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Reshaping the gut microbiota to ameliorate DSS-induced colitis using a novel synbiotic consisting of jujube powder and *Faecalibacterium prausnitzii*†

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Ulcerative colitis (UC) remains challenging to existing therapeutic approaches, which may have variable efficacy, side effects, and high relapse rates. Increasing evidence indicates the critical role of the gut microbiota in UC pathogenesis, making microbiota-targeted therapies promising for treatment. Here, we propose a novel synbiotic consisting of jujube powder and *Faecalibacterium prausnitzii* and confirm its effectiveness in ameliorating DSS-induced mouse colitis symptoms, including body weight loss, rectal bleeding, diarrhea, colon shortening, and histological damage. The synbiotic also enriched the diversity of the gut microbiota, in which anti-inflammatory microorganisms such as *Parasutterella* and *Parabacteroides* were increased while the potentially pathogenic and inflammation-associated microorganisms like *Clostridium_sensu_stricto_1* were suppressed. More importantly, the synbiotic helped to maintain immune homeostasis and inhibit the imbalance between Th1 and Th2 cells. The anti-inflammatory efficacy of the synbiotic was shown as a function of the ratio of jujube powder to *Faecalibacterium prausnitzii*. The aforementioned findings have shown the potential of this synbiotic in ameliorating colitis and are also helpful in deepening our understanding of the effects and mechanisms of the gut microbiota in UC therapy.

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1. Introduction

Ulcerative colitis (UC) is a kind of inflammatory bowel disease (IBD) with a high incidence and prevalence in high-income countries and an accelerating incidence in newly industrialized countries over the past decades.[1,2] UC is a chronic, recurrent, incurable, and lifelong disease, and moreover causes many gastrointestinal symptoms such as rectal bleeding and diarrhea,[3] along with multiple extraintestinal manifestations and complications including arthritis, skin lesions, and anemia,[2] all of which negatively impact long-term quality of life of patients. While the etiology of UC remains unclear, it is recognized that the pathophysiology of UC involves genetic susceptibility,[4] various environmental risk factors,[5] and host–microbiota interactions.[6] Dysbiosis of the gut microbiota is common in UC patients, as characterized by a lower diversity, a decreased abundance of beneficial microorganisms such as *Akkermansia*,[7] and an increased presence of harmful bacteria like *Enterobacteriaceae*.[8] Of particular note is the reduction of short-chain fatty acid (SCFA) producing bacteria such as *Faecalibacterium prausnitzii*, *Roseburia intestinalis*, and *Eubacterium rectale*,[9] which are conducive for preventing UC development. The damage to intestinal epithelial integrity and its barrier function is also observed in UC patients and animal models; thus bacteria are more likely to penetrate the damaged intestinal mucus layer and exacerbate the disease.[10] While the interplay between the gut microbiota and UC remains to be fully revealed, growing evidence supports that the gut microbiota may work as an effective target for UC therapy.

The importance of diet on the gut microbiota and, consequently, on gut health and disease, has been well documented.[11] The Western style diet, which is high in animal-derived and processed foods, has been identified as a risk factor for the development of IBD. In contrast, the Mediterranean diet, rich in plant-derived foods and seafood, has been associated with a reduced risk of IBD.[12,13] Synbiotics, which are combinations of live microorganisms and substrate(s), have demonstrated synergistic health benefits through targeted utilization by the host's microorganisms.[14] A synbiotic comprising *Bifidobacterium longum* and Synergy 1 appeared effective for patients with UC and Crohn's disease (CD).[15,16] In a mouse model of UC, two synbiotics formed with *Clostridium butyricum*

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and chitooligosaccharides, and *Bifidobacterium infantis* and xylooligosaccharides exhibited a relieving role, respectively.[17,18] A synbiotic comprising *Lactobacillus gasseri* 505 and a *Cudrania tricuspidata* leaf extract appeared effective in colitis-associated colorectal cancer treatment and protection.[19] Given all these efforts, a global understanding of the interaction among synbiotics and UC has remained far from adequate.

Ziziphus (jujube) is a traditional fruit of the *Rhamnaceae* family native to China, containing various biologically active ingredients including polysaccharides, phenolics, flavonoids, triterpenic acids, *etc*.[20,21] It is confirmed that jujube powder increases the abundance of *Lachnospiraceae*, promotes the production of SCFAs, and enhances the infiltration of $CD8^+$ T cells in the tumor microenvironment, thereby improving anti-PD-L1 efficiency against murine colon cancer.[22,23] Zhuang *et al.*[24] identified a promotive effect of jujube in a cyclophosphamide chemotherapy model. Jujube powder also boosted human serum albumin vaccination by increasing the abundance of *Coriobacteriaceae*, which is associated with SCFA and antibody production.[25] Additionally, jujube components such as polysaccharides and polyphenols have been shown to be helpful in reversing gut microbiota dysbiosis, enhancing SCFA production, inhibiting inflammatory pathways, and enhancing intestinal barrier function.[26–29] In particular, as a pivotal component, jujube polysaccharides have shown the potential for restoring the gut microbiota, increasing the abundance of beneficial bacteria, and suppressing harmful bacteria, thus leading to their numerous nutritious functions.[26,29] On the other hand, *Faecalibacterium prausnitzii* (*F. prausnitzii*) is frequently reported as a key microorganism in IBD. It has been observed that the abundance of *F. prausnitzii* decreases in IBD patients,[30] including those with CD[31] and UC.[32] The supplementation of *F. prausnitzii* protected against colitis in various mouse models,[33,34] indicating its anti-inflammatory properties. Furthermore, it has been identified that butyrate[35] and a 15 kDa protein derived from a single microbial anti-inflammatory molecule (MAM)[36] are the key anti-inflammatory components, inhibiting the NF-κB pathway and preventing colitis.

In this study, we developed a novel synbiotic by combining jujube powder with *F. prausnitzii* and examined its anti-inflammatory effectiveness in a dextran sulfate sodium (DSS)-induced colitis model in mice. The results confirmed that this novel synbiotic ameliorated colitis by alleviating weight loss, diarrhea, rectal bleeding, splenomegaly, colon shortening, and histopathological damage. The synbiotic also improved the diversity and structure of the gut microbiota, increased the abundance of beneficial microorganisms, and decreased the abundance of pathogenic bacteria associated with IBD. Moreover, the synbiotic inhibited the imbalance of immunity, as evidenced by a restored balance between Th1 and Th2 cells in mesenteric lymph nodes. The effects of the composition of jujube powder and *F. prausnitzii* on the anti-inflammatory benefits were also confirmed. All these results are conducive to the potential applications of this novel synbiotic in ameliorating colitis.

2 Materials and methods

2.1 Animals

Seven-week-old male C57BL/6J mice were provided by Vital River Laboratory Animal Technology Co., Ltd (Beijing, China) and housed in a pathogen-free animal facility at the Laboratory Animal Resources Center, Tsinghua University. The mice were maintained on a 12/12 hour light/dark cycle, with a temperature range of 22–26 °C, and provided with sterile pellet food and water *ad libitum*. Mice were subjected to adaptive feeding for a week before the treatments and divided into groups, randomly (n = 6). All animal protocols were approved by the Institutional Animal Care and Use Committee of Tsinghua University (no. 20-LZ1).

