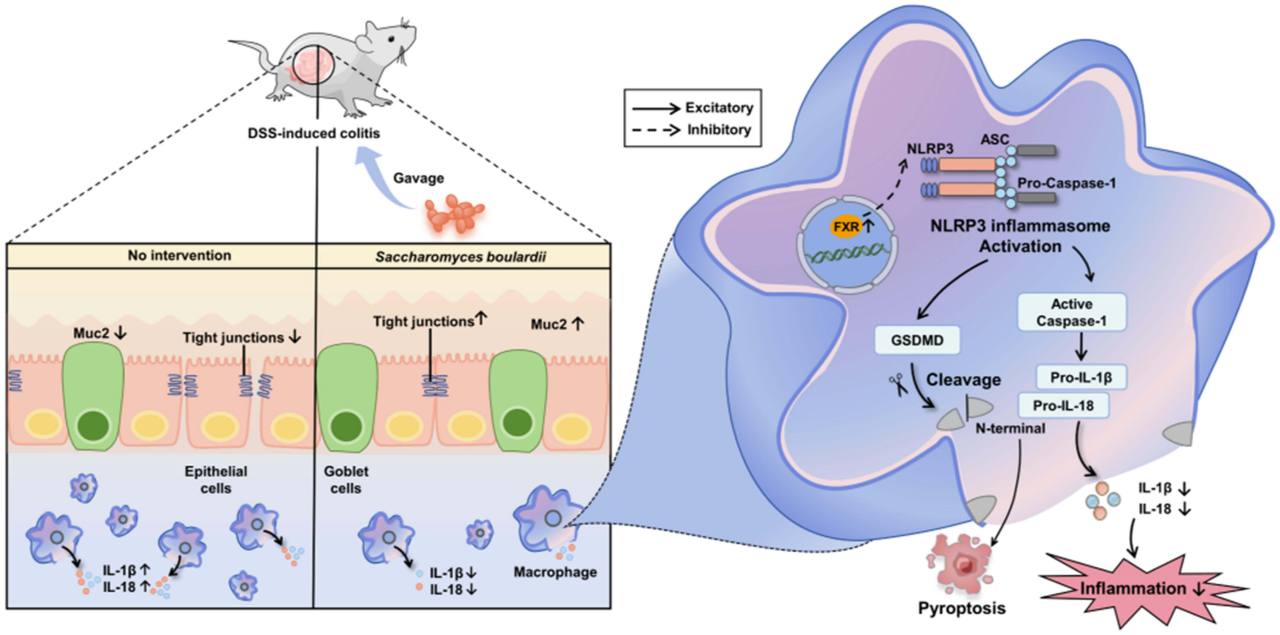
Keywords: *Saccharomyces boulardii*, Farnesoid X receptor, NLRP3 inflammasome, macrophages, pyroptosis, colitis

Introduction

Ulcerative colitis (UC), a quintessential inflammatory bowel disease (IBD), is characterized by mucosal inflammation localized to the mucosal layer, giving rise to a spectrum of clinical manifestations including abdominal pain, diarrhea, and/or tenesmus.[1] In recent years, there has been a surge in the global prevalence and incidence of UC. Nevertheless, the pathogenesis of UC remains poorly understood.[2] Several studies have suggested that immune system abnormalities, environmental implications, dysbiosis, genetic predisposition, and mucosal barrier dysfunction all contribute to UC development.[3,4] Despite the myriad pathogenic features associated with UC, managing UC poses considerable therapeutic challenges, particularly due to the limitations of conventional therapies, including high costs, tolerability issues,

Received: 20 November 2024
Accepted: 22 February 2025
Published: 4 March 2025

Graphical Abstract



toxicity, susceptibility to serious infections, and malignancy.[5,6] Therefore, innovative therapeutic strategies against UC are urgently needed.

Farnesoid X receptor (FXR) plays a crucial role in controlling immunological modulation, barrier function, and inflammatory responses.[7,8] Increasing evidence indicates the involvement of FXR in IBD. FXR activation could decrease intestinal permeability, intestinal inflammation, and cell permeability while preventing the loss of goblet cells.[8] Furthermore, the stimulation of FXR by its agonist exhibited protective effects in mice against deoxycholic acid-induced intestinal damage through inhibiting epithelial destruction and mucosal inflammatory cell infiltration.[9] Persuasive research indicated that FXR negatively influenced the activation of NOD-, LRR- and pyrin domain-containing 3 (NLRP3),[10,11] a key player in the pathogenesis of IBD and an important driver of intestinal homeostasis.[12] It has been reported that FXR plays a crucial role in sepsis by regulating the NLRP3 inflammasome.[10] It was observed that FXR negatively regulated the NLRP3 inflammasome, while FXR ligands like chenodeoxycholic acid and deoxycholic acid promoted NLRP3 activation. To determine whether FXR directly interacts with the NLRP3 inflammasome to inhibit its activation, experiments were conducted using FXR transfection and FXR knockdown models. The results indicated that FXR might physically interact with either NLRP3 or caspase-1, preventing the assembly of NLRP3 inflammasome components and consequently inhibiting its activation. Another study found that FXR inhibited NLRP3 activation through the PERK-CHOP pathway, which improved liver injury.[11] Activation of NLRP3 inflammasome promotes the maturation and secretion of IL-1β and IL-18, as well as the cleavage of gasdermin D (GSDMD), leading to pyroptosis, a type of programmed cell death.[13] Therefore, it is hypothesized that FXR-NLRP3 pathway might be involved in the development of UC.

Researchers have established the significance of gut microbiota in maintaining host health and influencing IBD development.[14] The fungus community, an essential component of the mammalian microbiota, plays a vital role in IBD.[15] *Saccharomyces boulardii* (Sb), a probiotic yeast extracted from litchi and mangostema, can be extremely tolerant of bile acids and resistant to low pH.[16] It is a unique species within the *Saccharomyces* genus, with a karyotype similar to that of the model yeast *Saccharomyces cerevisiae* (*S. cerevisiae*).[17] However, Sb differs from *S. cerevisiae* both physiologically and metabolically, notably by its inability to utilize galactose as a carbon source.[18] Unlike

S. cerevisiae, Sb has an optimal growth temperature of 37°C. In addition, it can withstand degradation by hydrolases and bile salts.[19] These characteristics enhance its survival in the gastrointestinal tract. To date, Sb is the only fungal probiotic and is widely applied in the treatment of gastrointestinal diseases,[20] serving as an adjuvant therapy for IBD. It exerts its effects through various mechanisms, including restoring normal microbiota,[21] producing antimicrobial substances,[22,23] interacting with pathogens,[24–26] and modulating immune responses.[27,28] Previous studies have shown that Sb could enhance gut barrier integrity and reduce levels of pro-inflammatory factors.[29,30] Additionally, Sb can sequester T helper cells in mesenteric lymph nodes, thereby mitigating inflammation.[28] Research by Bei Li et al demonstrated that Sb altered gut microbiota composition and promoted the synthesis of microbial metabolites.[31] Sb increased short-chain fatty acid (SCFA) levels, particularly butyrate, which lowered the incidence of diarrhea in patients receiving enteral nutrition.[32] Furthermore, studies have demonstrated that Sb treatment can reduce the incidence of relapses and improve symptoms in IBD patients who achieved more significant remissions during maintenance.[33,34] These findings highlight the potential of Sb as a promising treatment for UC.

Despite the promising pharmacological effects of Sb, the mechanism by which Sb alleviates UC remains poorly understood. Hence, the current study aimed at assessing the therapeutic properties of Sb and uncovering its precise mechanism.

Methods and Materials

Sb Cultivation and Supernatant Preparation

Lyophilized Sb CNCM-745 (Biocodex, France) was cultured in sterile Sabouraud dextrose broth (SDB) (0.1 g/10 mL) (Solarbio, China) for 24h at 33°C, 180 r/min, subculturing about 3 times. Then, it was centrifuged at 9000g at 4°C for 15 min for supernatant preparation (SbS). Supernatant was extracted and filtrated with 0.22-μm filters.

Cell Culture and Treatment

The human monocyte line THP-1 and the rat macrophage-like cell line RAW 264.7 were obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA). THP-1 cells were cultured in RPMI 1640 medium including 10% fetal bovine serum (FBS), 100 U/mL penicillin and streptomycin and should be administrated with 100nM phorbol 12-myristate 13-acetate (PMA) for 24~48 h to develop into macrophage-like cells at 37 °C under 5% CO_2. RAW 264.7 cells were cultivated in Dulbecco Minimum Essential Medium (DMEM) complemented with 10% FBS with 5% CO_2 at 37 °C. The cells were starved for 12~16 hours after reaching 60–70% confluency on each well of the plates. The starved cells were administered with a twofold dilution of SbS and SDB for 24h to test the effect of SbS on activating FXR, according to the previous study.[35] Additionally, to test the mechanism of SbS improving colitis, THP-1 cells were treated with FXR inhibitor guggulsterone (GS) (20 μM) (MCE, USA) for 18 h before being treated with SbS, SDB, LPS and nigericin. The levels of mRNA and protein expression were evaluated after all interventions were finished.

