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Lactobacillus rhamnosus from human breast milk ameliorates ulcerative colitis in mice *via* gut microbiota modulation†

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Gut microbiota imbalance is one of the major causes of ulcerative colitis (UC). *L. rhamnosus* SHA113 (LRS), a strain isolated from healthy human milk, influences the regulation of gut flora. This study aims to determine whether this strain can ameliorate UC by modulating gut microbiota. Mouse models of UC were established using C57BL/6Cnc mice with intragastric administration of 3.0% (w/v) dextran sodium sulfate (DSS). LRS was used to treat the mouse models of UC with 10^9 cfu mL^{-1} cell suspension *via* intragastric administration. To verify the effect of gut microbiota on UC, fecal microbiota collected from the mice after the treatment with LRS were also used to treat the UC mouse models (FMT). The severity of UC was evaluated based on body weight, colon length, disease activity index (DAI), and hematoxylin–eosin staining. The microbial composition was analyzed by 16S rRNA sequencing. The mRNA expression levels of cytokines, mucins, tight junction proteins, and antimicrobial peptides in the gastrointestinal tract were detected by quantitative real-time polymerase chain reaction. The short-chain fatty acid (SCFAs) in the cecal contents of all mice were quantitatively detected by gas chromatography and mass spectrometry. Both LRS and FMT exerted excellent therapeutic effects on UC, as evidenced by the reduction in body weight loss, colon length, and colon structural integrity, as well as the increase in the DAI (disease activity index). LRS and FMT treatments showed similar effects: (1) an increase of total SCFA production in the cecal contents and the abundance of gut microbial diversity and flora composition; (2) decreases in two genera (*Parabacteroides* and *Escherichia*/*Shigella*) related to the DAI and the enhancement of SCFAs and IL-10 positively related genera in the gut microbiota (*Bilophila*, *Roseburia*, *Akkermansia*, and *Bifidobacterium*); (3) downregulation of the expression of tumor necrosis factor-α, interleukin IL-6, and IL-1β, and upregulation of the expression of the anti-inflammatory cytokine IL-10; and (4) upregulation of the expression of mucins (Muc1–4) and tight junction protein ZO-1. Overall, *L. rhamnosus* SHA113 relieves UC *via* the regulation of gut microbiota: increases in SCFA-producing genera and decreases in UC-related genera. In addition, a single strain is sufficient to induce a significant change in the gut microbiota and exert therapeutic effects on UC.

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1. Introduction

Ulcerative colitis (UC) and Crohn's disease (CD)[1] are the two major forms of inflammatory bowel disease (IBD), a chronic intestinal inflammatory disease of the gastrointestinal tract. IBD has emerged as a public health challenge worldwide in the last decade.[2] IBD exhibits the highest prevalence in Western countries worldwide, reaching 0.5% of the general population and 10–30 per 100 000 persons.[3] In North America and Europe, more than 1.5 million and 2 million people suffer from IBD, respectively.[4] The incidence of IBD continues to increase in South America, Asia, Africa, and Eastern Europe.[5]

A recent study suggested that an increase in invasive flora and a decrease in protective flora in the intestine could be major reasons for the occurrence of UC.[6] The intestinal flora composition of patients with UC is significantly different from that of non-UC patients. The main manifestations include a reduction in the abundance of Firmicutes and an increase in the abundance of *Bacteroides* and *Actinomyces*.[7] Moreover, the

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abundance of *Bacteroides* and *Actinomyces* decreases in patients with colitis.[8] Thus, targeting intestinal flora is a potential approach to treat UC.

The mechanisms allowing probiotics to inhibit UC are generally attributed to the ability of probiotics to inhibit the colonization of pathogenic bacteria by competing for nutrients and common receptors or directly inhibiting pathogenic bacteria by producing bacteriocin or antitoxin proteases.[9] Probiotics maintain the normal intestinal flora, strengthen the mucosal barrier effect, suppress exposure to inflammatory signals, and adjust the unbalanced immune response of the immune system to inhibit host mucosal damage. The probiotics currently used to relieve colitis are *Lactobacillus* and *Bifidobacterium.*[10] The use of probiotics or fecal bacteria to change intestinal flora may be a new treatment strategy for UC.

L. rhamnosus is a probiotic approved by the Food and Drug Administration to be used in infant foods. Existing reports indicate that *L. rhamnosus* can adhere to and colonize the surface of the intestinal mucosa to form a biological barrier and thus hinder the invasion of the human intestinal tract by pathogenic microorganisms after entering the human digestive tract.[11] *L. rhamnosus* also metabolizes and produces bacteriostatic active substances in the intestinal tract, significantly reducing the viability of *Salmonella*, *Escherichia coli*, *Staphylococcus*, and other pathogens.[12] However, the use of *L. rhamnosus* as a treatment for UC has not been reported.

To evaluate the capability of *L. rhamnosus* in the amelioration of UC, *L. rhamnosus* SHA113, a strain previously isolated from human milk and proved to efficiently regulate gut microbiota, was used to treat UC in mice.

2. Materials and methods

2.1 Bacterial strains

L. rhamnosus SHA113 (coded as CCTCCM2017839), which was previously isolated from the breast milk of healthy women and stored at the China Typical Microorganism Preservation Center (Wuhan, Hubei Province, China),[13–16] was used in the study and is indicated as LRS in the subsequent sections. It was prepared from a 48 h static culture of SHA113 in the media of deMan, Rogosa, and Sharpe broth (Hopebio, Qingdao, China) at 37 °C without shaking.

2.2 Animal experiments

Forty male C57BL/6Cnc mice (aged 8 weeks and weighing 18–22 g) were obtained from Beijing Vital River Laboratory Animal Technology Limited Company, where they were maintained under specific pathogen-free conditions with a 12 h/12 h light/dark cycle; at a temperature of 21 °C ± 2 °C; at a humidity of 45%±10%; and with free access to food and water. All animal experiments were performed as per the guidelines of the Experimental Animal Care and Ethics Committee of Northwestern Polytechnical University. The approval number for the animal study was No. 202101002. After acclimatization for 1 week, the mice were randomly separated into four groups, with each group consisting of 10 mice. The animal experiments were divided into two parts, LRS intervention and fecal microbiota transplantation (FMT).

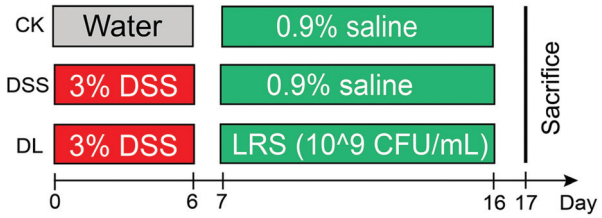

Fig. 1 Treatment scheme of LRS intervention.

The treatment of each group in the LRS intervention experiment is shown in Fig. 1: in this experiment, mice were fed freely and there was no difference in food intake among different groups. We weighed the mice before intragastric administration, and then intragastric administration of 10 μL g^{-1} of the body weight of mice. All the mice had free access to drinking water throughout the whole experiment. The mice in different groups were subjected to the treatments as follows: control group (CK), intragastric administration of 0.9% saline at days 7–16; mice in the model control group (DSS), provided water with 3.0% (w/v) dextran sodium sulfate (DSS with a molecular weight of 36–50 kDa, Shanghai Aladdin Biochemical Technology Co., Ltd) at days 0–6 and then intragastric administration of 0.9% saline at days 7–16; and mice in the LRS intervention group (DL), provided water with 3.0% (w/v) DSS at days 0–6 and then intragastric administration of 10^9 cfu mL^{-1} SHA113 (resuspended with 0.9% saline) at days 7–16.

An experiment involving FMT was performed to identify the capability of *L. rhamnosus* SHA113 influenced intestinal flora in the alleviation of colitis. For the experiments, gut microbiota-depleted DSS mice were reconstituted with fecal pellets from the DL group.[17] As shown in Fig. 2, the mice in the FMT intervention group (FDL) were provided water with 3.0% (w/v) DSS for 7 d (day 0 to day 6) and then subjected to intragastric administration of mixed antibiotics (metronidazole (1 g L^{-1}), vancomycin (1 g L^{-1}), and streptomycin (2 g L^{-1}) for 3 d (day 7 to day 9). Then, the mice received intragastric administration for 7 d (day 10 to day 16) by using the feces collected from DL groups. Before they were used, the feces were resuspended in 0.9% saline at a concentration of 0.125 g mL^{-1}. After the intervention, all mice were euthanized with an intraperitoneal injection of pelltobarbitalum natricum at 0.01–0.02 ml g^{-1} for different analyses on day 17.

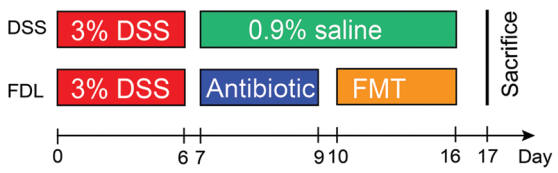

Fig. 2 Treatment scheme of FMT intervention.

2.4 Sample collection

During the experiments, all mice were fed freely and checked daily for morbidity and the food consumption and body weight were recorded. Every mouse was assigned scores daily for their pathological features, such as stool consistency, presence of blood stool, and body weight loss. Individual scores were combined to determine the disease activity index (DAI) of each mouse, which was calculated daily.[18] The maximum score was set to 12 based on a 0–4 scoring system by using the parameters listed in Table S1.†

At the end of all treatments, all mice were sacrificed after anesthesia using 0.1% pentobarbital sodium at the dosage of 40 mg kg^{-1} by intraperitoneal injection. A postmortem examination was performed to preliminarily examine the external surface, thoracic and abdominal cavities, and cecal contents of the mice. The spleen, kidneys, and liver were weighed after dissection. The samples for the test of bacterial flora in the cecal contents were collected and stored at −80 °C before they were subjected to 16S rRNA sequencing analysis. The colon tissues were collected and sliced into three sections. The proximal colon was used for the analysis of pro-inflammatory cytokines (kept in liquid nitrogen immediately), the middle portion for RNA isolation (kept in liquid nitrogen immediately), and the distal colon for histology (fixed in 4% paraformaldehyde).