2.2 Treatments

A mouse model of ulcerative colitis was established by administering 2.5% dextran sulfate sodium (DSS, molecular weight 36 000–50 000 Da, MP Biomedicals, Irvine, CA, USA) in drinking water for 7 days. The water was renewed every 2 days to avoid degradation of DSS. The mice recovered for 2 to 5 days after the cessation of DSS administration. *Ziziphus* (jujube) fruits that had been pre-dried were purchased from Ruoqiang County, Xinjiang Province. The fruits were denucleated and sliced into small pieces, and then dried at 50 °C for 24 h to remove most of the moisture. The dried pieces were subsequently ground into a fine powder (particle size < 10 μm) using an ultrafine pulverizer (Pls, Jinan, China) at −20 °C for 15 min.[23,24] The main components of jujube powder are listed in Table 1. The jujube powder was dissolved in sterile water and administered daily by gavage (800 mg kg^{-1}); this dose is approximately equivalent to a 60 kg adult consuming 3.89 g of jujube powder daily, converted based on body surface area factors.[37] Considering the yield of jujube powder (70.31 ± 6.81%, mean ± SEM, based on three independent measurements), this amount is equivalent to the consumption of one piece of jujube fruit per day by an adult weighing 60 kg, which falls within the typical food consumption range. *Faecalibacterium prausnitzii* (*F. prausnitzii*, strain A2-165, obtained from Mingzhou Biotechnology Co., Ltd (Ningbo, China)) was resuspended in phosphate buffered saline (PBS) and administered daily by gavage (200 μL of 2×10^9 colony forming units (CFUs) per mL per day). The groups receiving

Table 1 The main components of jujube powder. Components were measured using methods provided in the ESI.† Data are presented as mean ± SEM, based on three independent samples, each of which was technically repeated three times

Components	Proportion in percentage
Total sugar	92.66 ± 2.94
Reducing sugar	68.66 ± 0.85
Uronic acid	17.40 ± 0.44
Total phenol	1.33 ± 0.04
Total flavonoids	1.17 ± 0.01
Protein	0.74 ± 0.04

jujube powder and *F. prausnitzii* administration alone were recorded as the J group and the FP group, respectively. In the case of synbiotic administration, the jujube powder and *F. prausnitzii* were administered by gavage at the same dose, recorded as the J + FP group. The control group was abbreviated as the CTR group. To investigate the synbiotic formulation, the proportion of jujube powder and *F. prausnitzii* was altered by adjusting the dosage of *F. prausnitzii*, which was 200 μL of 2×10^7 CFU mL^{-1}, 2×10^8 CFU mL^{-1}, and 2×10^9 CFU mL^{-1} day^{-1}, respectively, corresponding to the abbreviations of the J + FP-L group, the J + FP-M group, and the J + FP-H group. All oral administrations were initiated concurrently with the administration of DSS and maintained throughout the duration of the experiment.

2.3 Assessment of DSS-induced colitis

The severity of DSS-induced colitis was measured daily using the disease activity index (DAI), including the evaluation of the body weight, stool consistency, and rectal bleeding of mice.[38] Specifically, it was scored as follows. The body weight change was scored as 0 when <1%, 1 when 1–5%, 2 when 5–10%, 3 when 10–15%, and 4 when >15%. The stool consistency was scored as 0 when normal, 1 when soft but formed, 2 when soft and deformed, 3 when mild diarrhea, and 4 when severe diarrhea. The rectal bleeding was scored as 0 when no bleeding, 2 when mild bleeding, and 4 when gross bleeding. The DAI was obtained by summing up the three scores.

2.4 Hematoxylin and eosin (H&E) staining and histological analysis

The distal colon of mice was fixed in 4% paraformaldehyde, embedded in paraffin, sliced, and stained with hematoxylin and eosin. The histological analysis was conducted according to a previous study.[39] In detail, it included the evaluation of inflammatory infiltration (0, none; 1, slight; 2, moderate; 3, severe), depth of injury (0, none; 1, mucosa; 2, mucosa and submucosa; 3, transmural), crypt damage (0, none; 1, basal one-third damage; 2, basal two-third damage; 3, only epithelium intact; 4, entire crypt and epithelium lost), goblet cell damage (0, none; 1, local loss; 2, loss of multiple sites; 3, massive loss; 4, all lost), and damage range (1, 1–25%; 2, 26–50%; 3, 51–75%; 4, 76–100%). The histological score was obtained by summing up the five scores.

2.5 Flow cytometry

The mice were sacrificed to obtain colon, mesenteric lymph nodes (MLNs), and spleen to prepare a single-cell suspension. The colon was cut into pieces, washed with PBS, and incubated with 1 mM dithiothreitol (DTT), 5 mM EDTA, and 15 mM HEPES in D-Hank's balanced salt solution (HBSS) at 37 °C for 30 min under gentle shaking. Then, the colon was digested with collagenase IV (1 mg ml^{-1}) and DNase I (20 μg ml^{-1}, Yeasen Biotech Co., Ltd, Shanghai, China) in RPMI 1640 medium supplemented with 10% FBS at 37 °C for 40 min. The single cells were obtained by filtration through a 70 μm nylon cell strainer (Corning, NY, USA). The MLNs and spleen were ground gently with a grinding rod and then filtered through a 70 μm nylon cell strainer. Red blood cell lysis buffer (Solarbio Science & Technology Co. Ltd, Beijing, China) was applied to remove the red blood cells in the spleen single cell suspension before staining. Live-dead dye was used to eliminate dead cells, and the anti-mouse CD16/32 antibody was used to block non-specific binding. For extracellular surface antigen staining, cells and antibodies were incubated in the dark at 4 °C for 30 minutes. For cytokine staining, cells were stimulated by the cell stimulation cocktail (plus protein transport inhibitors) from eBioscience, San Diego, USA, at 37 °C under 5% CO_2 for 4 h. For intercellular antigen staining, cells were fixed and permeabilized using the Foxp3/Transcription Factor Staining Buffer Set (eBioscience, San Diego, USA) according to the manufacturer's instructions. Then, cells and antibodies were also incubated in the dark at 4 °C for 30 minutes. All antibodies used are listed in the ESI Table S1.† Following staining, the cells were washed and resuspended in PBS for assays on the ID7000 spectral cell analyzer (Sony Corporation, Tokyo, Japan). All acquired data were analyzed using the ID7000 software.

2.6 16S rRNA sequencing and data analysis

Fecal samples were collected at the end of each experiment to analyze the gut microbiota. Total DNA was extracted using the QIAamp DNA Stool Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. The variable V3-V4 region of the 16S gene was amplified through the forward primer 338F (ACTCCTACGGGAGGCAGCAG) and reverse primer (GGACTACHVGGGTWTCTAAT). Subsequently, the sequencing was conducted using Illumina MiSeq (Majorbio Bio-Pharm Technology Co. Ltd, Shanghai, China). The high-quality sequences were clustered into operational taxonomic units (OTUs) using UPARSE (version 7.0.1090, **https://drive5.com/uparse/**) based on a similarity threshold of 97%. The subsequent bioinformatics analysis was conducted on the Majorbio cloud platform. The alpha diversity was calculated using Mothur (version 1.30.2, **https://mothur.org/wiki/calculators/**). Principal coordinates analysis (PCoA), partial least squares discriminant analysis (PLS-DA), and community composition and differential analysis were conducted using R (Version 3.3.1).