Animals Study

Seven-week-old female C57BL/6J mice, purchased from the Beijing Animal Research Center, China, were maintained in a specific pathogen-free (SPF) environment with a natural 12–12-h light/dark cycle with one week of adaptive feeding. All the mice were randomly assigned to one of the four groups: control, DSS, DSS + SDB, and DSS + SbS (n = 5~7 per group). The sample sizes for each group were determined based on prior studies.[36] Mice of control group were simply given sterile water. Mice of DSS group were continuously treated with 2.5% (w/v) DSS in sterile water for 7 days to trigger colitis. Mice in the DSS + SDB and DSS + SbS groups were given 400 μL SDB or SbS once daily for 14 days before and during DSS treatment, according to the previous study.[37] The experimental process was shown in Figure 1A. Disease activity index (DAI) was blindly observed during DSS treatment according to previous research.[38] The observers assessing the DAI were blinded to the treatment groups of the mice. Mice were labeled with codes, and the code was not revealed until after all DAI scores were recorded. After that, mice were sacrificed through cervical dislocation. The colonic tissue and feces were collected and frozen at −80°C for further assessment. This study and included experimental procedures were evaluated and approved by the Animal Ethical and Welfare Committee of Tianjin Medical University,

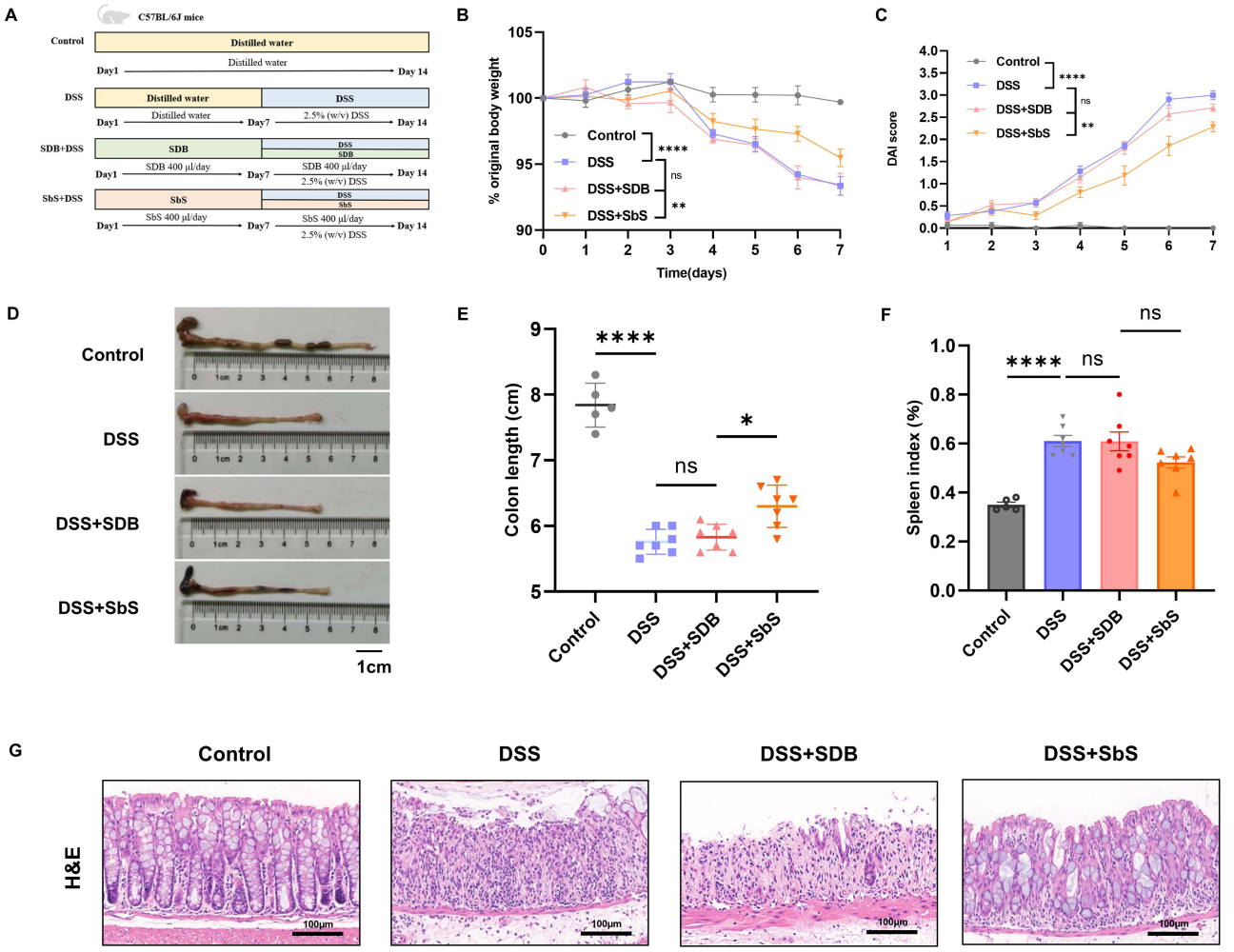


Figure 1 Effect of SbS on pathological symptoms of DSS-induced colitis. (**A**) Experimental process. (**B**) Percentage changes of body weight. (**C**) DAI score. (**D and E**) Colon photograph (**D**), Colon length (**E**) was measured. (**F**) The weights of spleen in different groups were recorded. (**G**) H&E staining of the colon (scale bar: 100 μm). All data were presented as mean±SEM. *P < 0.05, **P < 0.01, ****P<0.0001. ns, not significant.

Tianjin, China. Additionally, all procedures adhered to the National Institutes of Health guidelines for the care and use of laboratory animals.

Histological Assessment and Immunohistochemistry

Mice distal colon specimens were paraffin-embedded before being cut into 4 μm slices. H&E staining was utilized to assess the histology of intestine following deparaffinization and hydration. Then, the slices were observed under an optical microscope.

After deparaffinization and dehydration, the tissue sections were incubated for an overnight period at 4 °C with primary antibodies to MUC2 (Santa Cruz Biotechnology, Inc. Santa Cruz, USA). After 30 minutes of room temperature incubation with the secondary antibody, slices were counterstained with hematoxylin.

Immunofluorescence Staining

Fibroblast Growth Factor 15 (FGF 15) (ab229630, Abcam) in colon tissue was determined by immunofluorescent staining. Briefly, primary antibodies were attached to the sections and incubated at 4 ° C overnight after the fixation and embedding steps. After three PBS washes, slices were incubated with fluorochrome-conjugated secondary

antibodies. Eventually, 40.6-diamidino-2-phenylindole (DAPI) was added to slices for counterstaining the nuclear. A fluorescent microscope was used to view and photograph FGF15 fluorescence pictures (Lycra, USA).

Cleaved caspase-1 (c-caspase-1) in colon tissue was determined by double immunofluorescent staining. Briefly, primary antibodies: rabbit anti-cleaved-caspase1 antibody (1:100, #89332, Cell Signaling Technology) was applied to the sections and incubated overnight at 4 ° C after the fixation and embedding steps, while the macrophages received labeling with F4/80 in mice. Following PBST cleaning, DAPI was used to mount the slides, and a fluorescent microscope was utilized to view and capture the images.

Real-Time PCR Analysis

The total RNA of colon tissues and cell samples was extracted by using the RNeasy mini kit (Qiagen, USA). Then, cDNA was synthesized from collected RNA using the TIANScript RT Kit (TIANGEN, China) based on the manufacturer's guidelines. RT-PCR analysis was performed through primers (GENEWIZ, China) and TaqMan Gene Expression Master Mix. Relative mRNA expression of the gene was determined through $2^{-\Delta\Delta CT}$ method and adjusted to GAPDH levels. The primer sequences are listed in Table 1.

Table 1 Sequences of Primers Used for RT-qPCR

Gene		Gene Sequence
m-GAPDH	Forward primer Reverse primer	GGAGAAACCTGCCAAGTATG TGGGAGTTGCTGTTGAAGTC
m-IL-1β	Forward primer Reverse primer	ACGGACCCCAAAAGATGAAG TTCTCCACAGCCACAATGAG
m-IL-18	Forward primer Reverse primer	GCCTCAAACCTTCCAAATCAC GTTGTCTGATTCCAGGTCTCC
m-IL-6	Forward primer Reverse primer	CCAGTTGCCTTCTTGGGACT GGTCTGTTGGGAGTGGTATCC
m-TNF-α	Forward primer Reverse primer	CTTCTGTCTACTGAACTTCGGG CAGGCTTGTCACTCGAATTTTG
m-FXR	Forward primer Reverse primer	GGACGGGATGAGTGTGAAG TGAACTTGAGGAAACGGGAC
m-ZO-1	Forward primer Reverse primer	GGGCCATCTCAACTCCTGTA AGAAGGGCTGACGGGTAAAT
m-NLRP-3	Forward primer Reverse primer	ATTACCCGCCCGAGAAAGG TCGCAGCAAAGATCCACACAG
m-ASC	Forward primer Reverse primer	GGAGGGGTATGGCTTGGAG TGAGTGCTTGCCTGTGTTGGT
m-IFN-γ	Forward primer Reverse primer	GGAGGAACTGGCAAAAGGATGG CAGGTGTGATTCAATGACGCTTATG
m-Claudin-3 h-GAPDH	Forward primer Reverse primer Forward primer Reverse primer	CCTGTGGATGAACTGCGTG GTAGTCCTTGCGGTCGTAG CCCTTCATTGACCTCAACTACATGG CATGGTGGTGAAGACGCCAG
h-IL-1β	Forward primer Reverse primer	GCGGCCAGGATATAACTGACTTC TCCACATTCAGCACAGGACTCTC
h-IL-18	Forward primer Reverse primer	GATATGACTGATTCTGACTGTAG TACCTCTAGGCTGGCTAT

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Western Blot Analysis

Proteins from intestinal tissues and cells were isolated through using RIPA lysis buffer (containing PMSF). Proteins were added to the SDS gel electrophoresis system for electrophoresing to get separated proteins. Then, they were transferred to PVDF membrane (Invitrogen, USA). After being blocked with 10% skim milk or bovine serum albumin, membranes comprising proteins were incubated with primary anti-β-actin antibody (Cat#mAb3700, CST, 1:1000), anti-ZO-1 (Cat#A0659, ABclonal, 1:1000), anti-Claudin3 (Cat#83609, CST, 1:1000) antibodies, primary anti-FXR antibody (abs122163, Absin, 1:1200), anti-FGF15 (sc514647, Santa Cruze Biotechnology, 1:1000), anti-caspase-1 antibody (ab179515, Abcam, 1:1000), anti-GSDMD antibody (1:1000, ab209845, Abcam), anti-IL-1β antibody (#12242, Cell Signaling Technology,1:1000), and anti-IL-18 antibody (ab71495, Abcam, 1:1000) overnight at 4 °C. Subsequently, horseradish peroxidase-conjugated secondary antibodies were used to incubate. A chemiluminescence kit was used to detect protein band intensities and images were processed using Image J software.

Statistical Analysis

Data were expressed as mean ± SEM. Data normality was assessed using the Shapiro–Wilk test. *T*-test was conducted to compare differences between the two groups for normally distributed data. For multiple comparisons, a one-way analysis of variance (ANOVA) was performed. P value < 0.05 was considered to be statistically significant. All tests were analyzed using GraphPad Prism 8.0 software (San Diego, CA, USA).