2.5 Histopathology of intestinal tissue section structures

After the sacrifice of mice, the colon was immediately collected and stored in 4% paraformaldehyde at room temperature. Paraffin sections were dewaxed to water, stained with hematoxylin, and then dehydrated and sealed with eosin. Microscopic examination, image acquisition, and analyses were performed. Then, the full-scan organ images were taken to observe the structure of the intestinal tissue section using Case Viewer 2.4 software (The Digital Pathology Company, Germany).

2.6 Intestinal flora analysis

Cecal contents were collected from 6 mice per group for microbiome analysis. Bacterial genomic DNA was extracted from the prepared frozen cecal samples by using the QIAamp DNA Stool Mini Kit from Qiagen (Hilden, Germany) in accordance with the manufacturer's protocols. The bacterial 16S rDNA gene comprising V3–V4 hypervariable regions was amplified by PCR using barcoded primers adapted from existing universal primers of 341F and 806R. Subsequently, 16S rDNA high-throughput sequencing was performed on the Illumina MiSeq PE250 platform at Realbio Technology Co., Ltd (Shanghai, China). Trimmed high-quality reads from 24 cecal samples were clustered into 481 operational taxonomic units (OTUs), based on 97% sequence similarity. Taxonomic assignment of the representative sequence of each OTU was conducted by comparing with a customized collection of reference sequences based on the Ribosomal Database Project (RDP) at an 80% confidence level.[19,20] For the alpha diversity analysis, Shannon, Chao1, Simpson, and observed species indexes for each group were determined using the software QIIME. Differences in bacterial communities among different groups were determined by principal coordinate analysis (PCoA), based on weighted UniFrac distances and unweighted UniFrac distances.

2.7 Quantitative analysis of targeted short-chain fatty acids

For measurements of short-chain fatty acids in the cecal contents of mice, the collected cecal contents (50 mg) were mixed with 50 μL of internal standards (5% phosphoric acid, Sigma-Aldrich), 100 μL of a 125 μg mL^{-1} internal standard (isohexanoic acid) solution, and 400 μL of ether. The SCFAs were then extracted following the manufacturer's protocol (BioNovoGene Technology Co., Ltd, Suzhou, China). The obtained SCFA samples were measured using a TRACE 1310-ISQ LT GC–MS system (Thermo Fisher Scientific, USA). The used SCFA standards were a mixture of standard acetic acid, propionic acid, isobutyric acid, butyric acid, isovaleric acid, valeric acid, and caproic acid. All standards were purchased from Sigma-Aldrich (Shanghai, China).

2.8 Expression of proteins in the colonic tissue

The expression levels of proteins related to the intestinal barrier function (Muc1, Muc2, Muc3, Muc4, and ZO-1), inflammatory cytokines (IL-6, TNF-α, IL-1β, and IL-10), and antimicrobial peptides (S100A8 and Camp) in the colonic tissue were measured by quantitative real-time polymerase chain reaction analysis. The expression of the glyceraldehyde-3-phosphate dehydrogenase gene was also measured as the internal control. For measurements, the middle colonic tissue was homogenized, and total RNA was extracted using the TRIzol method (Servicebio, China). The obtained colonic RNAs were purified using the lithium chloride protocol to remove residual DSS contaminants. Complementary DNA was synthesized using reverse transcriptase kits (Thermo Fisher Scientific, USA). Real-time PCR was performed using the LightCycler 480 System (Roche, China) using SYBR Green (Sangon, China). The relative mRNA expression was analyzed using the comparative Ct method. The primers used in this study are listed in Table S2.†

2.9 Statistical analysis

The data of each group were collected from 6 randomly selected mice. All data were statistically analyzed using the software GraphPad Prism 8.0 (GraphPad Software Inc., CA, USA). Student's *t*-test (unpaired, two-tailed) was used to determine the levels of significance for comparison between two groups. Relative abundances of different microbiota were compared in the same group using the Wilcoxon test. Statistical significance among more than two groups was calculated using one-way ANOVA with Tukey's test. Statistical significance was indicated as follows: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ****, $P < 0.0001$; "ns", no significant. The results were reported as mean ± SD. Spearman's correlation between the bacteria and the UC-related parameters was conducted using the programming language R (V4.0.2, AT&T Bell Laboratories, New Zealand).

3. Results

3.1 LRS administration attenuates UC

Compared with the CK group, DSS administration caused significant reductions in body weight (Fig. 3a) and colon length (Fig. 3b) and resulted in considerably higher DAIs (Fig. 3c) in mice ($P < 0.05$). The DAI was obtained based on stool consistency, bloody stool, and body weight. This indicated that a mouse model of UC was successfully established. Compared with the DSS group, the LRS group showed significant increases in body weight and colon length and reductions in the DAI ($P < 0.05$). The DAI was reduced to that of the CK group. This finding indicated that LRS administration could efficiently treat DSS-induced UC.

H&E staining of the colon tissue (Fig. 3d) indicated that DSS administration caused the apparent loss of crypts, infiltration of mononuclear cells, and severe mucosal damage. These disease symptoms were largely reversed with DL treatments, as indicated by reduced inflammatory cell infiltration, relatively intact colonic architecture, and less mucosal damage. All results demonstrated that LRS administration significantly alleviated the DSS-induced pathogenic symptoms of UC.

3.2 Fecal microbial transplantation mitigates UC

As shown in Fig. 4, FMT treatment significantly ameliorated DSS-induced UC as evidenced by the markedly reversed weight loss (Fig. 4a) and significant relief of colon shortening (Fig. 4b), which were consistent with the reduction of the DAI index (Fig. 4c) ($P < 0.05$). H&E staining of the colon tissue (Fig. 4d) indicated that DSS administration caused the apparent loss of crypts, infiltration of mononuclear cells, and severe mucosal damage. These disease symptoms were largely reversed with FMT treatments, as indicated by reduced inflammatory cell infiltration, relatively intact colonic architecture, and less mucosal damage (FDL group). The results indicate that the changed intestinal microbiota play an important role in the therapeutic effects of *L. rhamnosus* SHA113 on UC.

3.3 Composition of intestinal flora

Shannon and Simpson indexes indicated no significant differences in the alpha diversity of intestinal flora among different groups (Fig. S1†). However, significantly lower Chao1 and observed species indexes were observed in the DSS group than in the CK group. These two indexes were significantly increased after administration with LRS and FMT ($P < 0.05$) (Fig. 5a and b).

Analysis of the landscape of gut microbiota indicates differences in the potential composition among the four groups. At the phylum level, the four groups shared similar taxonomic communities and exhibited relatively high abundances of Bacteroidetes, Firmicutes, and Proteobacteria (Fig. 5c). Compared with the CK group, the DSS group showed a significant decrease in Bacteroidetes and increases in Proteobacteria and the Firmicutes/Bacteroidetes (F/B) ratio ($P < 0.05$). Such changes were largely reversed to the CK group level after LRS and FMT administration (Fig. S2a and S2b†). The taxonomic composition was analyzed at the class/order/family level (Fig. S3a–S3c†), whereas the taxonomic communities were analyzed at the genus level (Fig. 5d).

For further understanding of the role of microbiome diversity, beta-diversity analysis was also performed to generate a PCoA plot by using unweighted-UniFrac distance and weighted-UniFrac distance algorithms (Fig. 5f). Apparent clustering separation among different OTUs revealed variations in the community structures in the four groups, suggesting the distinct composition of the communities.

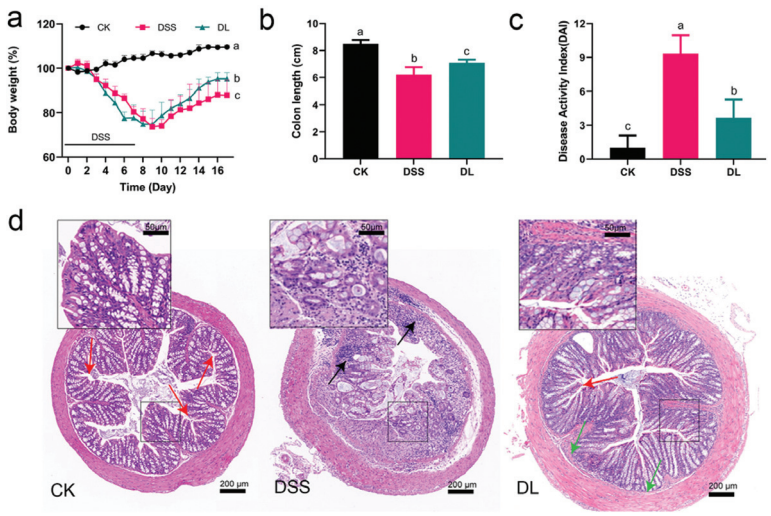

Fig. 3 Changes in the UC parameters of mice during the treatment with LRS. (a) Body weight. (b) Colon length. (c) Disease activity index. (d) Representative H&E staining microscopic images (scale bars 200 and 50 μm) of colon tissues from different groups. Different letters in each figure indicate significant differences in the data at $P < 0.05$, calculated by one-way ANOVA with Tukey's multiple comparison test. The red arrow denotes the crypt structure, the black arrow indicates inflammatory cell infiltration, and the green arrow denotes the mucosal layer. CK, the normal group; DSS, the UC model group without treatment; DL, the DSS model group treated with *L. rhamnosus* SHA113 cells (10^9 cfu mL^{-1}).

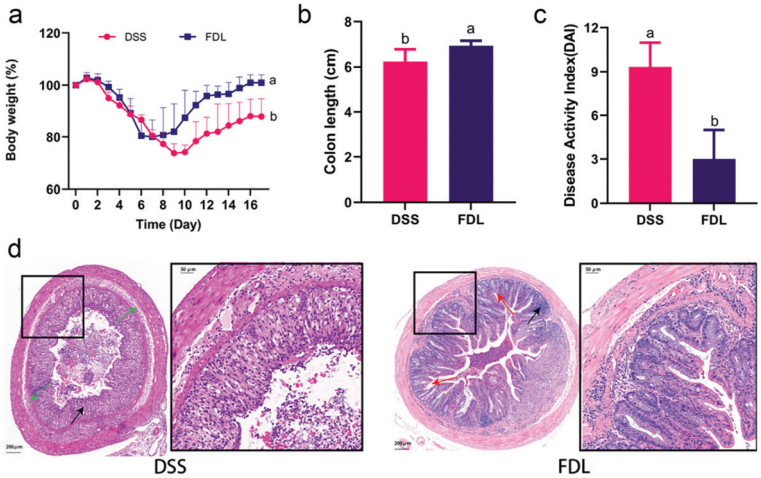

Fig. 4 Changes in the UC parameters of mice during the treatment with FMT. (a) Body weight. (b) Colon length. (c) Disease activity index. (d) Representative H&E staining microscopic images (scale bars 200 and 50 μm) of colon tissues from different groups. Different letters in each figure indicate significant differences in the data at $P < 0.05$, calculated by Student's *t*-test (unpaired, two-tailed). The red arrow denotes the crypt structure, the black arrow indicates inflammatory cell infiltration, and the green arrow denotes the mucosal layer. DSS, the UC model group without treatment; FDL, the DSS model group treated with FMT from *L. rhamnosus* SHA113 treated mice.