2.7 Statistical analysis

All statistical analyses were conducted using GraphPad Prism 9.3 (GraphPad Software Inc., San Diego, USA). A two-way analysis of variance (ANOVA) was applied to compare the weight and DAI differences among various groups of mice at different time points. One-way ANOVA was applied to compare the differences among more than two groups, provided that the data followed the Gaussian distribution with equal standard deviations. Tukey's or Dunnett's *post hoc* test was conducted following ANOVA. All data are presented as mean ± standard error of the mean (SEM). The annotation of ns indicates that the observed difference is not statistically significant. The

symbols *, **, ***, and **** represent $p < 0.05$, $p < 0.01$, $p < 0.001$, and $p < 0.0001$, respectively.

3 Results

3.1 The synbiotic consisting of jujube powder and *F. prausnitzii* ameliorated DSS-induced colitis

Mice were administered by gavage with the synbiotic, and jujube powder and *F. prausnitzii* at the same dose alone (Fig. 1A). Compared to the CTR group, the body weight of mice in all DSS-treated groups decreased and recovered after the cessation of DSS administration. Significant differences in body weight were observed between the DSS group and the J + FP group since day 9 ($p = 0.036836$ on day 9, 0.012793 on day 10, and 0.026645 on day 11), while there was no significant difference between the DSS group and other groups (Fig. 1B). Consistent with the trend of body weight changes, the disease activity index (DAI) of all groups treated with DSS presented an overall pattern of first increasing and then decreasing. The J + FP group displayed significantly lower DAI than the DSS group on days 5 and 10 ($p = 0.0010$ and 0.0138, respectively), and the FP group showed reduced DAI on day 5 ($p = 0.0491$) (Fig. 1C). The spleen index (SPI) was obtained through the ratio of spleen weight to body weight of mice, which was used to assess the degree of splenomegaly and inflammation. Through

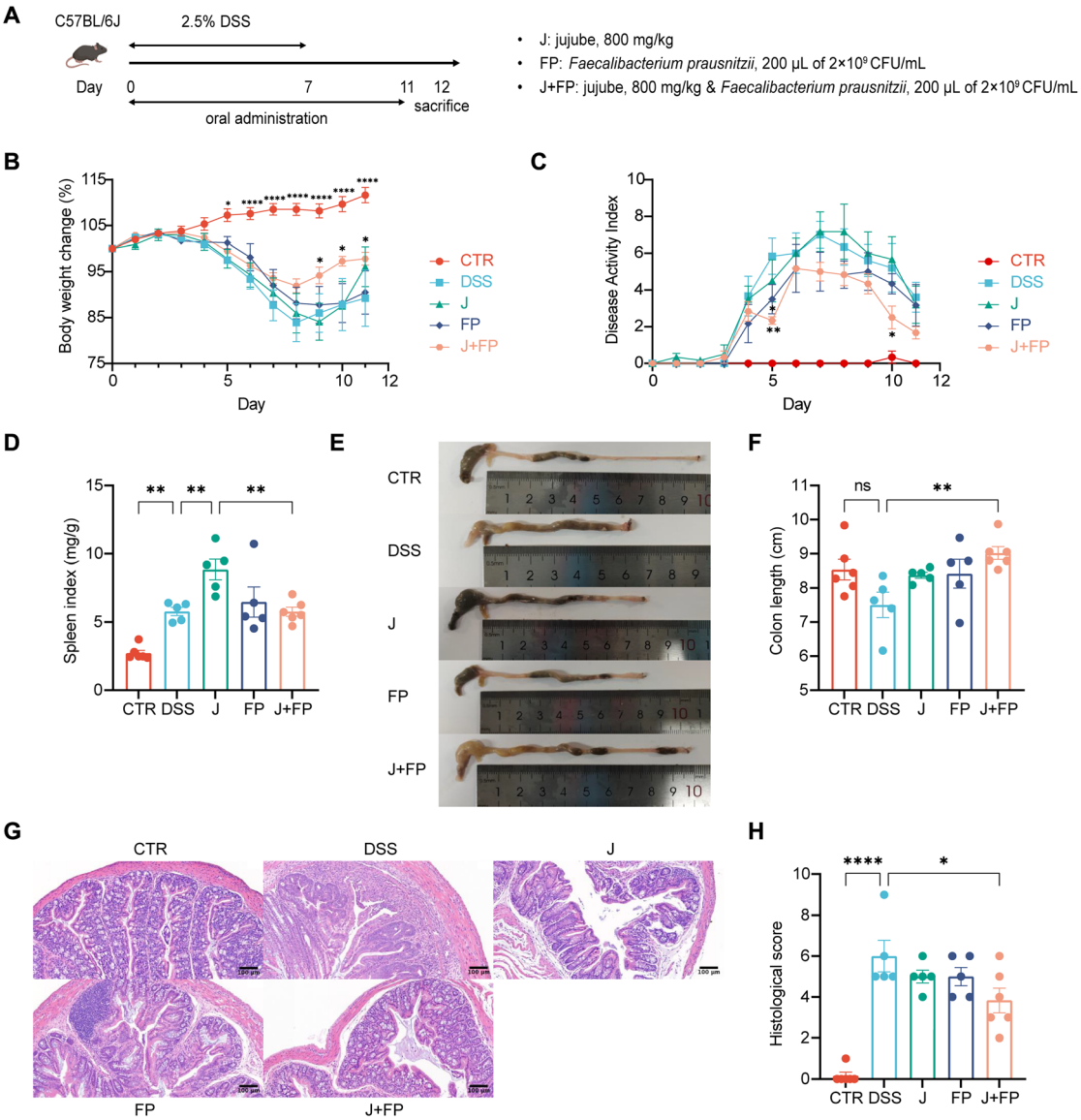

Fig. 1 Effects of jujube powder, *F. prausnitzii*, and the synbiotic on DSS-induced colitis. (A) The scheme of animal experimental design. (B) Body weight change (time effect: ****, treatment effect: ***, interaction effect: ****) and (C) disease activity index during the entire experiment process (time effect: ****, treatment effect: ****, interaction effect: ****). (D) Spleen index. (E) Representative pictures of the colon in different groups of mice. (F) Colon length. (G) Representative images of H&E staining in different groups of mice. (H) Histological score according to H&E staining. ns, no significance, $p > 0.05$, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.

analysis after sacrificing the mice, the SPI of the J group was notably higher than that of the DSS group (p = 0.0095), indicating that jujube powder is inclined to stimulate the immune response as reported in previous studies.[23–25] Although the SPI of the J + FP group did not decrease compared to the DSS group, the outcome of the synbiotic was superior to the J group, as evidenced by a marked decrease in SPI compared to the J group (p = 0.0087). This suggests the immune regulatory and inflammation inhibition function of *F. prausnitzii* (Fig. 1D). Pathological evaluation of colon tissue revealed that the J + FP group had a significantly milder degree of colon edema and longer colon length (p = 0.0047) (Fig. 1E and F). The histological score of the J + FP group was also notably decreased compared with the DSS group (p = 0.0163), manifested as a decrease in inflammatory cell infiltration, crypt damage, and loss of goblet cells (Fig. 1G and H). Overall, the synbiotic of jujube powder and *F. prausnitzii* showed an alleviation effect in DSS-induced colitis, offering greater relief from weight loss, diarrhea, rectal bleeding, splenomegaly, colon shortening, and histopathological changes than supplementation alone.