Results

SbS Treatment Ameliorated Symptoms of DSS-Induced Colitis

Mice treated with DSS or SDB were observed to exhibit weight loss over the last four days (Figure 1B), in contrast to those in the SbS group. Compared with the DSS group, considerably reduced DAI scores were observed in mice receiving SbS (Figure 1C), along with longer colon lengths (Figure 1D and E) and a decrease in spleen index (Figure 1F), as opposed to the DSS+SDB group. Moreover, histopathological analysis was conducted to assess the degree of intestinal injury and inflammation. Substantial damage was identified in the colitis mice compared to the control group, but inflammation was effectively ameliorated by SbS rather than SDB (Figure 1G). Collectively, these findings validated the therapeutic effects of SbS on DSS-induced colitis.

SbS Treatment Alleviated Inflammatory Responses and Preserved the Gut Barrier in DSS-Induced Mice

To assess the intensity of inflammatory response in DSS-induced mice, the levels of IL-6, TNF-α, and IFN-γ in colon tissue were quantified by RT-PCR analysis. Compared with the control group, the expressions of IL-6, TNF-α, and IFN-γ in mice exposed to DSS were strikingly increased, as well as in the DSS+SDB group (Figure 2A-C). However, the mRNA level of these pro-inflammatory cytokines significantly decreased following treatment with SbS. Tight junction proteins, such as ZO-1 and Claudin-3 are critical for preserving the gut barrier. Following SbS administration, the expression of ZO-1 (Figure 2D) and Claudin-3 (Figure 2E) at the gene level was markedly upregulated. Similar results were obtained via Western blot analysis, where increased protein levels of ZO-1 and Claudin-3 were observed after SbS treatment compared to the DSS group, while no significant changes were detected with SDB treatment (Figure 2F and G). MUC-2 protein, a mucin-associated protein essential for maintaining intestinal barrier function, was significantly increased in the number of MUC2-positive cells within each colonic crypt in the DSS+SbS group compared to both the DSS and DSS+SDB groups, as revealed by immunohistochemistry staining (Figure 2H). Overall, SbS exhibited effects on suppressing inflammatory responses and upholding the integrity of gut barrier in colitis.

SbS Treatment Activated FXR and Inhibited NLRP3 Expression in Colon Tissue

Growing evidence suggested a potential association between FXR and IBD.[39,40] Therefore, FXR expression in colon tissue was tested. A significant reduction in the mRNA levels of FXR following DSS or SDB administration was observed using RT-PCR, whereas an upregulation in FXR mRNA expression was detected after SbS treatment (Figure 3A). Consistently,

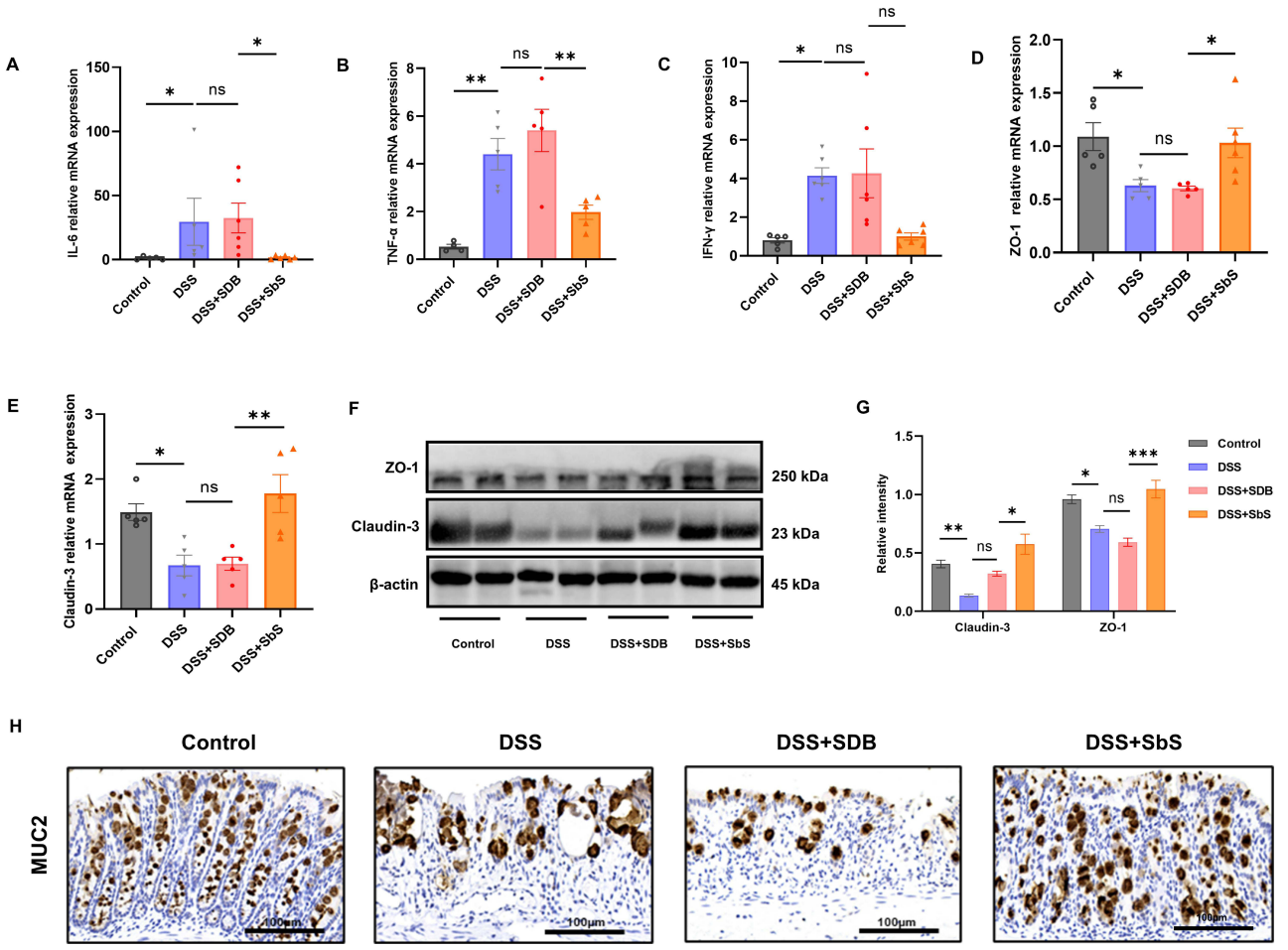

Figure 2 Inhibition of SbS administration on colonic inflammation and gut barrier destruction in DSS-induced mice. (**A-C**) The mRNA level of pro-inflammatory cytokines (IL-6, TNF-α, IFN-γ) in the colon tissue was detected by RT-PCR. (**D and E**) The expression of the intestinal tight junction proteins including ZO-1 and Claudin-3 were measured by RT-PCR. (**F and G**) Western blot was performed to measure the expression of Claudin-3 and ZO-1 in colon tissue, and the relative intensity was quantified by Image J software. (**H**) Immunohistochemical analysis of MUC-2 (scale bar: 100 μm). All data were presented as mean ±SEM. *P < 0.05, **P < 0.01, ***P < 0.001. ns, not significant.

the protein level of FXR was found to exhibit similar results (Figure 3D and E). Western blot results suggested that FGF 15, the target gene of FXR, increased after SbS administration, compared with the DSS and DSS+SDB group, indicating the activation of FXR by SbS (Figure 3D and E). Furthermore, immunofluorescence staining for FGF15 further confirmed these findings (Figure 3C). Hao et al found that the FXR agonist could exert anti-inflammatory effects by inhibiting the NLRP3 inflammasome signaling pathway.[10] Therefore, the expression of NLRP3 in colon tissue was tested. In mice exposed to DSS, a considerable rise in NLRP3 mRNA and protein levels was observed, which was subsequently downregulated following SbS administration but not after SDB treatment (Figure 3B, D, E). These findings suggested that SbS might alleviate colitis through the FXR-NLRP3 signaling pathway.

SbS Treatment Improved Pyroptosis of Intestinal Macrophages in Mice

Activation of NLRP3 inflammasome could activate protease caspase-1, causing gasdermin D-dependent pyroptosis.[41] Therefore, to determine whether pyroptosis occurred in intestinal macrophages or intestinal epithelial cells, we further investigated c-caspase-1, a key effector protein in pyroptosis process. In the lamina propria of the inflamed mucosa, an increase in c-caspase-1 expression was observed in $F4/80^{+}$ macrophages in the DSS and DSS+SDB groups, while a significant reduction in c-caspase-1 expression was detected following SbS treatment (Figure 4). Subsequently, the mRNA levels of ASC, IL-18, and IL-1β in colon tissue were examined. The mRNA expression of ASC was down-regulated after SbS administration (Figure 5A). Compared with the DSS group, a decrease in the expression of IL-1β and