As shown in Fig. 5e, relative to the CK group, the DSS group shows significant increases ($P < 0.05$) in 14 genera and decreases in 17 genera. LRS and FMT administration significantly reversed the increases in *Parabacteroides* and *Escherichia/Shigella*, among the genera exhibiting increases, and the decrease in *Roseburia* caused by DSS. This finding suggests that the changes in these three genera are the key factors that contribute to the therapeutic effect of LRS on UC. In addition to the effect of DSS, the administration of LRS and FMT also induced significant increases in the genera *Bilophila*, *Akkermansia*, and *Bifidobacterium* ($P < 0.05$). The increases in these three beneficial genera might also play an important role in the treatment of UC.

In addition to the aforementioned common reversed genera, 2 other DSS-increased genera (*Clostridium* XIVa and *Oscillibacter*) and 2 DSS-decreased genera (*Olsenella* and *Staphylococcus*) were also reversed by LRS administration. Six other DSS-increased genera (*Pseudoflavonifractor*, *Bacteroides*, *Butyricimonas*, *Clostridium* XlVb, *Helicobacter*, and *Klebsiella*) and 5 DSS-decreased genera (*Erysipelotrichaceae incertae sedis*, *Enterorhabdus*, *Prevotella*, *Odoribacter*, and *Parvibacter*) were reversed by FMT administration. Notably, 4 other DSS-increased genera and 7 DSS-decreased genera were neither reversed by LRS nor FMT administration, indicating that they are not crucial to the treatment of UC.

In addition, LRS administration also caused significant increases in 2 genera without DSS (*Flavonifractor* and *Parasutterella*) and large decreases in 4 genera without DSS (*Clostridium* IV, *Lachnospiracea incertae sedis*, *Ruminococcus*, and *Streptococcus*). Similarly, FMT administration also caused significant increases in 3 genera when not administered with DSS (*Blautia*, *Coprobacillus*, and *Faecalibacterium*) ($P < 0.05$) (Fig. 5e and S4a–S4c†).

Overall, FMT administration could reverse more changes in genera, compared with LRS administration. This finding is consistent with the aforementioned results indicating that compared with LRS administration, FMT administration slightly led to a slightly higher diversity of gut microbiota.

3.4 Production of targeted SCFAs in the cecal contents

The levels of different SCFAs (acetic acid, butyric acid, propionic acid, valeric acid, caproic acid, isobutyric acid, and isovaleric acid) in cecal samples were evaluated using a targeted metabolomics assay (Fig. 6). Relative to the CK group, the DSS group showed a significant decline in most SCFAs, except for isovaleric acid ($P < 0.05$). Relative to the DSS group, the groups administered with LRS and FMT showed significant increases in butyric acid, propionic acid, valeric acid, isobutyric acid, isovaleric acid, and total SCFAs to a level similar to or even higher than that of CK ($P < 0.05$). Compared with LRS administration, FMT administration also resulted in higher increases in butyric acid, valeric acid, and isobutyric acid. However, compared with the CK group, the FMT group showed decreases in acetic acid as DSS and caproic acid as DSS and LRS. A decrease in acetic acid is beneficial to human and animal health.[21–23] Therefore, compared with LRS administration, FMT administration can more efficiently reverse the adverse effects of UC on the SCFAs in the cecal contents.

3.5 Organ index, intestinal permeability, and cytokine expression

After sacrifice, the spleen, liver, kidneys, and thymus were separated and then weighed to calculate the index of each organ *versus* body weight. No significant difference in the thymus index was found among the four groups. The spleen, liver, and

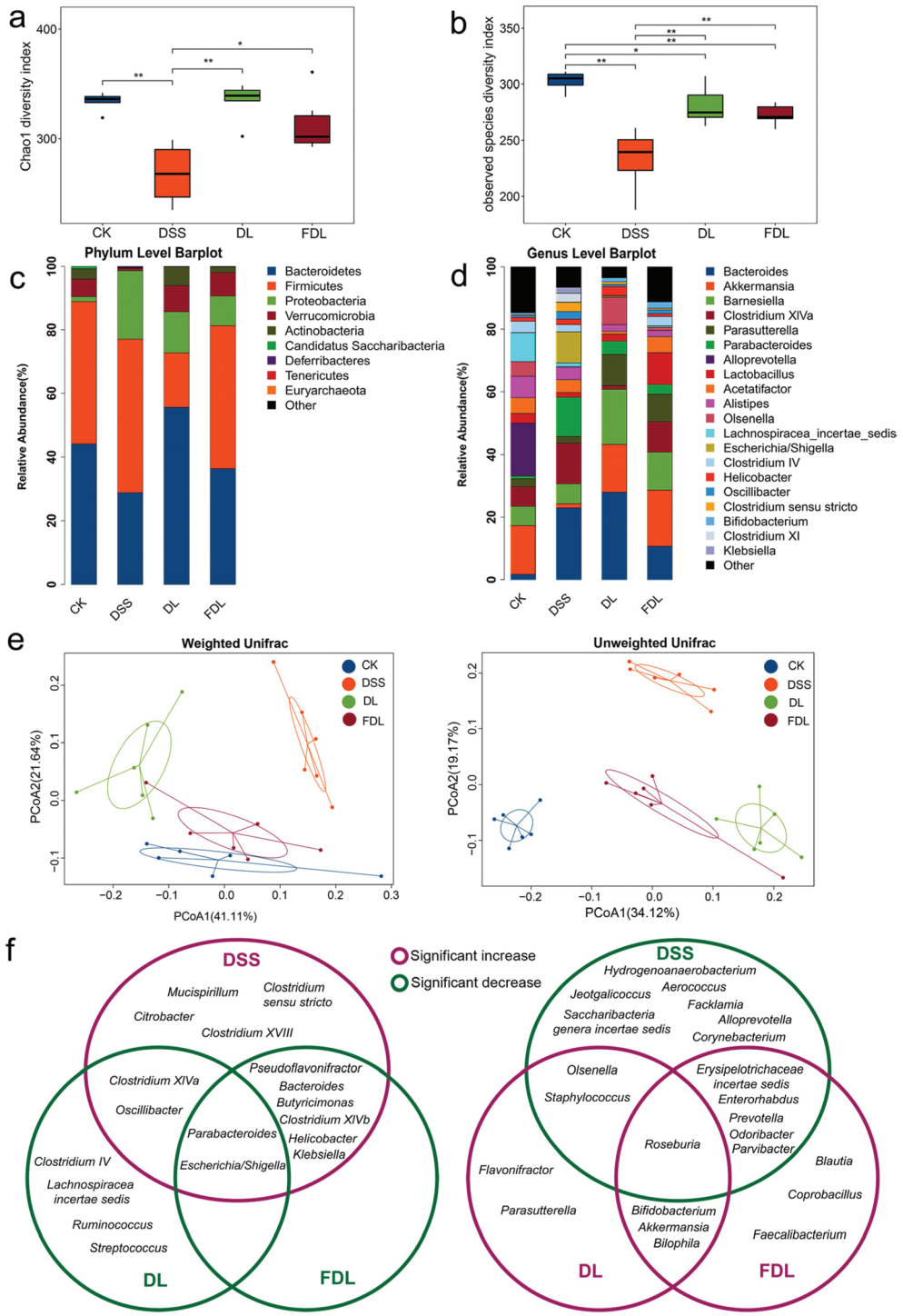

Fig. 5 Gut microbiota diversity and the composition of different groups. (a) Alpha diversity boxplot of the Chao index; (b) observed species diversity. Bar plots of the taxonomic composition at the phylum level (c) and the genus level (d). (e) PCoA using unweighted-UniFrac and weighted-UniFrac of beta diversity. (f) Differential bacteria ($P < 0.05$) at the genus level in the DSS group (compared with the CK group) and the DL and FDL groups (compared with the DSS group). (a–f) $n = 6$ mice per group. Statistical significance was calculated using one-way ANOVA with Tukey's multiple comparison test, and the significance was at $P < 0.05$.

kidneys of the DSS group showed considerable enlargement relative to those of the CK group. The intervention with LRS (DL group) and FMT (FDL group) exhibited similar efficiencies in alleviating this enlargement to a level similar to that in the CK group, except for the reduced effect of LRS on the spleen index (Fig. 7a).

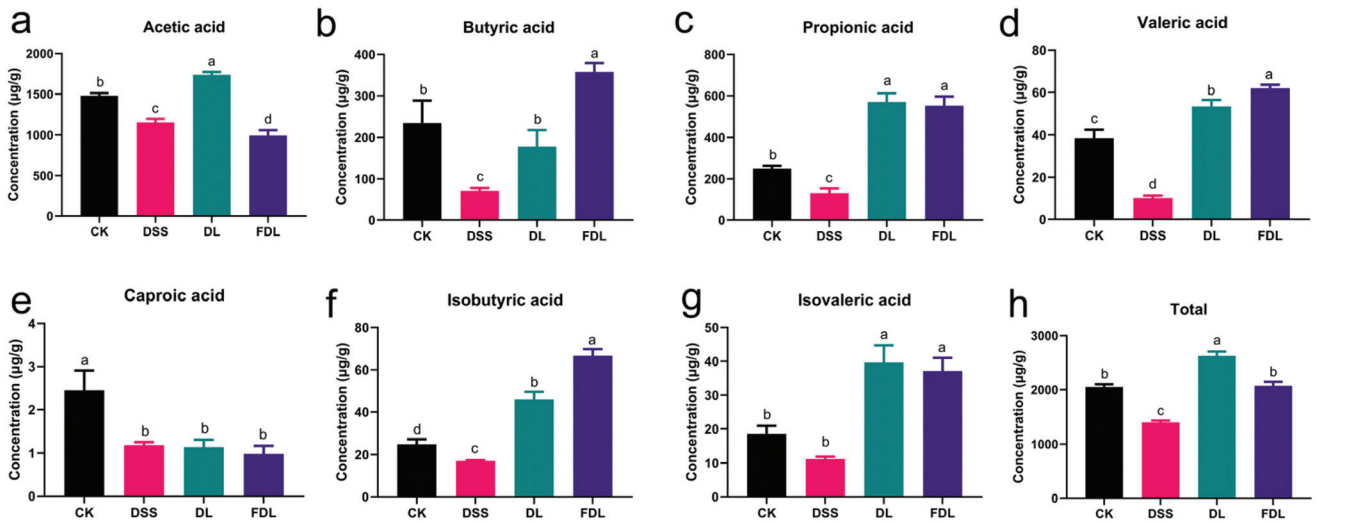

Fig. 6 SCFA content in the cecal samples. (a) Acetic acid; (b), butyric acid; (c) propionic acid; (d) valeric acid; (e) caproic acid; (f) isobutyric acid; (g) isovaleric acid; (h) total SCFAs. Data for each group are reported as the mean values of 6 mice. Bars denote the standard deviations of 6 replicates. The statistical significance was calculated using one-way ANOVA with Tukey's multiple comparison test. Different letters in each figure indicate significant differences in the data at $P < 0.05$.