3.2 The synbiotic reshaped the gut microbiota of DSS-induced colitis mice

Fecal samples were collected for 16S rRNA gene sequencing. The analysis of community alpha diversity showed that the DSS treatment reduced the species richness and diversity of the gut microbiota in mice, manifested as a trend of decreasing Sobs index, Chao index, ACE index, and Shannon index, as well as increasing Simpson index. It was noteworthy that the FP group showed a significant reduction in alpha diversity, as evidenced by lower Sobs index, Chao index, and ACE index than those of the DSS group, and a lower Shannon index, and a higher Simpson index than those of the CTR group (p = 0.0230, 0.0183, 0.0127, 0.0391, and 0.0255, respectively). In contrast, the J + FP group notably increased Sobs index, Chao index, and ACE index compared to the FP group (p = 0.0102, 0.0041, and 0.0046, respectively) (Fig. 2A). The beta diversity was evaluated through principal coordinates analysis (PCoA) and partial least squares discriminant analysis (PLS-DA). As shown by the clustering of the gut microbiota in each group, the microbial composition was altered by the administration of DSS, jujube, and *F. prausnitzii*. In particular, the PLS-DA revealed that the community composition of the J + FP group was more inclined to that of the CTR group on COMP2 compared to the J and the FP groups (Fig. 2B and C).

Furthermore, we evaluated the differences in the gut microbiota communities at various taxonomic levels. At the phylum level, there were changes in the abundance of multiple dominant populations, implying the dysbiosis of the gut microbiota. In particular, the FP group showed a trend of increased Firmicutes and decreased Bacteroidota abundance (Fig. S2†),

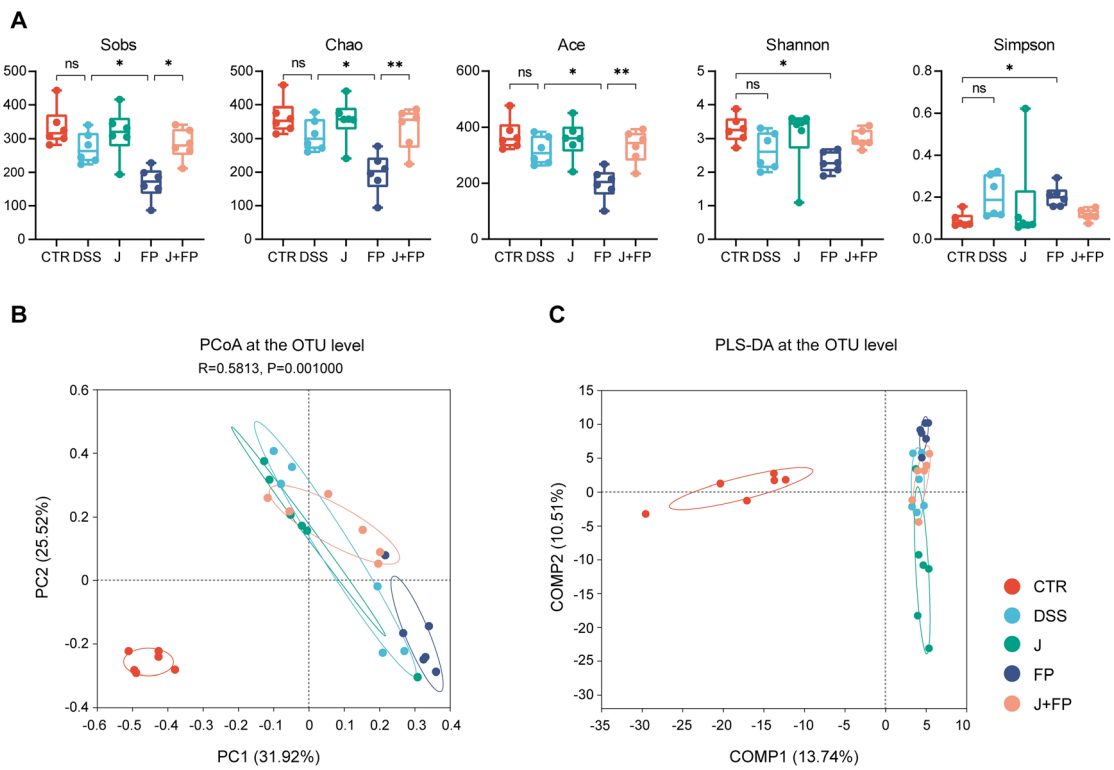

Fig. 2 The synbiotic restored the alpha diversity and altered the community structure of the gut microbiota. (A) Indexes to measure alpha diversity, including the Sobs index, Chao index, ACE index, Shannon index, and Simpson index, successively. (B) Principal coordinates analysis (PCoA) at the OTU level. (C) Partial least squares discriminant analysis (PLS-DA) at the OTU level. ns, no significance, $p > 0.05$, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.

which collectively resulted in a significantly higher ratio of Firmicutes to Bacteroidota (p = 0.0282). Conversely, the J + FP group reversed this alteration, significantly reducing the ratio (p = 0.0273) (Fig. 3A and B). The microorganisms with significant differences at the family level are illustrated in Fig. 3C. The administration of DSS decreased the abundance of *Muribaculaceae* and *Atopobiaceae*, while increases were noted in the abundance of *unclassified_o__Clostridia_UCG-014*, *Enterobacteriaceae*, and *Oscillospiraceae*. Compared to the DSS group, the administration of jujube powder was observed to increase the abundance of *Muribaculaceae*, *Atopobiaceae*, and *Prevotellaceae*, while simultaneously decreasing the abundance of *Enterobacteriaceae*. Both *F. prausnitzii* and the synbiotic decreased the abundance of *unclassified_o__Clostridia_UCG-014*, *Enterobacteriaceae*, and *Oscillospiraceae*. Moreover, the synbiotic also increased the abundance of *Atopobiaceae* and