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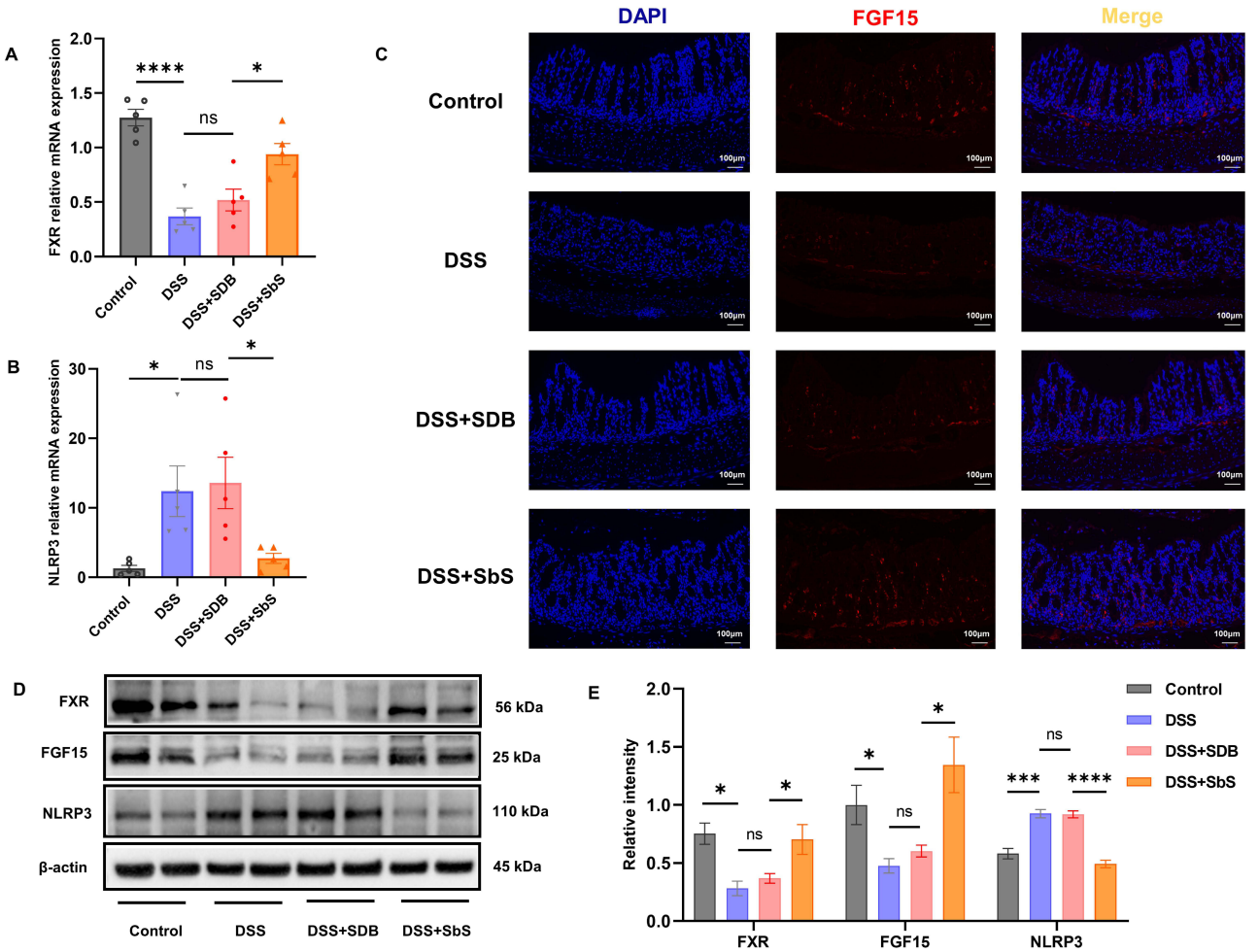

Figure 3 SbS administration activated FXR and inhibited NLRP3 expression in DSS-induced mice. (**A and B**) The mRNA levels of FXR and NLRP3 in the colon tissue were detected by RT-PCR. (**C**) The expression of colonic FGF15 was assessed by Immunofluorescence staining and visualized by fluorescence microscopy (red), the nucleus was counterstained with DAPI (blue) (scale bars: 100 μm). (**D and E**) Western blot analysis was applied to measure the expression of FXR, FGF15 and NLRP3, and the relative intensity was quantified by Image J software. All data were presented as mean ±SEM. *P < 0.05, ***P < 0.001, ****P<0.0001. ns, not significant.

IL-18, which were pro-inflammatory factors caused by pyroptosis, was observed following SbS treatment (Figure 5B and C). These findings were further confirmed at protein levels, where the expression of c-caspase-1, NT-GSDMD, and cleaved IL-1β and IL-18 examined by Western blot were upregulated in DSS group (Figure 5D-F). In contrast, a striking reduction in the expression of c-caspase-1, NT-GSDMD, and cleaved IL-1β and IL-18 was detected in mice treated with SbS compared with the DSS or DSS+SDB groups. Together, these results suggested that pyroptosis played an important role in colitis, potentially through its impact on macrophages, and SbS treatment could ameliorate intestinal macrophage pyroptosis.

SbS Treatment Improved Pyroptosis of Macrophages in Vitro

Having observed the augmentation of colonic FXR expression with SbS treatment, we proceeded to assess the protein levels of FXR in macrophages. Both THP-1 and RAW 264.7 cells exhibited elevated protein expression of FXR following SbS treatment (Figure 6A-D). Given that RAW 264.7 cells do not express ASC, a key requirement for NLRP3 inflammasome activation,[42] THP-1 cells were selected for subsequent experiments. To further investigate the role of macrophages in inflammation and the anti-inflammatory mechanism of SbS, the expression of FXR, NLRP3, and pyroptosis-related genes and proteins in THP-1 cells was scrutinized. Notably, increased mRNA levels of IL-1β and IL-18 were observed in THP-1 cells treated with LPS+nigericin (Figure 6E and F). In contrast, decreased expression of IL-1β and IL-18 at the gene level was observed following SbS administration. The protein levels of FXR, NLRP3, c-caspase-1, NT-GSDMD, and cleaved IL-1β and IL-18 were further

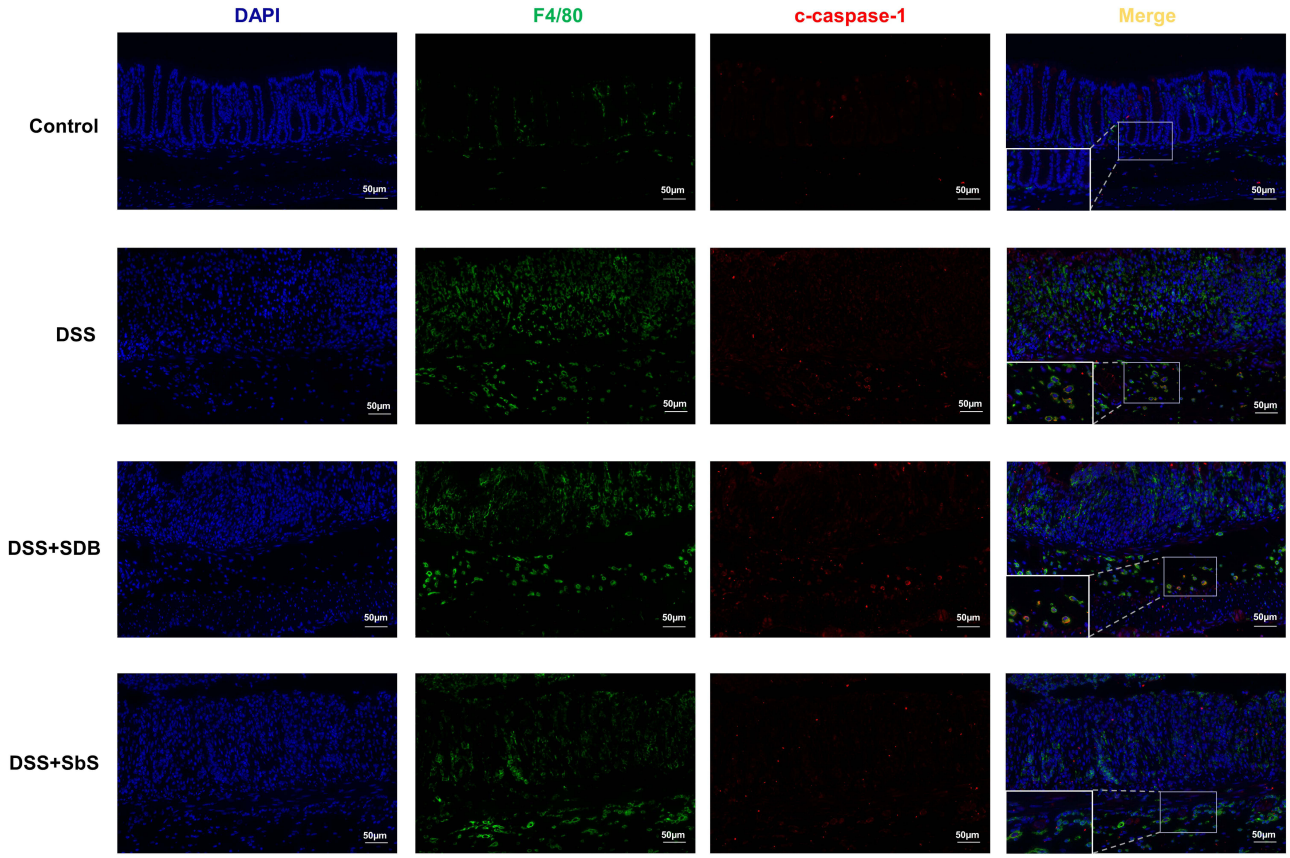

Figure 4 SbS administration decreased the expression of cleaved-caspase-1 of macrophages in colon tissue. Mice colon tissues were immunofluorescently labeled for the cell nucleus (blue), F4/80 (green), cleaved-caspase1 (red), and merge.

examined. The results indicated that NLRP3, c-caspase-1, NT-GSDMD, and cleaved IL-1β and IL-18 were upregulated, while FXR was downregulated at the protein level following LPS+nigericin treatment. However, SbS treatment led to an upregulation of FXR and a downregulation of NLRP3 and pyroptosis-related proteins expression (Figure 6G-J). The above evidence further confirmed that SbS could ameliorate pyroptosis in macrophages, which may be through the FXR-NLRP3 signaling pathway.

FXR Inhibitor Reversed the Therapeutic Effect of SbS

To further investigate the potential involvement of the FXR-NLRP3 signaling pathway in the therapeutic impact of SbS, THP-1 cells were pre-treated with GS, an FXR inhibitor. Compared with the LPS+nigericin and LPS+nigericin+SDB groups, reduced protein levels of NLRP3, c-caspase-1, NT-GSDMD, and cleaved IL-1β and IL-18 were observed following SbS treatment in THP-1 cells. In contrast, the therapeutic effect of SbS was found to be abolished by GS (Figure 7A-C). Increased expression of NLRP3, c-caspase-1, NT-GSDMD, and cleaved IL-1β and IL-18 at the protein level was detected following GS treatment. Therefore, FXR could negatively influence the expression of NLRP3 and indicators in the pyroptosis pathway while the therapeutic effects of SbS could be counteracted by GS.