As shown in Fig. 7b, compared with the CK group, DSS administration significantly increased the expression of the pro-inflammatory cytokines IL-1β, TNF-α, and IL-6, but decreased the expression of the anti-inflammatory cytokine IL-10 ($P < 0.05$). LRS and FMT administration efficiently reversed such changes, although not to the level similar to that in the CK group. Compared with LRS administration, FMT administration more efficiently reduced IL-1β expression, but less efficiently decreased TNF-α. These results indicated that LRS and FMT administration could inhibit the inflammation induced by UC.

The expression of mucins and antimicrobial peptides in the colon was also determined to evaluate whether LRS and FMT administration exerted protective effects on gut barrier functions. DSS administration significantly inhibited the expression of mucins (*Muc1*, *Muc2*, *Muc3*, and *Muc4*), barrier function protein (*ZO-1*), and antimicrobial peptides (*Camp* and *S100A8*) in the colonic tissue of the mice relative to those of the mice in the CK group ($P < 0.05$) (Fig. 7c and d). Such reductions in the expression of *Muc1*, *Muc2*, *Muc3*, *Muc4*, and *ZO-1* were reversed by LRS and FMT administration to varying extents. FMT administration further increased the expression of *Muc1* and *ZO-1* relative to those in the CK group, whereas LRS administration caused *Muc 2* to reach the same level as that in the CK group (Fig. 7c). Overall, FMT administration more efficiently improved the barrier function, compared with LRS administration.

In addition, LRS administration further increased the expression of the antimicrobial peptide *Camp*, whereas FMT administration inhibited the expression of *Camp* but significantly increased the expression of the antimicrobial peptide S100A8 ($P < 0.05$) (Fig. 7d). Both Camp and S100A8 exhibited anti-inflammatory activities during acute intestinal inflammation. The results indicated that FMT and LRS administration exerted different effects on the expression of antimicrobial peptides in the colonic tissue.

Considering all the results, FMT administration showed greater efficiency in reducing the spleen index and IL-1β, and increasing the barrier function; however, it exhibited less efficiency in enhancing the production of antimicrobial peptides and reducing the expression of the antimicrobial peptide *Camp*. These changes could be attributed to their different effects on changes in the gut microbiota and SCFAs.

3.6 Correlation between intestinal flora and UC indexes

A heatmap of Spearman's correlation between bacterial abundance and UC-related indexes (body weight; DAI; colon length; spleen, liver, kidney, and thymus indexes; expression levels of cytokines, antimicrobial peptides, and mucins) was generated to identify the potential correlation between the gut microbiota and UC-related indexes. The clustering results indicate that the bacterial genera were clearly divided into four groups.

As shown in Fig. 8, the genera in group A are positively correlated with the organ index, DAI, and pro-inflammatory factors, but negatively correlated with mucins, anti-inflammatory factors, and SCFAs. The abundances of these genera were significantly increased in the DSS group, suggesting that an increase in any of these bacteria could aggravate UC. The genera in group B mainly exhibited a positive correlation with the different SCFAs and IL-10 expression, suggesting that an increase in these genera can enhance the production of SCFAs in the cecal samples and the anti-inflammatory effect. The genera in group C primarily exhibited a negative correlation with the DAI indexes and pro-inflammatory cytokine expression and a positive correlation with the expression levels of mucins, colon length, and body weight. These results indi-

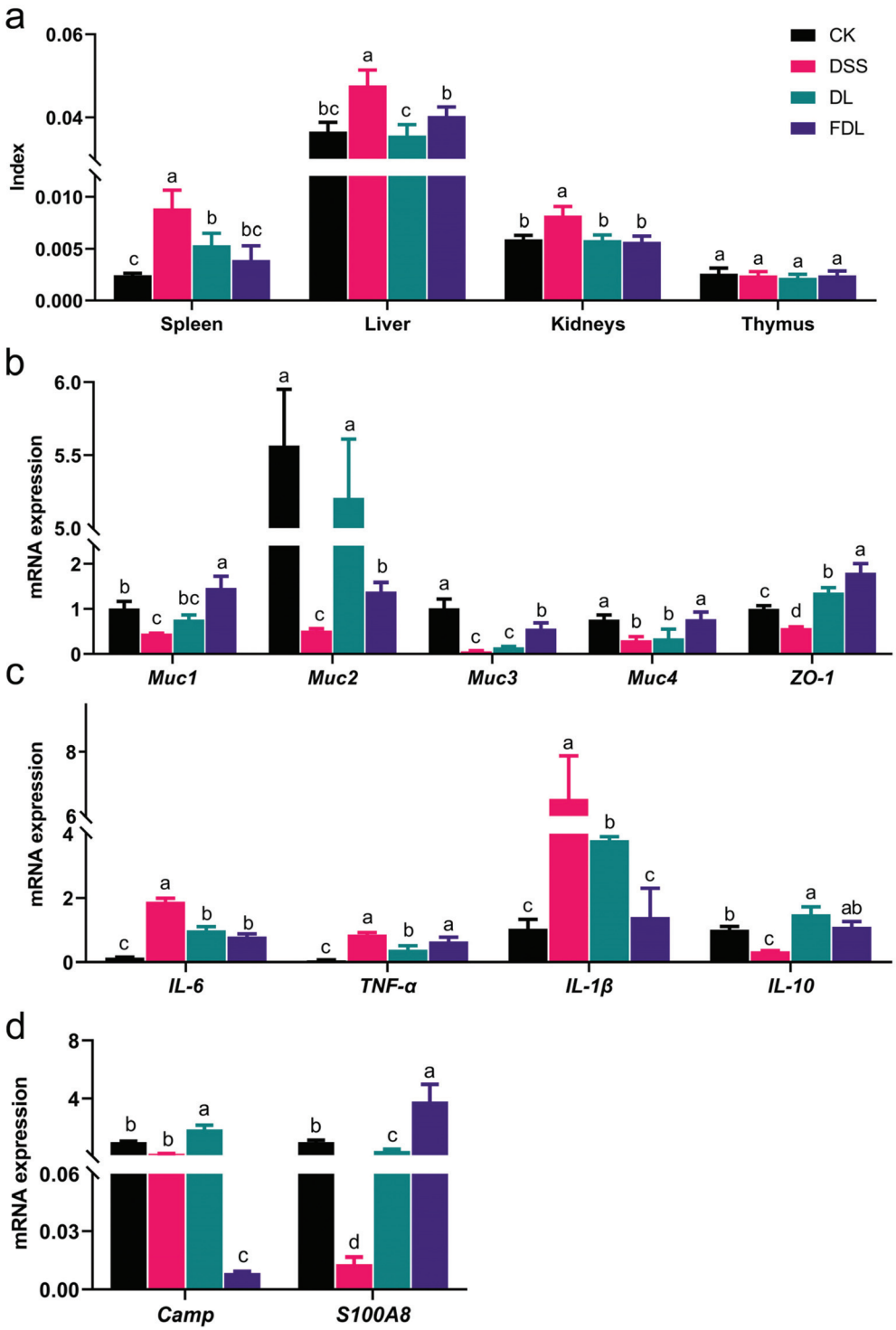

Fig. 7 Effects of LRS and FMT interventions on the organ index (a) and the colonic expression of genes related to the intestinal barrier function (b), inflammatory cytokines (c), and antimicrobial peptides (d). All data presented are the mean values for the 6 mice in each group. The bars indicate the standard deviations. Statistical significance is calculated using one-way ANOVA with Tukey's multiple comparison test. Different letters for the same parameter indicate significant differences among different groups at $P < 0.05$.

cate that an increase in genera exerts therapeutic effects on UC. The genera in group D are positively correlated with some SCFAs, the antimicrobial peptide S100A8, or the expression of mucins or ZO-1, indicating that they mainly exhibit antibacterial activities and restore the gut barrier function by producing anti-inflammatory peptides or SCFAs.

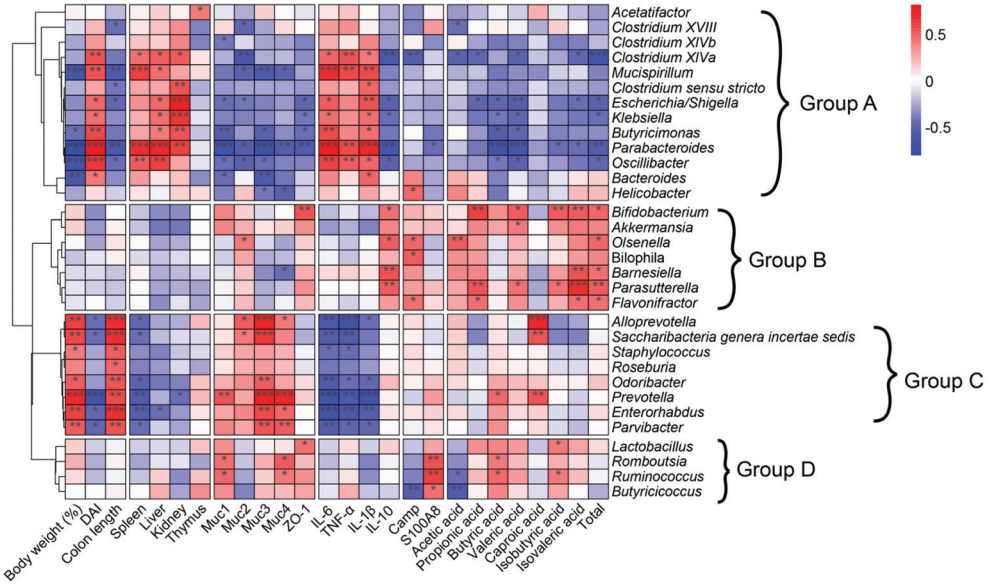

Fig. 8 Heatmap of Spearman's correlation between the bacteria and UC-related parameters. Significant difference determined at *P < 0.05; **P < 0.01; ***P < 0.001; ****P < 0.0001. Groups A, B, C, and D are formed based on the clustering results of the bacterial genera.