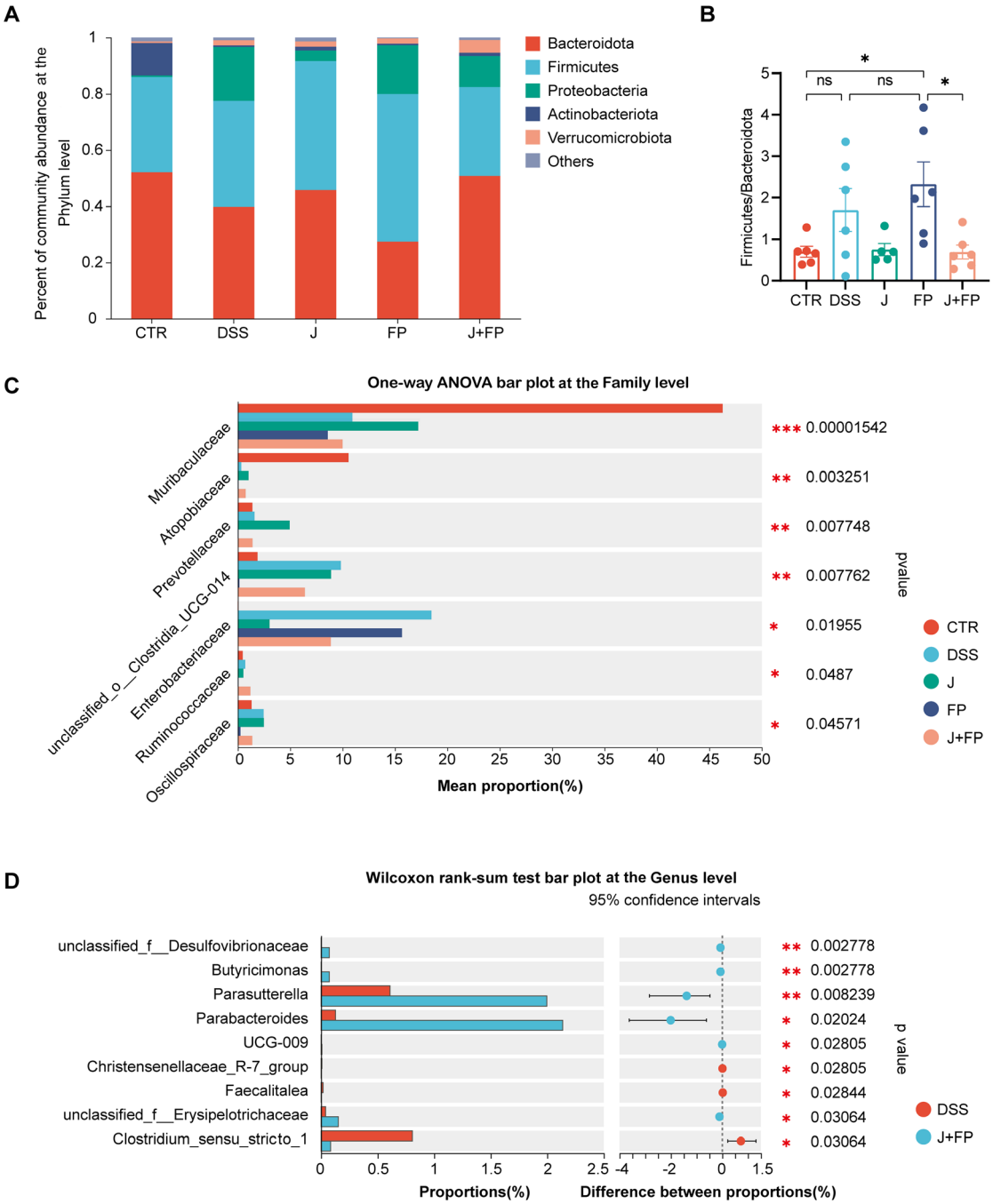

Fig. 3 Significantly different gut microbiota species at various taxonomic levels. (A) Percent of community abundance at the phylum level. (B) The ratio of Firmicutes to Bacteroidota. (C) Species with significant differences at the family level. (D) Wilcoxon rank-sum test at the genus level between the DSS group and the J + FP group. ns, no significance, $p > 0.05$, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.

Ruminococcaceae, balancing the impact of jujube powder and *F. prausnitzii* on the gut microbiota. At the genus level, *unclassified_f__Desulfovibrionaceae*, *Butyricimonas*, *Parasutterella*, *Parabacteroides*, *Butyricicoccaceae UCG-009*, and *unclassified_f__Erysipelotrichaceae* were enriched in the synbiotic group, while the DSS group presented the enrichment of *Christensenellaceae_R-7_group*, *Faecalitalea*, and *Clostridium_sensu_stricto_1* (Fig. 3D).

3.3 The composition of the synbiotics affected the alleviation on DSS-induced colitis

The proportion of jujube powder and *F. prausnitzii* in synbiotics was considered in the DSS-induced colitis model by altering the dose of *F. prausnitzii*, as shown in Fig. 4A. The J + FP-H group showed a significant alleviation of body weight loss in colitis mice since day 9 (p = 0.0307 on day 9 and 0.0267 on day 10). The J + FP-M group also showed a trend toward reducing weight loss, while in the J + FP-L group it was not that sufficient (Fig. 4B). Similarly, the J + FP-H group showed alleviation of colitis severity since day 9 according to the DAI (p = 0.0153 on day 9 and 0.0208 on day 10), and the J + FP-M group showed a similar DAI variation tendency, although it did not reach a significant degree (Fig. 4C). As for SPI, no significant elevation effect was shown (Fig. 4D). The colon length of the J + FP-M and J + FP-H groups was notably longer than in the DSS group (p = 0.0313 and p = 0.0099, respectively), indicating

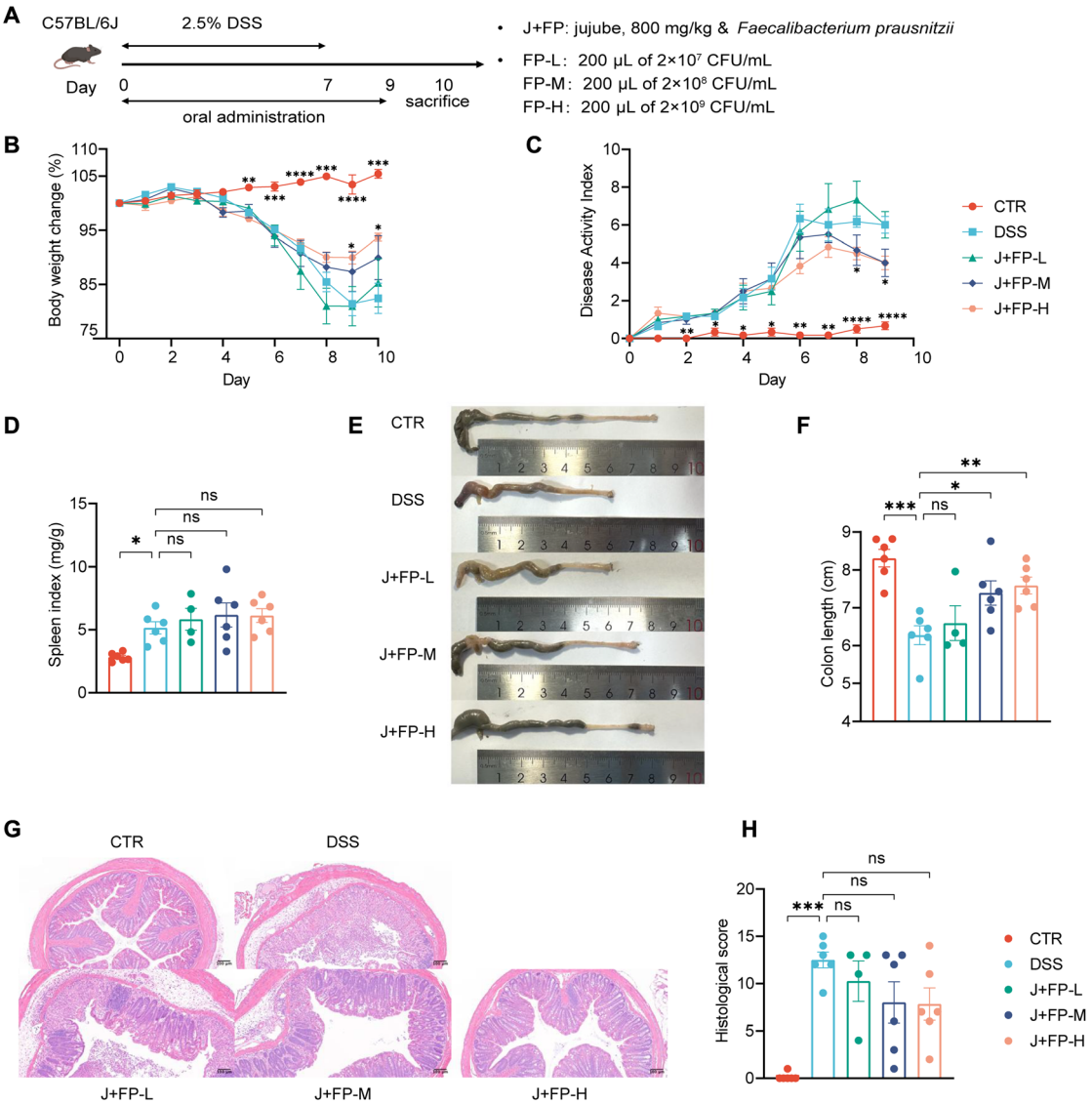

Fig. 4 The synbiotics consisting of different proportions of jujube powder and *F. prausnitzii* exerted different effects on DSS-induced colitis. (A) The scheme of animal experimental design. (B) Body weight change (time effect: ****, treatment effect: ****, interaction effect: ****) and (C) disease activity index during the entire experiment process (time effect: ****, treatment effect: ****, interaction effect: ****). (D) Spleen index. (E) Representative pictures of the colon in different groups of mice. (F) Colon length. (G) Representative images of H&E staining in different groups of mice. (H) Histological score according to H&E staining. ns, no significance, $p > 0.05$, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.