Discussion

In this study, we investigate the precise mechanism through which Sb can alleviate UC in both vitro and vivo models. Activation of FXR induced by SbS resulted in a reduction in the expression of NLRP3 and pyroptosis-associated genes and proteins, leading to decreased pyroptotic cell death in macrophages and a mitigated inflammatory response. This discovery identified Sb as a novel regulator of intestinal inflammation and highlighted FXR as a promising target, offering new insights into the precise prevention and treatment of UC.

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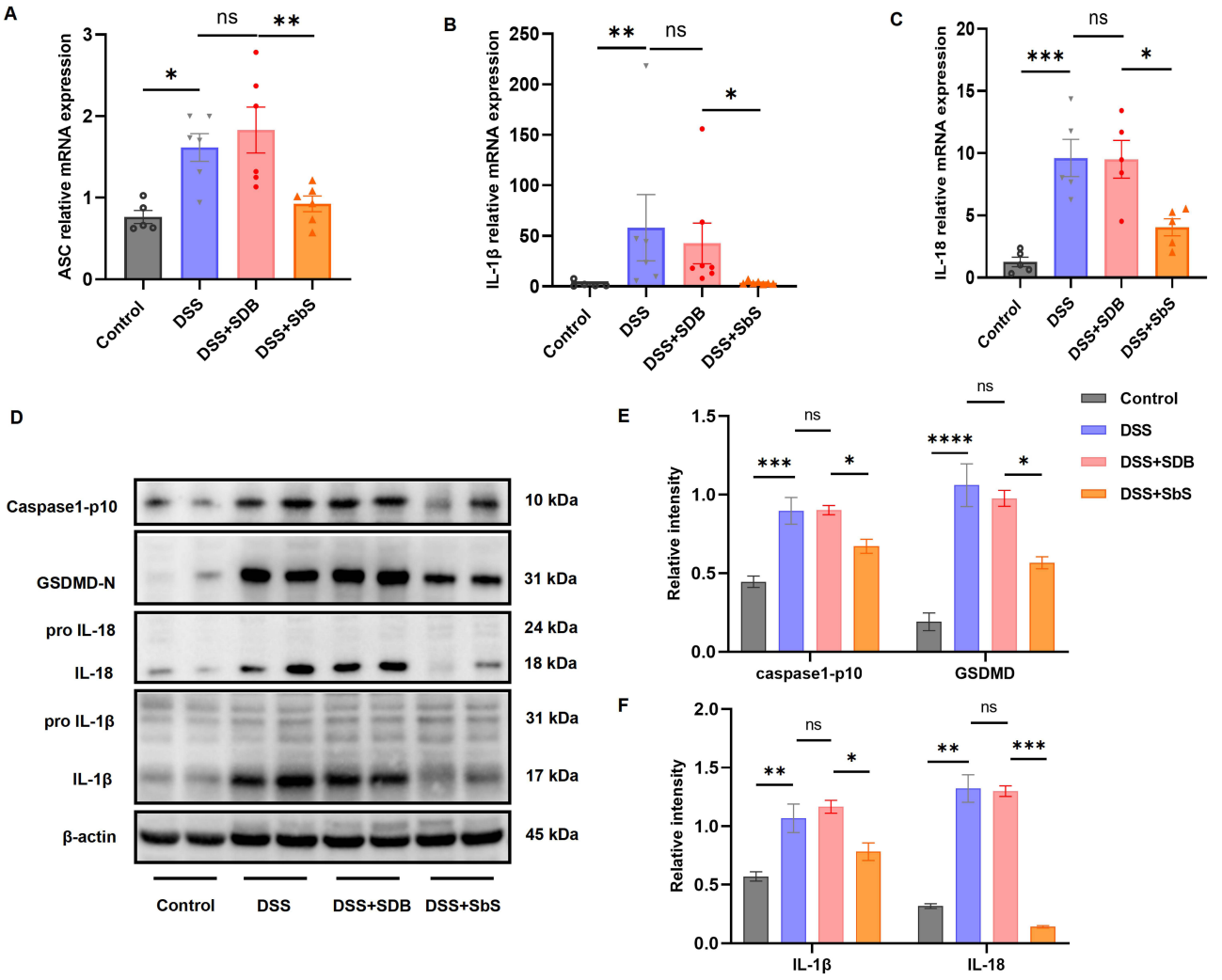

Figure 5 SbS administration reduced the expression of pyroptosis-related genes and proteins in colon tissue. (**A-C**) The mRNA expressions of ASC (**A**), IL-1β (**B**), IL-18 (**C**) were detected by RT-PCR. (**D-F**) The protein levels of caspase-1, GSDMD, IL-1β and IL-18 in the pyroptosis pathway were measured by Western blot and the relative intensity was quantified by Image J software. All data were presented as mean ±SEM. *P < 0.05, **P < 0.01, ***P < 0.001, ****P<0.0001. ns, not significant.

To date, conventional pharmacological therapies for UC have been accompanied by adverse reactions, such as tolerance, toxicity, serious infections, and malignancy.[5,6] Consequently, research efforts have shifted towards alternative colitis therapies. Probiotic therapy, including the use of Sb, has emerged as a viable option for IBD patients. In this study, SbS decreased the DAI score while increasing the body weight and colon length in mice exposed to DSS, indicating that SbS had a therapeutic effect on colitis symptoms. Dysfunction of the mucosal barrier consisting of epithelial tight junctions and a mucus layer results in bacterial translocation and inflammatory reactions.[3,43] Claudin-3 and ZO-1, tight-junction proteins, preserve intestinal epithelial integrity while many mucins like MUC-2 generated by colonic goblet cells form the thick mucus barrier.[44] Our study demonstrated that SbS could reduce pro-inflammatory factors and augment the expression of Claudin-3 and ZO-1 at gene and protein levels. Additionally, mice exposed to SbS exhibited a higher number of MUC-2 positive cells in each colonic crypt compared with other groups.

The inflammasome is a multimolecular complex composed of the nucleotide oligomerization domain (NOD)-like receptors (NLRs), ASC, and caspase-1.[45] Caspase-1 can be triggered in this multimolecular complex, causing cleavage of pro-inflammatory cytokines pro-IL-1β and pro-IL-18, as well as GSDMD, a protein that forms the pores. This process results in the release of bioactive IL-1β and IL-18 into the extracellular space and induces pyroptosis. Specifically, inflammasome assembly can initiate the autocatalytic processing of caspase-1, stimulating the cleavage of its substrates like pro-IL-1β, pro-IL-18, and GSDMD. Activated caspase-1 promotes the release of the N-terminal domain of GSDMD,

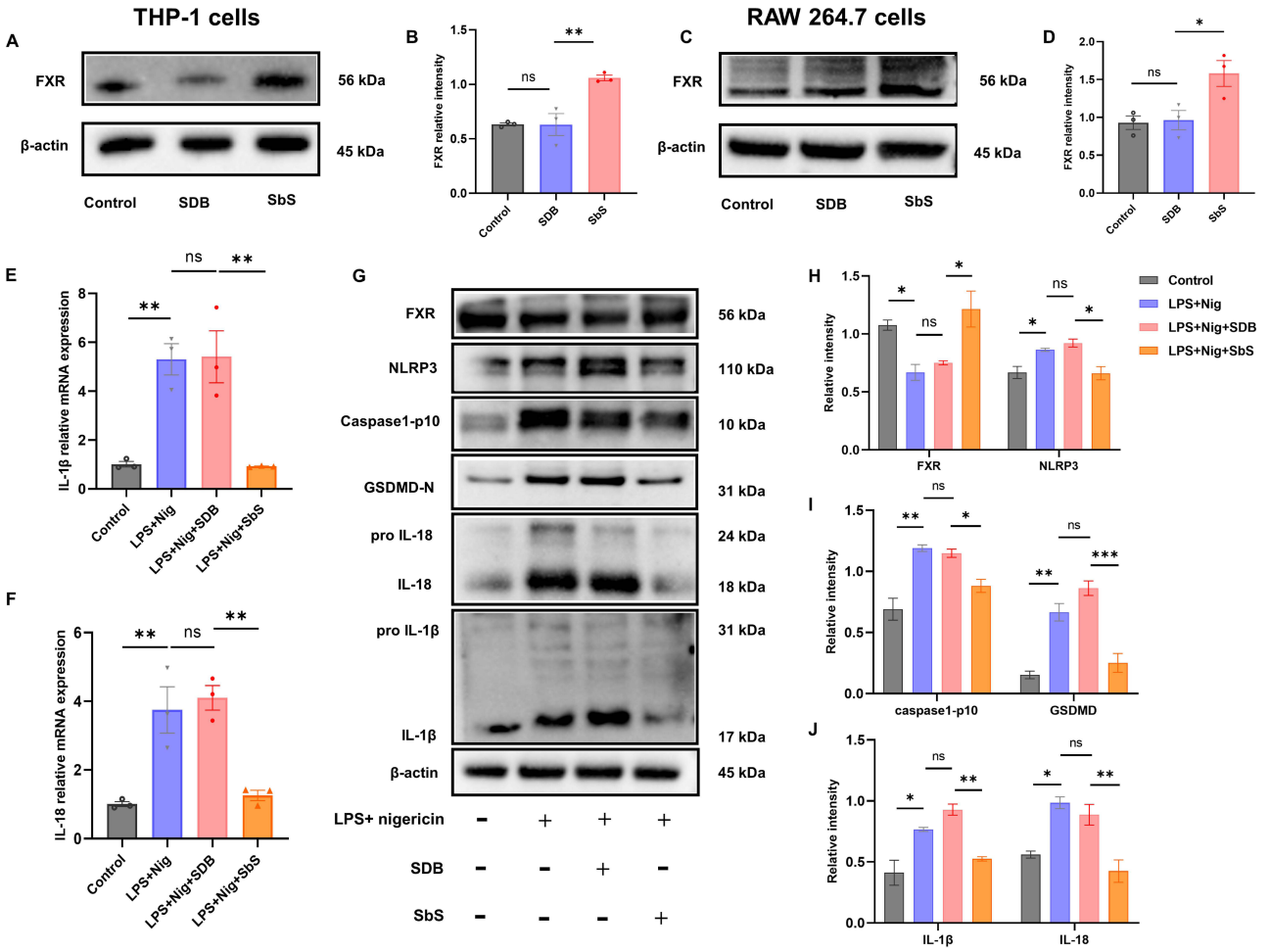

Figure 6 SbS administration ameliorated pyroptosis in THP-1 cells. (**A-D**) The expression level of FXR was measured by Western blot analysis in THP-1 cells (**A and B**) and RAW 264.7 cells (**C and D**) treated with SDB or SbS for 24 hours. (**E and F**) SDB or SbS was administrated for 24 hours in THP-1 cells before treated with 100ng/mL LPS for 4–6h and then 10μg/mL nigericin for 1 hour to induce pyroptosis of THP-1 cells. The mRNA levels of pro-inflammatory cytokines IL-1β (**E**) and IL-18 (**F**) in THP-1 cells were detected by real-time PCR. (**G-J**) The protein levels of FXR and the related indicators of pyroptosis pathway (NLRP3, caspase-1, GSDMD, IL-1β and IL-18) were measured by Western blot and the relative intensity was quantified by Image J software. All data were presented as mean ±SEM. *P < 0.05, **P < 0.01, ***P < 0.001. ns, not significant.