The correlation between different genera and UC-related indexes is summarized in Table S3.† The genera increased by DSS administration (group A) leads to an increase in the DAI of UC by reducing the expression of mucin genes and the tight junction protein ZO-1, upregulating the expression of pro-inflammatory cytokines and reducing the expression of the anti-inflammatory cytokine IL-10. The severity of UC is indicated by the enlargement of the organ index and decreases in colon length and body weight. Moreover, the abundances of these genera are negatively correlated with the SCFA contents in the cecal samples, suggesting that an increase in SCFAs can inhibit the growth of these pathogenic genera. However, the SCFAs positively correlated with the genera (group B) cannot significantly reduce the DAI. However, most of them are significantly correlated with an increase in IL-10 expression, and some of them are correlated with increases in the expression of Muc2, Muc4, or ZO-1. This finding indicated that these genera could suppress the growth of the pathogenic genera in group A by increasing IL-10 expression. However, the genera negatively correlated with the DAI (group C) could simultaneously induce increases in SCFAs (particularly caproic acid and butyric acid), mucin genes, colon length, and body weight, and reduce the expression of pro-inflammatory cytokines. The genera producing the antimicrobial peptide S100A8 (group D) could also increase the content of some SCFAs (butyric acid and isobutyric acid) and the expression of mucin genes. However, not all significantly changed genera were regulated by LRS or FMT.

3.6 Correlation between SCFAs and UC indexes

The correlation between the SCFA contents and UC indexes is summarized in Fig. 9. The contents of butyric acid, valeric acid, isobutyric acid, propionic acid, and isovaleric acid are positively correlated with the expression levels of IL-10, ZO-1, and mucins, but negatively correlated with the DAI and the expression levels of IL-6 and IL-1β, indicating their importance

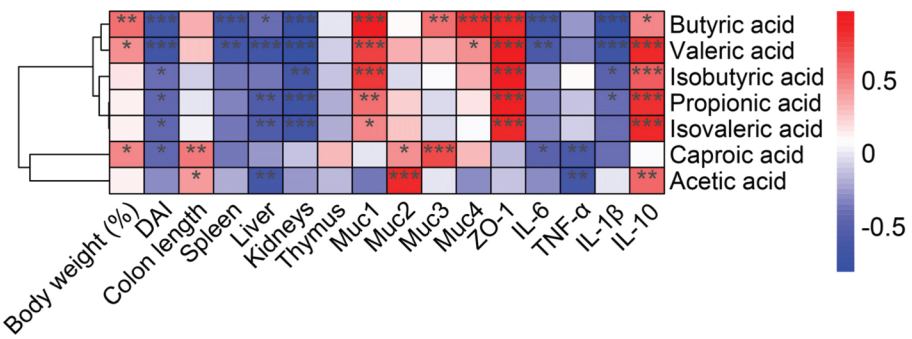

Fig. 9 Heatmap of Spearman's correlation between SCFAs and UC indexes. Significant differences at *P < 0.05; **P < 0.01; ***P < 0.001; ****P < 0.0001.

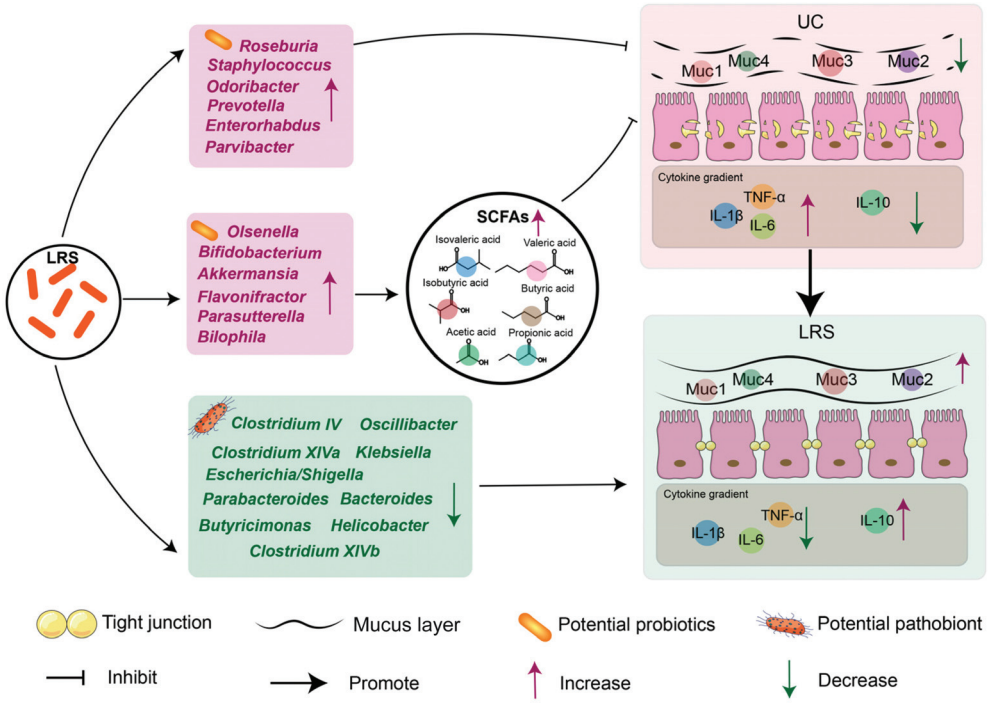

Fig. 10 Overall mechanisms of LRS for the treatment of UC.

in anti-inflammation and restoration of the gut barrier function during the treatment of UC. Notably, the contents of caproic acid and acetic acid are negatively correlated with the expression levels of TNF-α and the DAI and positively correlated with the expression levels of mucins and colon length. This result varies from those of other SCFAs. Comparatively, caproic acid was more negatively correlated with the DAI, and acetic acid was more negatively correlated with the liver index. Caproic acid may be an important SCFA in the treatment of UC. This finding has been rarely reported.

3.6 Overall mechanisms of LRS for the treatment of UC

Considering that the genera are significantly regulated by LRS or FMT, it leads to the conclusion that LRS administration reduces the abundance of DAI-related pathogenic genera and enhances the SCFA-producing genera, inducing increases in the expression of IL-10 and the production of SCFAs, as well as an improvement in the gut barrier function. The influenced change in the gut microbiota (FMT) can directly treat UC. These are summarized in Fig. 10. The decrease in pathogenic *Escherichia/Shigella* and the increase in SCFA-producing bacteria *Akkermansia*, *Bilophila*, and *Bifidobacterium* may be the key mechanisms underlying the therapeutic effects of LRS. The change in gut microbiota increased the expression of mucins and ZO-1 in the gut barrier and the anti-inflammatory factors. The increases in intestinal SCFAs may induce the expression of IL-10, promote the growth of therapeutic genera, and inhibit the pathogenic genera. The therapeutic effects can be enhanced by gut microbiota changes caused by LRS administration, as evidenced by the more significant effects of FMT than those of LRS.

4. Discussion

In this study, *L. rhamnosus* SHA113, a strain isolated from healthy human breast milk, was first used to treat DSS-induced UC in mice. The results indicated that *L. rhamnosus* SHA113 exerted a superior therapeutic effect on UC, as evidenced by reductions in body weight loss, DAI, colon length, and the integrity of the colon structure. FMT was used to confirm that the mechanisms were attributable to the change in gut microbiota. Many single strains have been reported to treat diseases *via* the regulation of intestinal microbiota, such as *Lactobacillus plantarum* NA136 for nonalcoholic fatty liver disease,[24] *L. plantarum* LIP-1 for hyperlipidaemic rats,[25] *B. pseudocatenulatum* LI09 and *B. catenulatum* LI10 for liver injury,[26] and *S. boulardii* for UC carcinogenesis.[27] FMT was also used to alleviate experimental colitis in mice[28] and pediatric UC cases of clinical patients.[29] Modulation of gut mucosal microbiota has been proved as a mechanism of probiotic-based adjunctive therapy for UC,[30] which is consistent with the results of the current study. *L. rhamnosus* LRa05 regulates gut microbiota to improve lipid accumulation in mice fed with a high-fat diet by regulating the intestinal microbiota, reducing glucose content, and promoting carbohydrate metabolism in the liver.[31] The present study is the first to report on the capability of *L. rhamnosus* to exert therapeutic effects on UC *via* the regulation of gut microbiota. This finding indicated

that the administration of a single strain, *L. rhamnosus* SHA113, is sufficient to cause a change in the gut microbiota to treat UC. Therefore, this strain has the potential to be developed as a microbial drug for the treatment of UC.

Using DSS to induce the occurrence of UC provides reliability to the results. The construction of animal models often determines the success or failure of experiments. Numerous animal models have been developed to characterize the complexity of the pathogenesis of IBD, delineate the underlying molecular mechanisms, and evaluate potential human therapeutics.[32] DSS, as a chemical agent for inducing experimental UC, is widely used because of its rapidity, simplicity, reproducibility, controllability, and many similarities with UC in humans.[33,34] The mechanism by which DSS induces intestinal inflammation is yet to be determined, but damage to the epithelial monolayer lining in the large intestine is a possible mechanism, which allows the dissemination of pro-inflammatory intestinal contents (bacteria and their products) into the underlying tissue.[35] In the current study, 3.0% DSS was used to successfully induce UC, and a certain process was determined as the mechanism of DSS-induced UC. The results showed that DSS disrupted the diversity and composition of gut microbiota and the intestinal tissue structure, increased the expression levels of the pro-inflammatory factors IL-6, TNF-α, and IL-1β, and decreased the expression levels of the anti-inflammatory factor IL-10 and mucins. Moreover, the expression levels of the two anti-microbial peptides Camp and S100A8[36] decreased.