that the colon shortening caused by DSS was reversed (Fig. 4E and F). As for the histopathology score, the synbiotics, particularly in the J + FP-M and J + FP-H groups (Fig. 4G and H), contributed an improvement, although the variation degree was not significant. This may be due mainly to sample heterogeneity, suggesting that sampling numbers should be increased in the studies in photographic evaluation. These results also show that the alleviating effects on DSS-induced colitis were affected by the proportion of jujube powder and *F. prausnitzii* in the synbiotic.

3.4 The synbiotics regulated immune response and mitigated Th1/Th2 imbalance caused by DSS-induced colitis

Innate immune cells in the colon and MLNs were evaluated by flow cytometry. The results showed that innate immunity was activated in mice with colitis. Total myeloid cells and neutrophils significantly increased in the colon (Fig. 5A and C). In the MLNs, dendritic cells decreased significantly, possibly due to their migration to the colon for antigen presentation. The increase in the infiltration of monocytes and macrophages indicates an inflammatory response (Fig. 5G, I and J). Compared to the DSS group, the synbiotic J + FP-L increased the myeloid cells in MLNs (p = 0.0401) (Fig. 5F), while the synbiotic J + FP-M increased the monocytes in MLNs (p = 0.0385) (Fig. 5I). As for macrophages in MLNs, the synbiotic J + FP-H showed an inhibition functionality, although the variation did not reach a significant degree (Fig. 5J). The above results suggest that, from the innate immunity perspective, the formulation of the synbiotic should be optimized, which merits further investigation.

Adaptive immune cells in secondary lymphoid organs were also assessed, including the MLNs and spleen. Adaptive immune imbalance occurred in the MLNs, which are close to the inflamed colon. Total B cells and T cells of colitis mice were upregulated and downregulated, respectively, while the J + FP-H group restored the levels of total B cells and T cells (p = 0.0019 and 0.0248, respectively) (Fig. 6A–C). Both $CD4^+$ T cells and $CD8^+$ T cells decreased in the MLNs in colitis mice, with no obvious change in the relative proportion (Fig. 6D and Fig. S5A, B†). The use of the synbiotic J + FP-H notably increased the proportion of $CD4^+$ T cells in the MLNs (p = 0.0008). The J + FP-M group showed a similar trend to the

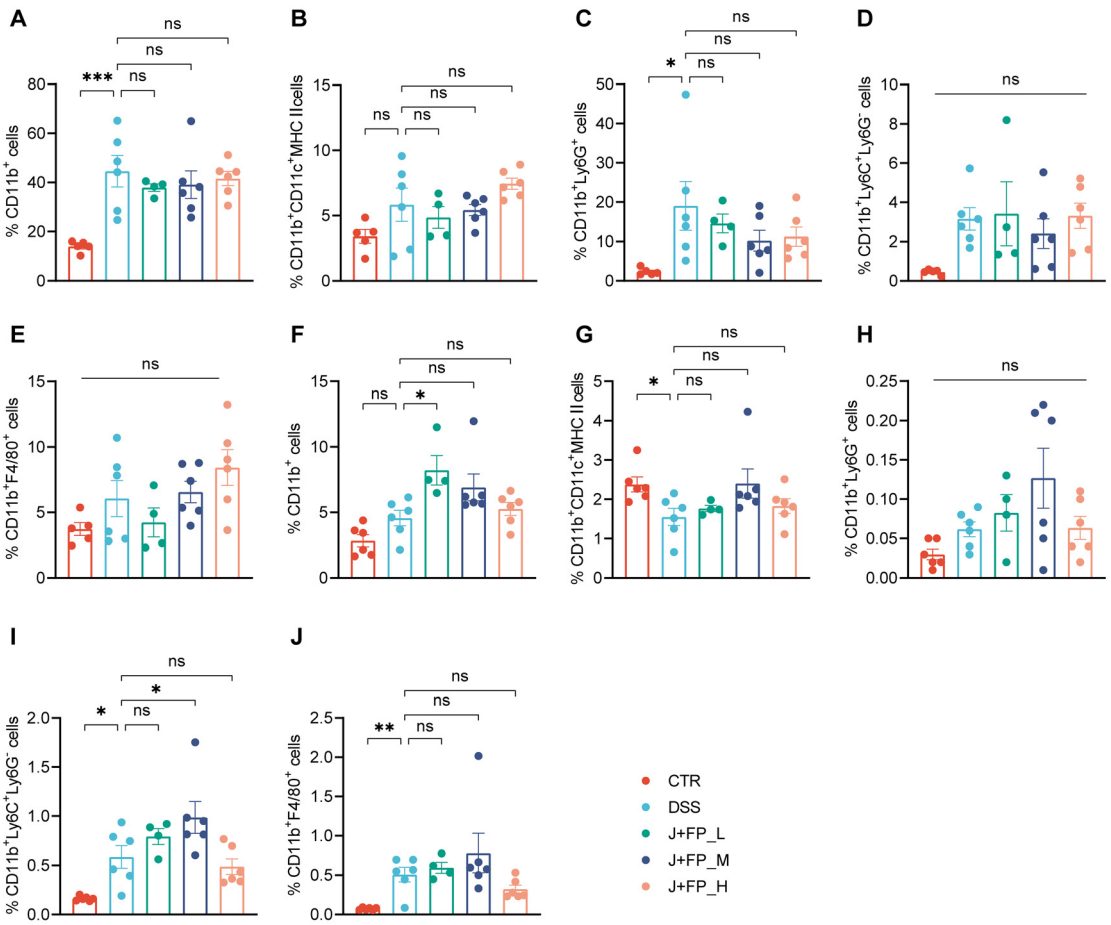

Fig. 5 Synbiotics had no significant effect on the infiltration of myeloid cells in colitis. Statistical results of (A) myeloid cells, (B) dendritic cells, (C) neutrophils, (D) monocytes, and (E) macrophages in the colon. Statistical results of (F) myeloid cells, (G) dendritic cells, (H) neutrophils, (I) monocytes, and (J) macrophages in MLNs. Statistical analyses of these cells were based on the proportion of cells to $CD45^+$ T cells. ns, no significance, $p > 0.05$, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.