leading to conformational changes that result in the formation of oligomeric pores in the cell membrane. These pores allow the mature IL-1β and IL-18 to be released extracellularly while triggering pyroptosis.[46–49] Of all the NLRs described, NLRP3 inflammasome has garnered the greatest attention. Baseline expression levels of NLRP3 are typically low and must be upregulated for inflammasome activation to occur.[50,51] Increasing studies have highlighted the pivotal role of the NLRP3 inflammasome in the occurrence and development of IBD.[52–54] Researchers have reported the suppressive effect of FXR on NLRP3 activation.[10,11,55] FXR, initially identified in the 1990s due to its activation by the farnesol metabolite, is predominantly expressed in the liver and intestine.[56] It is reported that FXR is a therapeutic target for IBD and acts by regulating innate lymphocytes.[39] In our study, we found that FXR played a role in the regulation of the NLRP3 inflammasome in both colon tissue and macrophages. A decrease in FXR expression was detected accompanied by an increase in NLRP3 inflammasome expression in DSS-induced mice, showing an antagonistic link between FXR and NLRP3 inflammasome, consistent with previous studies.[57,58] However, few studies have investigated the precise relationship between FXR and NLRP3. A study published in 2022 reported an increase in NLRP3, caspase-1, and IL-1β at both the gene and protein levels in colitis mice, while FXR expression was found to be reduced.[58] Nigakinone was shown to enhance FXR expression and decrease the levels of NLRP3, caspase-1, and IL-1β. Therefore, researchers concluded that Nigakinone might exert anti-inflammatory effects by inhibiting the NLRP3

https://doi.org/10.2147/JIR.S504957

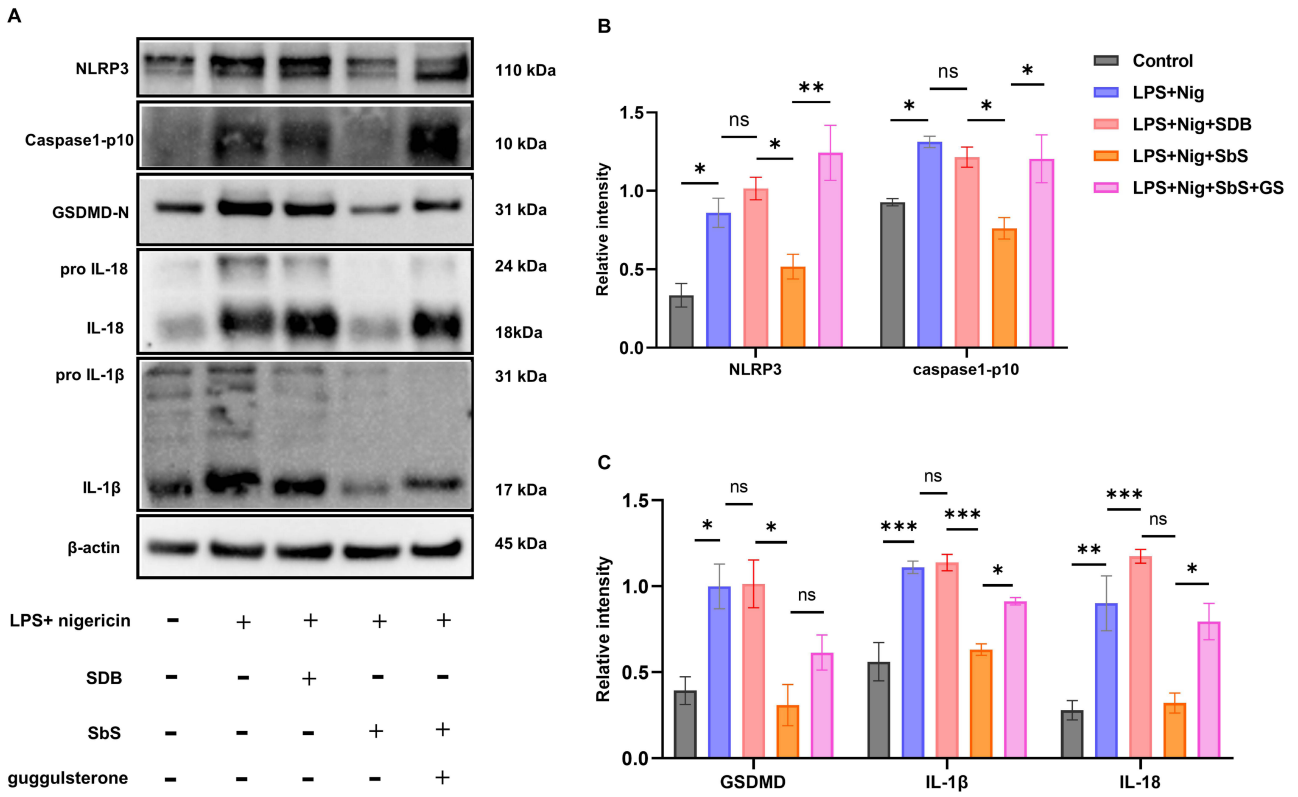

Figure 7 FXR inhibitor abolished the therapeutic effect of SbS. To induce pyroptosis in THP-1 cells, FXR inhibitor (GS) was administered to THP-1 cells for 18 hours, followed by the administration of SDB or SbS for 24 hours, 100ng/mL LPS for 4–6 hours, and 10μg/mL nigericin for 1 hour. (**A-C**) The protein levels of NLRP3, caspase-1, GSDMD, IL-1β and IL-18 in the pyroptosis pathway were measured by Western blot and the relative intensity was quantified by Image J software. All data were presented as mean ±SEM. *P < 0.05, **P < 0.01, ***P<0.001. ns, not significant.

signaling pathway. However, they did not clarify the exact relationship between FXR and NLRP3. Another research group reported that FXR in macrophages had a direct negative regulatory effect on the activation of the NLRP3 inflammasome.[10] They isolated macrophages from $FXR^{-/-}$ mice and their wild-type littermates to assess responses to inflammasome activation. The results showed that $FXR^{-/-}$ macrophages exhibited increased sensitivity to caspase-1 and IL-1β activation, with elevated basal levels of active caspase-1 and mature IL-1β compared to wild-type mice. In the present study, the negative association between FXR and NLRP3 inflammasome was further validated in THP-1 cells of induced pyroptosis via LPS+nigericin treatment. We explored the precise interaction between FXR and NLRP3 in THP-1 cells using an FXR inhibitor (GS). Our findings indicated that the expression of NLRP3 and pyroptosis-related proteins were upregulated in GS-treated THP-1 cells compared to those treated with SbS, suggesting FXR adversely impacted the activation of NLRP3 inflammasome. Genetic knockout or knockdown models can be used to further confirm the negative association between FXR and NLRP3 inflammasome. From the above discussion, we elucidated that SbS could activate FXR and then lead to NLRP3 inflammasome inhibition resulting in reduced pyroptosis, which can alleviate colitis.

However, it must be pointed out that there are also limitations in this study. First, we did not investigate how SbS interacted with FXR. A postbiotic is defined as a "preparation of inanimate microorganisms and/or their components that confers a health benefit on the host", according to the International Scientific Association of Probiotics and Prebiotics consensus statement.[59] Researchers have reported that short-chain fatty acids, proteins and other uncharacterized molecules secreted by Sb could be potential postbiotics.[60–63] Recently, our research team have demonstrated that SbS can effectively alleviate colitis.[63] We conducted further investigations to elucidate the specific bioactive components within the SbS responsible for this effect. We found that the SbS-mediated activation of the epidermal growth factor receptor (EGFR) signaling pathway could be abrogated by treatment with proteinase K or heat killed, suggesting that the bioactive components is likely a protein. Through a series of experiments, we were able to isolate a protein from the SbS,