A change in gut microbiota is vital to successfully treat UC by LRS administration. The microbiome in UC is different from that in healthy individuals, as indicated by the alpha diversity boxplot (which evaluates species richness or the presence of the taxa), β-diversity (determines the presence and abundance of the taxa and how they are related to the community), the relative abundance of the flora, the contents of probiotics and harmful bacteria, and the composition of the intestinal flora.[37,38] Dysbiosis (a state of imbalance or altered composition or altered function of the microbiota, leading to altered host–microbe interactions) occurs when harmful microbes overtake the beneficial ones, which is particularly observed in diseased states.[39–42] The proportions of several butyrate-producing bacteria, such as those of the genera *Roseburia*, *Coprococcus*, and *Ruminococcus*, were significantly reduced; meanwhile, the pathogens *Escherichia*/*Shigella* and *Enterococcus* were prevalent in patients with IBD.[43] An increase in *Escherichia*/*Shigella* and a decrease in *Faecalibacterium* indicate the onset of a newly diagnosed untreated inflammatory bowel disease associated with an increase in pro-inflammatory cytokines and a decrease in anti-inflammatory bacteria.[44] A decrease in a single species of *Clostridium* subcluster XIVa was observed in UC patients.[45] Remission of UC by FMT was associated with the proportions of *Clostridium* clusters IV and XIVa in the patients.[46] In the current study, *Escherichia*/*Shigella* and *Clostridium* subcluster XIVa were increased, whereas the genus *Roseburia* was decreased in DSS-induced UC mouse models. Microbial dysbiosis (mainly characterized by an increased abundance in *Escherichia*/*Shigella*) and a skewed negative correlation between *Escherichia*/*Shigella* and the phylum Firmicutes are two characteristics of inflamed mucosae in patients with UC.[47] This observation is consistent with previous studies on mice and patients. In addition, an increase in cytotoxin-producing *Klebsiella* was also found. However, some genera that were positively correlated with the remission of UC (*Oscilibacter*, *Parabacteroides*, *Butyricimonas*, *Bacteroides* and *Mucispirillum*) were increased in DSS-induced mice. In contrast to the previous reports, these genera were negatively correlated with mucins, SCFAs, and IL-10, but positively correlated with the DAI and pro-inflammatory cytokines. Moreover, except for *Mucispirillum*, these increased genera in the DSS-induced group were reduced by LRS and/or FMT administration.

Targeted regulation of the microbiota by the administration of probiotics has been successfully used in certain intestinal diseases.[48] In the present study, the DSS-treated group exhibited low alpha diversity, and LRS and FMT could reverse the changes. Relative to the CK group, the DSS-treated group presented an increase in the abundance of Firmicutes and Proteobacteria, as well as a decrease in the abundance of Bacteroidetes at the phylum level. The reason is that *L. rhamnosus* SHA113 belongs to Firmicutes. The Firmicutes/Bacteroidetes (F/B) ratio increased significantly, and studies have shown that changes in the F/B ratio are often related to the occurrence and development of diseases.[49] The increase in the relative abundance of Firmicutes has been reported as the remission of UC by FMT in mice.[28] This finding is consistent with the results of the current study.

L. rhamnosus SHA113 alleviated UC by increasing the abundance of beneficial bacteria and decreasing the abundance of harmful bacteria. The inner mucus layer contained no bacteria in the healthy control group. In patients with UC, the mucus layer was damaged, and the permeability of the intestine increased. Consequently, the microbiota interacted with the epithelial surface that was inaccessible under normal conditions, causing inflammatory reactions.[50,51] The administration of FMT significantly increased the genera (*Prevotella*, *Enterorhabdus*, and *Parvibacter*) that were positively correlated with the expression of mucins and negatively correlated with the expression of inflammatory cytokines and the DAI in mice. The abundance of the SCFA-producing genera *Bifidobacterium*, *Akkermansia*, *Olsenella*, and *Flavonifractor* was largely increased by LRS and FMT administration. However, LRS administration also prompted an increase in the opportunistic pathogen *Parasutterella*. The difference in the change in gut microbiota may be the key reason that LRS and FMT caused different remissions of inflammation and gut barrier damage in view of the expression of mucins and ZO-1. The therapeutic effects of FMT on UC were noted in the previous studies.[52,53] Therefore, the influenced change in the gut microbiota, the decrease in the genera *Escherichia* and *Shigella*, and the increase in the SCFA-producing genera *Roseburis*, *Bifidobacterium*, and *Akkermansia* are key factors that contribute to the therapeutic effect of *L. rhamnosus* SHA113. However, in contrast to the pre-

vious studies, LRS and FMT administration prompted an increase in the lipopolysaccharide-producing bacteria *Bilophila* and a decrease in *Parabacteroides*, which were reported to be associated with the remission of the patient's condition.[29]

SCFAs are metabolites of the intestinal flora, and can provide energy for the colon cells, regulate the pH value in the intestinal lumen, participate in host immune regulation and enhance the intestinal barrier function. Among the SCFAs, butyric acid plays a particularly important role in relieving UC. Butyrate has been shown to inhibit the expression of pro-inflammatory factors and promote the expression of tight junction proteins.[54,55] The use of butyrate preparations can help relieve UC. The present study determined the contents of SCFAs (acetic acid, propionic acid, butyric acid, caproic acid, isobutyric acid, isovaleric acid, and valeric acid) in mouse cecal contents to explore the effects of the two treatments on SCFAs. The contents of 7 SCFAs in the feces of the mice in the DSS group were significantly reduced, which is consistent with previous research.[54] After the intervention, the contents of 6 SCFAs (except for caproic acid) in the contents of cecum in mice were significantly increased, which was consistent with the pathological results. This increase indicates that *L. rhamnosus* SHA113 can further alleviate colitis in mice by increasing the content of intestinal SCFAs.

The anti-inflammatory effect is one of the major reasons for the successful treatment of UC by LRS administration. The enlargement of immune organs is a manifestation of inflammation in the body.[56] In the present study, LRS and FMT alleviated the enlargement of the spleen, liver, and kidneys in mice with DSS-induced enteritis. This finding showed that the degree of inflammation was alleviated to a certain extent. Moreover, TNF-α is a major pro-inflammatory cytokine involved in IBD. TNF-α can induce the expression of other pro-inflammatory cytokines (IL-6, IL-1β, *etc*.). It is required for leukocyte attachment and infiltration *via* the intestinal mucosa, acting as a critical step in the inflammation and tissue injury encountered in IBD.[57,58] Numerous studies have been conducted on the use of TNF-α as a therapeutic target against IBD.[59,60] In the present study, the LRS-treated group exhibited lower expression levels of TNF-α, IL-6, and IL-1β, suggesting that LRS uses TNF-α as a therapeutic target to alleviate UC. IL-10 is an anti-inflammatory cytokine known to critically maintain intestinal immune homeostasis.[61,62] This cytokine is crucial in preventing the progression of IBD.[63] In the present study, the DSS-treated group showed a lower expression of IL-10, compared with the LRS-treated group. This result suggests that LRS relieves inflammation in UC by affecting the pathway where IL-10 is located.

The induction of mucin expression is another major reason for the successful treatment of UC by LRS administration. Mucus forms a physical barrier that prevents microorganisms and noxious substances from reaching the surface of the epithelium.[64] The major building blocks of the mucus consist of high-molecular-weight glycoproteins called mucins (Muc1–Muc21).[65] Mucins are essential in the treatment of UC.[66,67] Various probiotics (*Bifidobacterium*, *Enterococcus faecium*, *Lactobacillus*, *Saccharomyces*, *Bacillus*, *etc*.) can relieve UC by increasing the expression of mucins.[61] In the present study, *L. rhamnosus* SHA113 increased the expression levels of *Muc1*, *Muc2*, *Muc3*, and *Muc4* in the colon tissue. FMT exhibited similar results. ZO-1, as a tight junction protein, is vital in the intestinal barrier. One of the pathogenesis of UC is the destruction of intestinal barrier, which leads to the entry of harmful bacteria and induces enteritis.[68–70] In the present study, the results showed that the expression of ZO-1 in the DSS group decreased significantly, which was consistent with the previous findings.[71,72] However, after the administration of LRS and FMT, the expression of ZO-1 increased and reached a significant level. Camp and S100A8 usually exert anti-inflammatory effects.[36] The expression levels of them significantly decreased in the DSS group, but significantly increased after LRS and FMT intervention, potentially contributing to the anti-inflammatory effect of LRS.

Overall, *L. rhamnosus* SHA113 alleviates UC by increasing the expression of mucins, anti-inflammatory cytokines, and anti-inflammatory peptides; changing the structure of intestinal flora; and increasing the content of intestinal SCFAs. The increases in *Bacteroides*, *Oscillibacter*, *Clostridium* XVIII, *Escherichia*/*Shigella*, *Butyricimonas*, and *Parabacteroides* and the decreases in *Alloprevotella*, *Prevotella*, *Roseburia*, *Odoribacter*, and *Parvibacter*, among others, were related to the occurrence of UC. The reductions in mucins, anti-inflammatory factors, and SCFAs might be also related to the development of UC. The decrease in the genera *Escherichia*/*Shigella* and the increase in the SCFA-producing genera *Roseburis*, *Bifidobacterium*, and *Akkermansia* are identified as the key factors that contribute to the therapeutic effect of *L. rhamnosus* SHA113.

Author contributions

Chunmei Jiang, Dongyan Shao and Junling Shi contributed to the conception of the study; Bing Pang and Han Jin performed the experiment; Bing Pang and Han Jin contributed significantly to the analysis and manuscript preparation; Bing Pang performed the data analyses and wrote the manuscript; and Ning Liao and Junjun Li helped perform the analysis with constructive discussions.

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Conflicts of interest

There are no conflicts of interest to declare.

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References

1 T. M. Kadayat, S. Banskota, P. Gurung, G. Bist, T. B. T. Magar, A. Shrestha, J. A. Kim and E. S. Lee, Discovery and structure-activity relationship studies of 2-benzylidene-2,3-dihydro-1H-inden-1-one and benzofuran-3(2H)-one derivatives as a novel class of potential therapeutics for inflammatory bowel disease, *Eur. J. Med. Chem.*, 2017, **137**, 575–597.