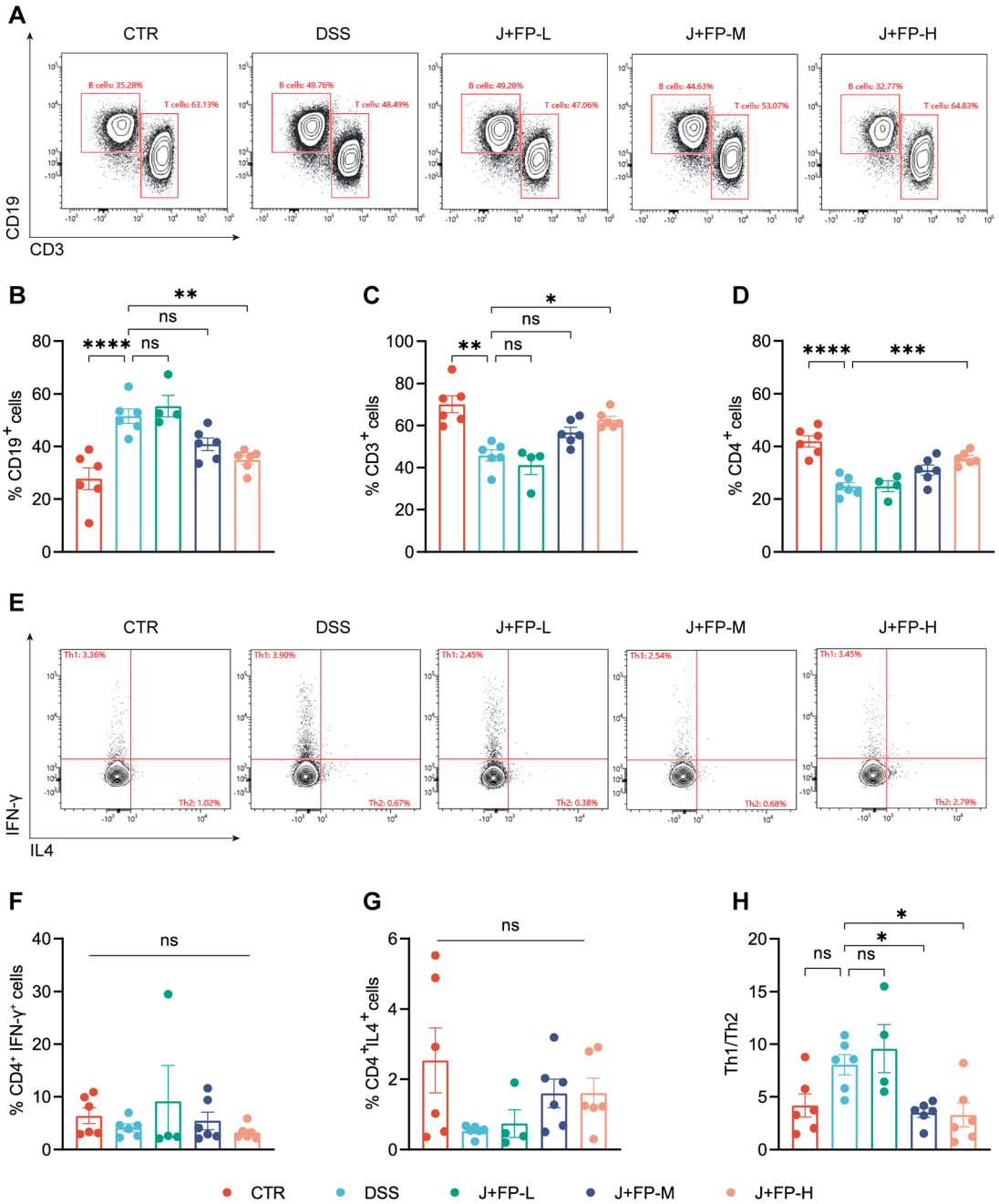

Fig. 6 The synbiotics mitigated the adaptive immune imbalance in the mesenteric lymph nodes. (A) Comparison of representative flow cytometry assays and (B) statistical results of B cells and (C) T cells in the MLNs. (D) Statistical results of $CD4^+$ T cells in the MLNs. (E) Comparison of representative flow cytometry assays and (F) statistical results of Th1 cells, (G) Th2 cells, and (H) the ratio of Th1 cells to Th2 cells in the MLNs. Statistical analyses of B cells, T cells, and $CD4^+$ T cells were based on the proportion of cells to $CD45^+$ T cells. Statistical analyses of Th1 and Th2 cells were based on the proportion of cells to $CD4^+$ T cells. ns, no significance, $p > 0.05$, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.

J + FP-H group in restoring B cells, T cells, and $CD4^+$ T cells in the MLNs, although the variation did not reach a significant degree. Of particular note is the restoration of the balance between T helper 1 (Th1) cells and T helper 2 (Th2) cells, which was distorted by the DSS treatment, primarily due to the reduction of Th2 cells. Both the J + FP-M and J + FP-H groups increased Th2 cells, thereby recovering Th1/Th2 balance (p = 0.0284, and 0.0212, respectively) (Fig. 6E–H). It was observed that Th17 cells and Treg cells also increased in the MLNs of colitis mice, while the impact of the synbiotics was not significant (Fig. S5C–E†). We also examined adaptive immune response in the spleen (Fig. S6A–K†). Similarly, an increase in B cells and reductions in total T cells, $CD4^+$ T cells, and $CD8^+$ T cells were observed in the spleen of colitis mice. However, the changes in regulatory T cells and helper T cells in the spleen were not pronounced, which may due to spleen being

located far from the inflammatory sites. Compared to the significant changes in MLNs, the effects of the synbiotics on spleen immune cells were marginal. It was concluded from the overall responses of the MLNs and spleen that the synbiotics help to regulate immune homeostasis towards recovery of the Th1/Th2 balance for DSS-induced colitis.

4 Discussion

The benefits of synbiotics on health and disease are widely recognized, alongside those of probiotics and prebiotics.[14] Further evidence is required to elucidate the effects and the underpinning mechanisms of synbiotics. Here, we found that a novel synbiotic consisting of jujube powder and *F. prausnitzii* exerted an alleviating effect on a DSS-induced mouse colitis model. Moreover, the synbiotic reshaped the gut microbiota and inhibited imbalanced immune response, suggesting its potential as an intervention in IBD.

Although the health benefits of jujube and *F. prausnitzii* have been extensively reported,[20,22–28,33–36] studies on developing synbiotics combining these two components are rare. Jing *et al.*[40] reported that a synbiotic composed of jujube and *Lactobacillus rhamnosus* GG enhanced the therapeutic effects of the cancer vaccine on MC38 murine colon tumor by reshaping the gut microbiota. He *et al.*[41] studied the alleviation of the combination of arabinogalactan, *Bifidobacterium longum*, and *F. prausnitzii* on insulin-resistant mice, demonstrating a stronger effect than using alone. In this study, we proposed and demonstrated the efficacy of a novel synbiotic composed of jujube powder and *F. prausnitzii*, which significantly mitigated DSS-induced colitis in mice, alleviating weight loss, diarrhea, rectal bleeding, splenomegaly, colon shortening, and ulcerative lesions (Fig. 1B–H). As observed in previous studies,[26–28,36,37,40] both jujube and *F. prausnitzii* independently alleviated colitis. Here we obtained a greatly enhanced outcome by combining them into a synbiotic. Through varying the proportion of jujube powder and *F. prausnitzii*, the differences in the effectiveness of different formulations of the synbiotic in alleviating colitis were shown (Fig. 4B–H).