identified as thioredoxin (Trx), which can activate the EGFR pathway by promoting EGFR ligand generation, thereby ameliorating colitis. Additionally, Trx also exhibited antioxidant properties that contributed to its anti-inflammatory effects. This current study, however, takes a markedly different approach from our previous work. Here, we delved into the FXR-NLRP3 signaling axis, and delineated how SbS suppressed NLRP3 inflammasome activation through FXR stimulation, thereby attenuating pyroptosis in macrophages. These two investigations thus explore completely distinct molecular mechanisms, leading to divergent cellular effects in the modulation of inflammation. It is important to note that while our team has validated the potential key role of Trx derived from SbS in alleviating colitis, this does not preclude the possibility of other bioactive molecules within SbS contributing to the observed effects. However, both Sb and SbS, or other molecules, may experience degradation as they pass through the gastrointestinal tract due to exposure to gastric acid and pepsin, resulting in the decrease of retention time and concentration in the gut. Therefore, a delivery system is needed to minimize their loss in the gastrointestinal environment. Engineered therapeutic approaches hold promise as a locally acting treatment strategy. This localized approach may minimize the systemic side effects often associated with therapeutics administered systemically. A study suggested that the method enhanced the retention time and concentration of Sb in the gut, ultimately improving therapeutic outcomes.[64] Thus, engineering methods could serve as effective carriers for drugs, offering significant therapeutic potential. Whether through engineering or other nanomaterials approaches, these strategies may enhance the therapeutic efficacy of SbS and its derived postbiotics. Future research on SbS will focus on identifying and extracting the active postbiotics it produces. Through in-depth analysis of the critical molecular constituents, we aim to lay the groundwork for further research and potential applications. Second, this study has primarily focused on the FXR-NLRP3 signaling pathway as the mechanism by which SbS alleviates colitis, but other pathways may also play a role. For instance, SbS might affect the Toll-like receptor (TLR) signaling pathway, which is crucial for pathogen recognition and immune response initiation.[65–68] SbS could also modulate the production of SCFAs by the gut microbiota,[32,69,70] contributing to its anti-inflammatory effects. To further validate the significance of the FXR-NLRP3 pathway in mediating SbS's effects on colitis, we could employ genetic knockout or knockdown models. Additionally, comprehensive gene expression profiling and proteomic analysis can be conducted. By comparing the gene and protein expression profiles of cells or tissues treated with SbS in $FXR^{-/-}$ mice and wild-type mice, we can identify changes specifically related to the FXR-NLRP3 pathway and distinguish them from alterations in alternative pathways. In addition, we selected only one DSS-induced colitis model, which may provide limited insights into the mechanisms of human UC. Therefore, future research will establish various colitis models, including 2,4,6-trinitrobenzenesulfonic acid (TNBS)-induced colitis and IL-10 knockout mice, to obtain more pronounced and accurate experimental results. Meanwhile, we did not clarify how FXR regulated the NLRP3 inflammasome. Han et al found that FXR inhibited NLRP3 inflammasome activation through the PERK-CHOP pathway,[11] while Hao et al demonstrated that FXR negatively regulated NLRP3 inflammasome by directly interacting with NLRP3 and caspase-1.[10] Further investigations are needed to explore how FXR regulates the NLRP3 inflammasome.

Conclusions

In conclusion, our research demonstrated that SbS, supernatant of Sb, could ameliorate colitis symptoms, inflammatory responses and gut barrier damage. The therapeutic efficacy of SbS in colitis mitigation likely stems from its modulation of FXR activation, resulting in the suppression of NLRP3 inflammasome, thereby mitigating pyroptosis in colon tissue and macrophages. Subsequent research could yield more accurate targets for Sb alleviating UC in the future, thereby contributing to advancements in the field.

Acknowledgments

This work was supported by National Natural Science Foundation of China (grant Number: 82070545 and 82270574) and Tianjin Medical University General Hospital Fund for Distinguished Young Scholars (22ZYYJQ02).

Disclosure

The authors report no conflicts of interest in this work.

References

1. Feuerstein JD, Moss AC, Farraye FA. Ulcerative Colitis. *Mayo Clin Proc*. 2019;94(7):1357–1373. doi:10.1016/j.mayocp.2019.01.018
2. Kobayashi T, Siegmund B, Le Berre C, et al. Ulcerative colitis. *Nat Rev Dis Primers*. 2020;6(1):74. doi:10.1038/s41572-020-0205-x
3. Ungaro R, Mehandru S, Allen PB, Peyrin-Biroulet L, Colombel JF. Ulcerative colitis. *Lancet*. 2017;389(10080):1756–1770. doi:10.1016/S0140-6736(16)32126-2
4. Axelrad JE, Cadwell KH, Colombel JF, Shah SC. The role of gastrointestinal pathogens in inflammatory bowel disease: a systematic review. *Therap Adv Gastroenterol*. 2021;14:17562848211004493. doi:10.1177/17562848211004493
5. Singh S, Allegretti JR, Siddique SM, Terdiman JP. AGA technical review on the management of moderate to severe ulcerative colitis. *Gastroenterology*. 2020;158(5):1465–1496e1417. doi:10.1053/j.gastro.2020.01.007
6. Solitano V, Parigi TL, Ragaini E, Danese S. Anti-integrin drugs in clinical trials for inflammatory bowel disease (IBD): insights into promising agents. *Expert Opin Investig Drugs*. 2021;30(10):1037–1046. doi:10.1080/13543784.2021.1974396
7. Vavassori P, Mencarelli A, Renga B, Distrutti E, Fiorucci S. The bile acid receptor FXR is a modulator of intestinal innate immunity. *J Immunol*. 2009;183(10):6251–6261. doi:10.4049/jimmunol.0803978
8. Gadaleta RM, van Erpecum KJ, Oldenburg B, et al. Farnesoid X receptor activation inhibits inflammation and preserves the intestinal barrier in inflammatory bowel disease. *Gut*. 2011;60(4):463–472. doi:10.1136/gut.2010.212159
9. Xu M, Shen Y, Cen M, et al. Modulation of the gut microbiota-farnesoid X receptor axis improves deoxycholic acid-induced intestinal inflammation in mice. *J Crohns Colitis*. 2021;15(7):1197–1210. doi:10.1093/ecco-jcc/jjab003
10. Hao H, Cao L, Jiang C, et al. Farnesoid X receptor regulation of the NLRP3 inflammasome underlies cholestasis-associated sepsis. *Cell Metab*. 2017;25(4):856–867e855. doi:10.1016/j.cmet.2017.03.007
11. Han CY, Rho HS, Kim A, et al. FXR inhibits endoplasmic reticulum stress-induced NLRP3 inflammasome in hepatocytes and ameliorates liver injury. *Cell Rep*. 2018;24(11):2985–2999. doi:10.1016/j.celrep.2018.07.068
12. Zhen Y, Zhang H. NLRP3 Inflammasome and Inflammatory Bowel Disease. *Front Immunol*. 2019;10:276. doi:10.3389/fimmu.2019.00276
13. Wang L, Hauenstein AV. The NLRP3 inflammasome: mechanism of action, role in disease and therapies. *mol Aspects Med*. 2020;76:100889. doi:10.1016/j.mam.2020.100889
14. Ni J, Wu GD, Albenberg L, Tomov VT. Gut microbiota and IBD: causation or correlation? *Nat Rev Gastroenterol Hepatol*. 2017;14(10):573–584. doi:10.1038/nrgastro.2017.88
15. Iliev ID, Funari VA, Taylor KD, et al. Interactions between commensal fungi and the C-type lectin receptor Dectin-1 influence colitis. *Science*. 2012;336(6086):1314–1317. doi:10.1126/science.1221789
16. Pais P, Almeida V, Yilmaz M, Teixeira MC. Saccharomyces boulardii: what makes it tick as successful probiotic? *J Fungi (Basel)*. 2020;6(2). doi:10.3390/jof6020078
17. Khatri I, Tomar R, Ganesan K, Prasad GS, Subramanian S. Complete genome sequence and comparative genomics of the probiotic yeast Saccharomyces boulardii. *Sci Rep*. 2017;7(1):371. doi:10.1038/s41598-017-00414-2
18. Abdel-Haq R, Schlachetzki JCM, Boktor JC, et al. A prebiotic diet modulates microglial states and motor deficits in α-synuclein overexpressing mice. *ELife*. 2022;11: e81453.
19. Kabluchko T, Bomko T, Nosalskaya T, Martynov A, Osolodchenko T. In the gastrointestinal tract exist the protective mechanisms which prevent overgrowth of pathogenic bacterial and its incorporation. *Ann Mechnikov Inst*. 2017;1:28–33.
20. Plaza-Diaz J, Ruiz-Ojeda FJ, Gil-Campos M, Gil A. Mechanisms of action of probiotics. *Adv Nutrition*. 2019;10(suppl_1):S49–S66. doi:10.1093/advances/nmy063
21. McFarland LV. Use of probiotics to correct dysbiosis of normal microbiota following disease or disruptive events: a systematic review. *BMJ Open*. 2014;4(8):e005047. doi:10.1136/bmjopen-2014-005047
22. Wu X, Vallance BA, Boyer L, et al. Saccharomyces boulardii ameliorates Citrobacter rodentium-induced colitis through actions on bacterial virulence factors. *Am J Physiol Gastrointest Liver Physiol*. 2008;294(1):G295–G306. doi:10.1152/ajpgi.00173.2007
23. Czerucka D, Roux I, Rampal P. Saccharomyces boulardii inhibits secretagogue-mediated adenosine 3',5'-cyclic monophosphate induction in intestinal cells. *Gastroenterology*. 1994;106(1):65–72. doi:10.1016/S0016-5085(94)94403-2
24. Tiago FCP, Martins FS, Souza ELS, et al. Adhesion to the yeast cell surface as a mechanism for trapping pathogenic bacteria by Saccharomyces probiotics. *J Med Microbiol*. 2012;61(Pt 9):1194–1207. doi:10.1099/jmm.0.042283-0
25. Gedek BR. Adherence of Escherichia coli serogroup O 157 and the Salmonella typhimurium mutant DT 104 to the surface of Saccharomyces boulardii. *Mycoses*. 1999;42(4):261–264. doi:10.1046/j.1439-0507.1999.00449.x
26. Pontier-Bres R, Munro P, Boyer L, et al. Saccharomyces boulardii modifies Salmonella typhimurium traffic and host immune responses along the intestinal tract. *PLoS One*. 2014;9(8):e103069. doi:10.1371/journal.pone.0103069
27. Fidan I, Kalkanci A, Yesilyurt E, et al. Effects of Saccharomyces boulardii on cytokine secretion from intraepithelial lymphocytes infected by Escherichia coli and Candida albicans. *Mycoses*. 2009;52(1):29–34. doi:10.1111/j.1439-0507.2008.01545.x
28. Dalmasso G, Cottrez F, Imbert V, et al. Saccharomyces boulardii inhibits inflammatory bowel disease by trapping T cells in mesenteric lymph nodes. *Gastroenterology*. 2006;131(6):1812–1825. doi:10.1053/j.gastro.2006.10.001
29. Wang C, Li W, Wang H, et al. Saccharomyces boulardii alleviates ulcerative colitis carcinogenesis in mice by reducing TNF-α and IL-6 levels and functions and by rebalancing intestinal microbiota. *BMC Microbiol*. 2019;19(1):246. doi:10.1186/s12866-019-1610-8
30. Terciolo C, Dobric A, Ouaissi M, et al. Saccharomyces boulardii CNCM I-745 restores intestinal barrier integrity by regulation of E-cadherin recycling. *J Crohns Colitis*. 2017;11(8):999–1010. doi:10.1093/ecco-jcc/jjx030
31. Li B, Zhang H, Shi L, et al. Saccharomyces boulardii alleviates DSS-induced intestinal barrier dysfunction and inflammation in humanized mice. *Food Funct*. 2022;13(1):102–112. doi:10.1039/D1FO02752B
32. Schneider S-M, Girard-Pipau F, Filippi J, et al. Effects of Saccharomyces boulardii on fecal short-chain fatty acids and microflora in patients on long-term total enteral nutrition. *World J Gastroenterol*. 2005;11(39):6165–6169. doi:10.3748/wjg.v11.i39.6165
33. Guslandi M, Giollo P, Testoni PA. A pilot trial of Saccharomyces boulardii in ulcerative colitis. *Eur J Gastroenterol Hepatol*. 2003;15(6):697–698. doi:10.1097/00042737-200306000-00017