2 S. C. Ng, H. Y. Shi, N. Hamidi, F. E. Underwood, W. Tang, E. I. Benchimol, R. Panaccione, S. Ghosh, J. C. Y. Wu, F. K. L. Chan, J. J. Y. Sung and G. G. Kaplan, Worldwide incidence and prevalence of inflammatory bowel disease in the 21st century: a systematic review of population-based studies, *Lancet*, 2018, **390**, 2769–2778.

3 G. G. Kaplan, The global burden of IBD: from 2015 to 2025, *Nat. Rev. Gastroenterol. Hepatol.*, 2015, **12**, 720–727.

4 J. Burisch, T. Jess, M. Martinato and P. L. Lakatos, The burden of inflammatory bowel disease in Europe, *J. Crohns Colitis*, 2013, **7**, 322–337.

5 G. P. Ramos and K. A. Papadakis, Mechanisms of Disease: Inflammatory Bowel Diseases, *Mayo Clin. Proc.*, 2019, **94**, 155–165.

6 Y. Mishima, A. Oka, B. Liu, J. W. Herzog, C. S. Eun, T. J. Fan, E. Bulik-Sullivan, I. M. Carroll, J. J. Hansen, L. Chen, J. E. Wilson, N. C. Fisher, J. P. Ting, T. Nochi, A. Wahl, J. V. Garcia, C. L. Karp and R. B. Sartor, Microbiota maintain colonic homeostasis by activating TLR2/MyD88/PI3K signaling in IL-10-producing regulatory B cells, *J. Clin. Invest.*, 2019, **130**, 3702–3716.

7 A. Alam, G. Leoni, M. Quiros, H. Wu, C. Desai, H. Nishio, R. M. Jones, A. Nusrat and A. S. Neish, The microenvironment of injured murine gut elicits a local pro-restitutive microbiota, *Nat. Microbiol.*, 2016, **1**, 15021.

8 S. Duranti, F. Gaiani, L. Mancabelli, C. Milani, A. Grandi, A. Bolchi, A. Santoni, G. A. Lugli, C. Ferrario, M. Mangifesta, A. Viappiani, S. Bertoni, V. Vivo, F. Serafini, M. R. Barbaro, A. Fugazza, G. Barbara, L. Gioiosa, P. Palanza, A. M. Cantoni, G. L. de'Angelis, E. Barocelli, N. de'Angelis, D. van Sinderen, M. Ventura and F. Turroni, Elucidating the gut microbiome of ulcerative colitis: bifidobacteria as novel microbial biomarkers, *FEMS Microbiol. Ecol.*, 2016, **92**, fiw191.

9 T. Takamura, D. Harama, S. Fukumoto, Y. Nakamura, N. Shimokawa, K. Ishimaru, S. Ikegami, S. Makino, M. Kitamura and A. Nakao, Lactobacillus bulgaricus OLL1181 activates the aryl hydrocarbon receptor pathway and inhibits colitis, *Immunol. Cell Biol.*, 2011, **89**, 817–822.

10 B. O. Schroeder, G. M. H. Birchenough, M. Stahlman, L. Arike, M. E. V. Johansson, G. C. Hansson and F. Backhed, Bifidobacteria or fiber protects against diet-Induced microbiota-mediated colonic mucus deterioration, *Cell Host Microbe*, 2018, **23**, 27–40.

11 J. Kolinska, M. Zakostelecka, Z. Zemanova, V. Lisa, J. Golias, H. Kozakova and B. Dvorak, Cellular differentiation of non-transformed intestinal epithelial cells is regulated by Lactobacillus rhamnosus and L. casei strains, *Physiol. Res.*, 2018, **67**, 261–273.

12 F. R. L. Rolim, K. M. O. dos Santos, S. C. de Barcelos, A. S. do Egito, T. S. Ribeiro, M. L. da Conceição, M. Magnani, M. E. G. de Oliveira and R. D. R. D. Queiroga, Survival of Lactobacillus rhamnosus EM1107 in simulated gastrointestinal conditions and its inhibitory effect against pathogenic bacteria in semi-hard goat cheese, *LWT–Food Sci. Technol.*, 2015, **63**, 807–813.

13 N. Li, B. Pang, J. Li, G. Liu, X. Xu, D. Shao, C. Jiang, B. Yang and J. Shi, Mechanisms for Lactobacillus rhamnosus treatment of intestinal infection by drug-resistant Escherichia coli, *Food Funct.*, 2020, **11**, 4428–4445.

14 N. Li, B. Pang, G. Liu, X. Zhao, X. Xu, C. Jiang, B. Yang, Y. Liu and J. Shi, Lactobacillus rhamnosus from human breast milk shows therapeutic function against foodborne infection by multi-drug resistant Escherichia coli in mice, *Food Funct.*, 2020, **11**, 435–447.

15 G. Liu, B. Pang, N. Li, H. Jin, J. Li, W. Wu, C. Ai, C. Jiang and J. Shi, Therapeutic effect of Lactobacillus rhamnosus SHA113 on intestinal infection by multi-drug-resistant Staphylococcus aureus and its underlying mechanisms, *Food Funct.*, 2020, **11**, 6226–6239.

16 M. S. R. Rajoka, H. M. Mehwish, M. Siddiq, H. B. Zhao, J. Zhu, L. Yan, D. Y. Shao, X. G. Xu and J. L. Shi, Identification, characterization, and probiotic potential of *Lactobacillus rhamnosus* isolated from human milk, *LWT-Food Sci. Technol.*, 2017, **84**, 271–280.

17 W. Zhang, G. Zou, B. Li, X. Du, Z. Sun, Y. Sun and X. Jiang, Fecal Microbiota Transplantation (FMT) Alleviates Experimental Colitis in Mice by Gut Microbiota Regulation, *J. Microbiol. Biotechnol.*, 2020, **30**, 1132–1141.

18 Y.-J. Liu, B. Tang, F.-C. Wang, L. Tang, Y.-Y. Lei, Y. Luo, S.-J. Huang, M. Yang, L.-Y. Wu, W. Wang, S. Liu, S.-M. Yang and X.-Y. Zhao, Parthenolide ameliorates colon inflammation through regulating Treg/Th17 balance in a gut microbiota-dependent manner, *Theranostics*, 2020, **10**, 5225–5241.

19 R. C. Edgar, UPARSE: highly accurate OTU sequences from microbial amplicon reads, *Nat. Methods*, 2013, **10**, 996–998.

20 P. F. Kemp and J. Y. Aller, Bacterial diversity in aquatic and other environments: what 16S rDNA libraries can tell us, *FEMS Microbiol. Ecol.*, 2004, **47**, 161–177.