The structure of the gut microbiota was altered in DSS-induced acute colitis and the synbiotic played a restorative and reconstructive role, as shown by 16S rRNA sequencing and microbial analysis (Fig. 2A–C). As shown in our previous work,[22,23] jujube powder contains polysaccharides and dietary fibers, which can serve as prebiotics regulating the microbiota and exerting health benefits in enhancing the treatment efficiency of murine colon cancer. In this study, we also observed the prebiotic effect of jujube powder in the mouse colitis model, as evidenced by the recovery of the gut microbiota alpha diversity and the ratio of Firmicutes to Bacteroidetes. Considering the complicacy of components in jujube powder, which contains non-prebiotic components such as glucose, identification and quantitative analysis of prebiotics and their function should be included in further studies. The supplementation of *F. prausnitzii* alone reduced the alpha diversity of the gut microbiota, probably owing to competition with other gut microbes or other ecological effects at a high dose. Similarly, other probiotics have been reported to reduce alpha diversity in previous studies. Jing *et al.*[40] found that the alpha diversity of the gut microbiota was reduced during the administration of *Lactobacillus rhamnosus* GG in tumor vaccine studies. It was also described that probiotics delayed the post-antibiotic reconstitution of indigenous microbes in both murine models and clinical patients.[42,43] However, this unfavorable impact on the gut microbiota ecology of *F. prausnitzii* was counterbalanced by the combination of jujube powder, exhibiting significantly elevated alpha diversity in the synbiotic group. This more balanced microbial community may contribute to the mechanism by which the synbiotic is more effective in alleviating colitis. Similarly, the synbiotic significantly reversed the increase in the ratio of Firmicutes to Bacteroidetes caused by *F. prausnitzii* (Fig. 3A and B), whose deviation serves as a key indicator of gut microbiota dysbiosis in IBD.[7]

The administration of jujube powder, *F. prausnitzii*, and the synbiotic increased the abundance of probiotics, particularly strains that have been identified in previous studies to utilize carbohydrates to produce SCFAs, thereby exerting anti-inflammatory and other health benefits, including *Muribaculaceae*,[44] *Atopobiaceae*,[45] *Ruminococcaceae*,[46] *Butyricimonas*,[47] *Parasutterella*,[48] and *Parabacteroides.*[49] Simultaneously, the abundance of potentially pathogenic and inflammation-associated microorganisms decreased, including *unclassified_o__Clostridia_UCG-014*,[50] *Enterobacteriaceae*,[8] *Oscillospiraceae*,[51] and *Clostridium_sensu_stricto_1*[52] (Fig. 3C and D). While the individual contributions of jujube powder and *F. prausnitzii* were distinct in different gut microbiota families, the synbiotic bridged the gap, serving as an overall intermediary. 16S data showed the abundance at the family level. *F. prausnitzii* belongs to the family of *Oscillospiraceae* in taxonomy. The FP group had a lower abundance of *Oscillospiraceae*, while the addition of jujube powder increased the level of *Oscillospiraceae*, indicating the promotion effect of jujube powder on *F. prausnitzii* colonization (Fig. 3C).

F. prausnitzii is capable of directly utilizing simple carbohydrates such as glucose and fructose[53] and complex carbohydrates such as inulin.[54] Moreover, *F. prausnitzii* can be promoted by the cross-feeding of metabolites from other microorganisms, *e.g.,* the glucuronic acid pathway metabolized by *Parabacteroides* species[55] and the acetate pathway produced by bifidobacteria degrading fructan.[56] Jujube powder is rich in carbohydrates including glucose, fructose, oligosaccharides, and polysaccharides. Previous studies have shown that jujube powder was fermented by the gut microbiota, which not only promoted SCFA production but also strengthened the pathways of amino acids and bile acids.[24,25,40] It is possible that monosaccharides and polysaccharides of jujube powder were fermented by *F. prausnitzii* to produce anti-inflammatory SCFAs. These saccharides may also be metabolized by indigenous microbiota into acetate favorable in cross-feeding and the growth of *F. prausnitzii.* We thus conjecture that jujube powder

and *F. prausnitzii* may hold the potential of forming a synergistic synbiotic. It is also possible that jujube powder and *F. prausnitzii* may function separately as complementary synbiotics, in which jujube functions on reshaping a favorable microbial structure and metabolism whereas *F. prausnitzii* plays the major role in anti-inflammation, jointly enhancing the alleviation outcome of colitis. Additional *in vitro* and *in vivo* experiments are needed to determine the interactions between jujube powder and *F. prausnitzii*, and the cross-feeding that occurs between *F. prausnitzii* and indigenous microbiota.

Flow cytometry of immune cells in the colon, MLNs, and spleen revealed that the synbiotics modulated immune responses triggered by DSS-induced colitis. The excessive activation of $CD11b^+$ myeloid cells is indicative of inflammation.[57] Our study showed that myeloid cells increased at different levels in the colon and MLNs of DSS-induced colitis, including myeloid cells, neutrophils, monocytes, and macrophages derived from the myeloid layer, indicating local inflammation of the colon and response of peripheral lymphoid organs. The synbiotics did not significantly alleviate the infiltration of myeloid cells, possibly because innate immunity was undergoing a natural recovery process in the late stage of inflammation after DSS discontinuation (Fig. 5A–J). The expansion of B cells has been confirmed to be associated with exacerbated colitis.[58] Our results also supported this conclusion, as evidenced by significantly increased B cells in the MLNs and spleen of DSS-induced colitis mice (Fig. 6A and B and Fig. S6A†). The administration of the synbiotic J + FP-H downregulated the level of B cells in the MLNs, helping in mucosal healing (Fig. 6B). Müller *et al.* demonstrated that activated $CD4^+$ T cells increased in the gut of patients with active IBD.[59] Here we found a significant decrease of total T cells, $CD4^+$ T cells, and $CD8^+$ T cells in the MLNs and spleen (Fig. 6A, C, D and Fig. S5A and S6B–D†), which may indicate the migration of activated T cells from secondary lymphoid organs to the inflammatory site. The synbiotic J + FP-H increased $CD4^+$ T cells in the MLNs, indicating its effects in regulating adaptive immune response. The changes and functions of Th2 cells in IBD are controversial. Britton *et al.*[60] found that more Th2 cells were induced in germ-free mice by receiving microbiota from individuals with IBD. In contrast, Fan *et al.*[61] presented that chronic colitis could be ameliorated by increasing Th2 anti-inflammatory response. Here we found that Th2 cells in the MLNs were downregulated by DSS treatment, and the synbiotics J + FP-M and J + FP-H restored Th2 cells of DSS-induced colitis mice to recover the Th1/Th2 balance (Fig. 6G and H), indicating the potential of synbiotics in regulating adaptive immune homeostasis.

5 Conclusions

This study proposed a new synbiotic consisting of jujube powder and *Faecalibacterium prausnitzii*, which exerted alleviating effects by reshaping a more balanced gut microbiota in a DSS-induced colitis mouse model. This synbiotic inhibited systemic inflammatory response by preventing immune imbalance. Remarkably, the anti-inflammatory benefits of the synbiotic were dependent on the specific formulation. All these findings suggest that this novel synbiotic possesses the potential to facilitate the management of IBD and could be developed as a new dietary intervention strategy, complementing existing probiotics and prebiotics.

Author contributions

Yalin Zhou: conceptualization, investigation, formal analysis, and writing – original draft. Zhenghuan Yang: investigation and formal analysis. Huiren Zhuang: methodology and investigation. Tianhao Chen: investigation. Sherlyn Sze Ning Koay: investigation and writing – review & editing. Ran Li: investigation. Guoqiang Jiang: funding acquisition, supervision, and writing – review and editing. Zheng Liu: funding acquisition, supervision, and writing – review and editing.

Data availability

All data needed to evaluate the conclusions are present in the paper. The raw sequences of 16S rRNA are deposited in the SRA database of NCBI with BioProject ID PRJNA1155818. Additional data related to this paper may be requested from the authors.

Conflicts of interest

The authors declare that they have no competing interests.

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