34. Guslandi M, Mezzi G, Sorghi M, Testoni PA. Saccharomyces boulardii in maintenance treatment of Crohn's disease. *Dig Dis Sci*. 2000;45 (7):1462–1464. doi:10.1023/A:1005588911207
35. Gu Y, Wang C, Qin X, et al. Saccharomyces boulardii, a yeast probiotic, inhibits gut motility through upregulating intestinal serotonin transporter and modulating gut microbiota. *Pharmacol Res*. 2022;181:106291. doi:10.1016/j.phrs.2022.106291
36. Wang C, Chu Q, Dong W, et al. Microbial metabolite deoxycholic acid-mediated ferroptosis exacerbates high-fat diet-induced colonic inflammation. *Mol Metabol*. 2024;84:101944. doi:10.1016/j.molmet.2024.101944
37. Hoffmann TW, Pham H-P, Bridonneau C, et al. Microorganisms linked to inflammatory bowel disease-associated dysbiosis differentially impact host physiology in gnotobiotic mice. *ISME J*. 2016;10(2):460–477. doi:10.1038/ismej.2015.127
38. Huang L, Zheng J, Sun G, et al. 5-Aminosalicylic acid ameliorates dextran sulfate sodium-induced colitis in mice by modulating gut microbiota and bile acid metabolism. *Cell mol Life Sci*. 2022;79(8):460. doi:10.1007/s00018-022-04471-3
39. Fu T, Li Y, Oh TG, et al. FXR mediates ILC-intrinsic responses to intestinal inflammation. *Proc Natl Acad Sci U S A*. 2022;119(51):e2213041119. doi:10.1073/pnas.2213041119
40. Li S, Zhuge A, Chen H, et al. Sedanolide alleviates DSS-induced colitis by modulating the intestinal FXR-SMPD3 pathway in mice. *J Adv Res*. 2024.
41. Huang Y, Xu W, Zhou R. NLRP3 inflammasome activation and cell death. *Cell mol Immunol*. 2021;18(9):2114–2127. doi:10.1038/s41423-021-00740-6
42. W-t H, Wan H, Hu L, et al. Gasdermin D is an executor of pyroptosis and required for interleukin-1β secretion. *Cell Res*. 2015;25(12):1285–1298. doi:10.1038/cr.2015.139
43. Parikh K, Antanaviciute A, Fawkner-Corbett D, et al. Colonic epithelial cell diversity in health and inflammatory bowel disease. *Nature*. 2019;567 (7746):49–55. doi:10.1038/s41586-019-0992-y
44. van der Post S, Jabbar KS, Birchenough G, et al. Structural weakening of the colonic mucus barrier is an early event in ulcerative colitis pathogenesis. *Gut*. 2019;68(12):2142–2151. doi:10.1136/gutjnl-2018-317571
45. Barnett KC, Li S, Liang K, Ting JP. A 360° view of the inflammasome: mechanisms of activation, cell death, and diseases. *Cell*. 2023;186 (11):2288–2312. doi:10.1016/j.cell.2023.04.025
46. Xia S, Zhang Z, Magupalli VG, et al. Gasdermin D pore structure reveals preferential release of mature interleukin-1. *Nature*. 2021;593:7860):-607–611. doi:10.1038/s41586-021-03478-3
47. Shi J, Zhao Y, Wang K, et al. Cleavage of GSDMD by inflammatory caspases determines pyroptotic cell death. *Nature*. 2015;526(7575):660–665. doi:10.1038/nature15514
48. Liu X, Zhang Z, Ruan J, et al. Inflammasome-activated gasdermin D causes pyroptosis by forming membrane pores. *Nature*. 2016;535:7610):-153–158. doi:10.1038/nature18629
49. Ding J, Wang K, Liu W, et al. Pore-forming activity and structural autoinhibition of the gasdermin family. *Nature*. 2016;535(7610):111–116. doi:10.1038/nature18590
50. Szabo G, Petrasek J. Inflammasome activation and function in liver disease. *Nat Rev Gastroenterol Hepatol*. 2015;12(7):387–400. doi:10.1038/nrgastro.2015.94
51. Guo H, Callaway JB, Ting JP. Inflammasomes: mechanism of action, role in disease, and therapeutics. *Nat Med*. 2015;21(7):677–687. doi:10.1038/nm.3893
52. Zhang Y, Tao M, Chen C, et al. BAFF blockade attenuates DSS-induced chronic colitis via inhibiting NLRP3 Inflammasome and NF-κB Activation. *Front Immunol*. 2022;13:783254. doi:10.3389/fimmu.2022.783254
53. Liu X, Fang Y, Lv X, et al. Deubiquitinase OTUD6A in macrophages promotes intestinal inflammation and colitis via deubiquitination of NLRP3. *Cell Death Differ*. 2023;30(6):1457–1471. doi:10.1038/s41418-023-01148-7
54. Weber S, Sitte S, Voegele AL, et al. NLRP3 inhibition leads to impaired mucosal fibroblast function in patients with inflammatory bowel diseases. *J Crohns Colitis*. 2023; 18(3):446–461.
55. Bao H, Li H, Jia Y, et al. Ganoderic acid A exerted antidepressant-like action through FXR modulated NLRP3 inflammasome and synaptic activity. *Biochem Pharmacol*. 2021;188:114561. doi:10.1016/j.bcp.2021.114561
56. Fiorucci S, Zampella A, Ricci P, Distrutti E, Biagioli M. Immunomodulatory functions of FXR. *mol Cell Endocrinol*. 2022;551:111650. doi:10.1016/j.mce.2022.111650
57. Sun X, Chen Z, Yu L, et al. Bacteroides dorei BDX-01 alleviates DSS-induced experimental colitis in mice by regulating intestinal bile salt hydrolase activity and the FXR-NLRP3 signaling pathway. *Front Pharmacol*. 2023;14:1205323. doi:10.3389/fphar.2023.1205323
58. Liu F, Yao Y, Wang Q, et al. Nigakinone alleviates DSS-induced experimental colitis via regulating bile acid profile and FXR/NLRP3 signaling pathways. *Phytother Res*. 2023;37(1):15–34. doi:10.1002/ptr.7588
59. Salminen S, Collado MC, Endo A, et al. The International Scientific Association of Probiotics and Prebiotics (ISAPP) consensus statement on the definition and scope of postbiotics. *Nat Rev Gastroenterol Hepatol*. 2021;18(9):649–667. doi:10.1038/s41575-021-00440-6
60. Calvigioni M, Bertolini A, Codini S, et al. HPLC-MS-MS quantification of short-chain fatty acids actively secreted by probiotic strains. *Front Microbiol*. 2023;14:1124144. doi:10.3389/fmicb.2023.1124144
61. Canonici A, Pellegrino E, Siret C, et al. Saccharomyces boulardii improves intestinal epithelial cell restitution by inhibiting αvβ5 integrin activation state. *PLoS One*. 2012;7(9):e45047. doi:10.1371/journal.pone.0045047
62. Buts JP, Dekeyser N, Stilmant C, Delem E, Smets F, Sokal E. Saccharomyces boulardii produces in rat small intestine a novel protein phosphatase that inhibits Escherichia coli endotoxin by dephosphorylation. *Pediatr Res*. 2006;60(1):24–29. doi:10.1203/01.pdr.0000220322.31940.29
63. Qin X, Zhao Q, Zhao Q, et al. A Saccharomyces boulardii-derived antioxidant protein, thioredoxin, ameliorates intestinal inflammation through transactivating epidermal growth factor receptor. *Pharmacol Res*. 2024;208:107372. doi:10.1016/j.phrs.2024.107372
64. Heavey MK, Hazelton A, Wang Y, et al. Targeted delivery of the probiotic Saccharomyces boulardii to the extracellular matrix enhances gut residence time and recovery in murine colitis. *Nat Commun*. 2024;15(1):3784. doi:10.1038/s41467-024-48128-0
65. Justino PFC, Franco AX, Pontier-Bres R, et al. Modulation of 5-fluorouracil activation of toll-like/MyD88/NF-κB/MAPK pathway by Saccharomyces boulardii CNCM I-745 probiotic. *Cytokine*. 2020;125:154791. doi:10.1016/j.cyto.2019.154791
66. Ko H, Kim BS, Lee YE, et al. Anti-inflammatory effects of Gingerenone A through modulation of toll-like receptor signaling pathways. *Eur. J. Pharmacol*. 2024;983:176997. doi:10.1016/j.ejphar.2024.176997

67. Onyishi CU, Desanti GE, Wilkinson AL, et al. Toll-like receptor 4 and macrophage scavenger receptor 1 crosstalk regulates phagocytosis of a fungal pathogen. *Nat Commun*. 2023;14(1):4895. doi:10.1038/s41467-023-40635-w
68. Yang J-Y, Kim M-S, Kim E, et al. Enteric viruses ameliorate gut inflammation via toll-like receptor 3 and toll-like receptor 7-mediated interferon-β production. *Immunity.* 2016;44(4):889–900. doi:10.1016/j.immuni.2016.03.009
69. Li Y, Ali I, Lei Z, et al. Effect of a multistrain probiotic on feline gut health through the fecal microbiota and its metabolite SCFAs. *Metabolites*. 2023;13(2):228. doi:10.3390/metabo13020228
70. Chang PV, Hao L, Offermanns S, Medzhitov R. The microbial metabolite butyrate regulates intestinal macrophage function via histone deacetylase inhibition. *Proc Natl Acad Sci USA*. 2014;111(6):2247–2252. doi:10.1073/pnas.1322269111