21 H. A. Murad, H. M. Abdallah and S. S. Ali, Mentha longifolia protects against acetic-acid induced colitis in rats, *J. Ethnopharmacol.*, 2016, **190**, 354–361.
22 S. M. Bastaki, E. Adeghate, N. Amir, S. Ojha and M. Oz, Menthol inhibits oxidative stress and inflammation in acetic acid-induced colitis in rat colonic mucosa, *Am. J. Transl. Res.*, 2018, **10**, 4210–4222.
23 B. B. Colombo, V. Fattori, C. F. S. Guazelli, T. H. Zaninelli, T. T. Carvalho, C. R. Ferraz, A. J. C. Bussmann, K. W. Ruiz-Miyazawa, M. M. Baracat, R. Casagrande and W. A. Verri Jr., Vinpocetine Ameliorates Acetic Acid-Induced Colitis by Inhibiting NF-κB Activation in Mice, *Inflammation*, 2018, **41**, 1276–1289.
24 Z. Zhao, L. Chen, Y. Zhao, C. Wang, C. Duan, G. Yang, C. Niu and S. Li, Lactobacillus plantarum NA136 ameliorates nonalcoholic fatty liver disease by modulating gut microbiota, improving intestinal barrier integrity, and attenuating inflammation, *Appl. Microbiol. Biotechnol.*, 2020, **104**, 5273–5282.
25 J. J. Song, W. J. Tian, L. Y. Kwok, Y. L. Wang, Y. N. Shang, B. Menghe and J. G. Wang, Effects of microencapsulated Lactobacillus plantarum LIP-1 on the gut microbiota of hyperlipidaemic rats, *Br. J. Nutr.*, 2017, **118**, 481–492.
26 D. Fang, D. Shi, L. Lv, S. Gu, W. Wu, Y. Chen, J. Guo, A. Li, X. Hu, F. Guo, J. Ye, Y. Li and L. Li, Bifidobacterium pseudocatenulatum LI09 and Bifidobacterium catenulatum LI10 attenuate D-galactosamine-induced liver injury by modifying the gut microbiota, *Sci. Rep.*, 2017, **7**, 8770.
27 C. Wang, W. Li, H. Wang, Y. Ma, X. Zhao, X. Zhang, H. Yang, J. Qian and J. Li, 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**, 246.
28 W. Zhang, G. Zou, B. Li, X. Du, Z. Sun, Y. Sun and X. Jiang, Fecal Microbiota Transplantation (FMT) Alleviates Experimental Colitis in Mice by Gut Microbiota Regulation, *J. Microbiol. Biotechnol.*, 2020, **30**, 1132–1141.
29 A. Quagliariello, F. Del Chierico, S. Reddel, A. Russo, A. Onetti Muda, P. D'Argenio, G. Angelino, E. F. Romeo, L. Dall'Oglio, P. De Angelis, L. Putignani and C. All The Other Fmt Opbg Committee, Fecal Microbiota Transplant in Two Ulcerative Colitis Pediatric Cases: Gut Microbiota and Clinical Course Correlations, *Microorganisms*, 2020, **8**, 1486.
30 P. Chen, H. Xu, H. Tang, F. Zhao, C. Yang, L. Y. Kwok, C. Cong, Y. Wu, W. Zhang, X. Zhou and H. Zhang, Modulation of gut mucosal microbiota as a mechanism of probiotics-based adjunctive therapy for ulcerative colitis, *Microb. Biotechnol.*, 2020, **13**, 2032–2043.
31 M. Sun, T. Wu, G. Zhang, R. Liu, W. Sui, M. Zhang, J. Geng, J. Yin and M. Zhang, Lactobacillus rhamnosus LRa05 improves lipid accumulation in mice fed with a high fat diet via regulating the intestinal microbiota, reducing glucose content and promoting liver carbohydrate metabolism, *Food Funct.*, 2020, **11**, 9514–9525.
32 B. Chassaing and A. Darfeuille-Michaud, The commensal microbiota and enteropathogens in the pathogenesis of inflammatory bowel diseases, *Gastroenterology*, 2011, **140**, 1720–1728.
33 S. Wirtz, V. Popp, M. Kindermann, K. Gerlach, B. Weigmann, S. Fichtner-Feigl and M. F. Neurath, Chemically induced mouse models of acute and chronic intestinal inflammation, *Nat. Protoc.*, 2017, **12**, 1295–1309.
34 P. M. Munyaka, M. F. Rabbi, E. Khafipour and J. E. Ghia, Acute dextran sulfate sodium (DSS)-induced colitis promotes gut microbial dysbiosis in mice, *J. Basic Microbiol.*, 2016, **56**, 986–998.
35 S. Shigemori, T. Watanabe, K. Kudoh, M. Ihara, S. Nigar, Y. Yamamoto, Y. Suda, T. Sato, H. Kitazawa and T. Shimosato, Oral delivery of Lactococcus lactis that secretes bioactive heme oxygenase-1 alleviates development of acute colitis in mice, *Microb. Cell Fact.*, 2015, **14**, 189.
36 H. W. Koon, D. Q. Shih, J. Chen, K. Bakirtzi, T. C. Hing, I. Law, S. Ho, R. Ichikawa, D. Zhao, H. Xu, R. Gallo, P. Dempsey, G. Cheng, S. R. Targan and C. Pothoulakis, Cathelicidin signaling via the toll-like receptor protects against colitis in mice, *Gastroenterology*, 2011, **141**, 1852–1863.
37 M. Bejaoui, H. Sokol and P. Marteau, Targeting the Microbiome in Inflammatory Bowel Disease: Critical Evaluation of Current Concepts and Moving to New Horizons, *Dig. Dis.*, 2015, **33**(Suppl 1), 105–112.
38 E. Bellaguarda and E. B. Chang, IBD and the gut microbiota–from bench to personalized medicine, *Curr. Gastroenterol. Rep.*, 2015, **17**, 15.
39 P. A. Engen, S. J. Green, R. M. Voigt, C. B. Forsyth and A. Keshavarzian, The Gastrointestinal Microbiome: Alcohol Effects on the Composition of Intestinal Microbiota, *Alcohol Res.*, 2015, **37**, 223–236.
40 E. Sherwin, T. G. Dinan and J. F. Cryan, Recent developments in understanding the role of the gut microbiota in brain health and disease, *Ann. N. Y. Acad. Sci.*, 2018, **1420**, 5–25.
41 Q. Zhu, R. Gao, Y. Zhang, D. Pan, Y. Zhu, X. Zhang, R. Yang, R. Jiang, Y. Xu and H. Qin, Dysbiosis signatures of gut microbiota in coronary artery disease, *Physiol. Genomics*, 2018, **50**, 893–903.
42 I. Aggeletopoulou, C. Konstantakis, S. F. Assimakopoulos and C. Triantos, The role of the gut microbiota in the treatment of inflammatory bowel diseases, *Microb. Pathog.*, 2019, **137**, 103774.
43 L. P. Chen, W. Wang, R. Zhou, S. C. Ng, J. Li, M. F. Huang, F. Zhou, X. Wang, B. Shen, M. A. Kamm, K. C. Wu and B. Xia, Characteristics of fecal and mucosa-associated microbiota in Chinese patients with inflammatory bowel disease, *Medicine*, 2014, **93**, e51.
44 L. T. Thorkildsen, F. C. Nwosu, E. Avershina, P. Ricanek, G. Perminow, S. Brackmann, M. H. Vatn and K. Rudi, Dominant fecal microbiota in newly diagnosed untreated inflammatory bowel disease patients, *Gastroenterol. Res. Pract.*, 2013, **2013**, 636785.

45 K. Takeshita, S. Mizuno, Y. Mikami, T. Sujino, K. Saigusa, K. Matsuoka, M. Naganuma, T. Sato, T. Takada, H. Tsuji, A. Kushiro, K. Nomoto and T. Kanai, A Single Species of Clostridium Subcluster XIVa Decreased in Ulcerative Colitis Patients, *Inflamm. Bowel Dis.*, 2016, **22**, 2802–2810.
46 N. G. Rossen, S. Fuentes, M. J. van der Spek, J. G. Tijssen, J. H. Hartman, A. Duflou, M. Löwenberg, G. R. van den Brink, E. M. Mathus-Vliegen, W. M. de Vos, E. G. Zoetendal, G. R. D'Haens and C. Y. Ponsioen, Findings from a randomized controlled trial of fecal transplantation for patients with ulcerative colitis, *Gastroenterology*, 2015, **149**, 110–118.
47 J. Xu, N. Chen, Z. Wu, Y. Song, Y. Zhang, N. Wu, F. Zhang, X. Ren and Y. Liu, 5-Aminosalicylic Acid Alters the Gut Bacterial Microbiota in Patients With Ulcerative Colitis, *Front. Microbiol.*, 2018, **9**, 1274.
48 A. C. Ford, E. M. Quigley, B. E. Lacy, A. J. Lembo, Y. A. Saito, L. R. Schiller, E. E. Soffer, B. M. Spiegel and P. Moayyedi, Efficacy of prebiotics, probiotics, and synbiotics in irritable bowel syndrome and chronic idiopathic constipation: systematic review and meta-analysis, *Am. J. Gastroenterol.*, 2014, **109**, 1547–1561; quiz 1546, 1562.
49 S. V. Lynch and O. Pedersen, The Human Intestinal Microbiome in Health and Disease, *N. Engl. J. Med.*, 2016, **375**, 2369–2379.
50 C. Becker, M. F. Neurath and S. Wirtz, The Intestinal Microbiota in Inflammatory Bowel Disease, *ILAR J.*, 2015, **56**, 192–204.
51 D. Sheehan and F. Shanahan, The Gut Microbiota in Inflammatory Bowel Disease, *Gastroenterol. Clin. North Am.*, 2017, **46**, 143–154.
52 S. C. Ganal-Vonarburg and C. U. Duerr, The interaction of intestinal microbiota and innate lymphoid cells in health and disease throughout life, *Immunology*, 2020, **159**, 39–51.
53 G. Morris, M. Berk, A. F. Carvalho, J. R. Caso, Y. Sanz and M. Maes, The Role of Microbiota and Intestinal Permeability in the Pathophysiology of Autoimmune and Neuroimmune Processes with an Emphasis on Inflammatory Bowel Disease Type 1 Diabetes and Chronic Fatigue Syndrome, *Curr. Pharm. Des.*, 2016, **22**, 6058–6075.
54 M. Sun, W. Wu, Z. Liu and Y. Cong, Microbiota metabolite short chain fatty acids, GPCR, and inflammatory bowel diseases, *J. Gastroenterol.*, 2017, **52**, 1–8.
55 B. Dalile, L. Van Oudenhove, B. Vervliet and K. Verbeke, The role of short-chain fatty acids in microbiota-gut-brain communication, *Nat. Rev. Gastroenterol. Hepatol.*, 2019, **16**, 461–478.
56 M. F. Neurath, Resolution of inflammation: from basic concepts to clinical application, *Semin. Immunopathol.*, 2019, **41**, 627–631.
57 K. A. Papadakis and S. R. Targan, Role of cytokines in the pathogenesis of inflammatory bowel disease, *Annu. Rev. Med.*, 2000, **51**, 289–298.
58 C. M. Olesen, M. Coskun, L. Peyrin-Biroulet and O. H. Nielsen, Mechanisms behind efficacy of tumor necrosis factor inhibitors in inflammatory bowel diseases, *Pharmacol. Ther.*, 2016, **159**, 110–119.
59 M. A. Aardoom, G. Veereman and L. de Ridder, A Review on the Use of Anti-TNF in Children and Adolescents with Inflammatory Bowel Disease, *Int. J. Mol. Sci.*, 2019, **20**, 2529.
60 D. Pugliese, C. Felice, A. Papa, A. Gasbarrini, G. L. Rapaccini, L. Guidi and A. Armuzzi, Anti TNF-α therapy for ulcerative colitis: current status and prospects for the future, *Expert Rev. Clin. Immunol.*, 2017, **13**, 223–233.
61 C. Neumann, A. Scheffold and S. Rutz, Functions and regulation of T cell-derived interleukin-10, *Semin. Immunol.*, 2019, **44**, 101344.
62 L. Zhu, T. Shi, C. Zhong, Y. Wang, M. Chang and X. Liu, IL-10 and IL-10 Receptor Mutations in Very Early Onset Inflammatory Bowel Disease, *Gastroenterol. Res.*, 2017, **10**, 65–69.
63 P. J. Koelink, F. M. Bloemendaal, B. F. Li, L. Westera, E. W. M. Vogels, M. van Roest, A. K. Gloudemans, A. B. van 't Wout, H. Korf, S. Vermeire, A. A. Te Velde, C. Y. Ponsioen, G. R. A. M. D'Haens, J. S. Verbeek, T. L. Geiger, M. E. Wildenberg and G. R. van den Brink, Anti-TNF therapy in IBD exerts its therapeutic effect through macrophage IL-10 signalling, *Gut*, 2020, **69**, 1053–1063.
64 S. Cornick, A. Tawiah and K. Chadee, Roles and regulation of the mucus barrier in the gut, *Tissue Barriers*, 2015, **3**, e982426.
65 G. Petrou and T. Crouzier, Mucins as multifunctional building blocks of biomaterials, *Biomater. Sci.*, 2018, **6**, 2282–2297.
66 D. D. Eichele and K. K. Kharbanda, Dextran sodium sulfate colitis murine model: An indispensable tool for advancing our understanding of inflammatory bowel diseases pathogenesis, *World J. Gastroenterol.*, 2017, **23**, 6016–6029.
67 H. Taman, C. G. Fenton, I. V. Hensel, E. Anderssen, J. Florholmen and R. H. Paulssen, Transcriptomic Landscape of Treatment-Naïve Ulcerative Colitis, *J. Crohns Colitis*, 2018, **12**, 327–336.
68 K. F. Castro-Ochoa, H. Vargas-Robles, S. Chánez-Paredes, A. Felipe-López, R. I. Cabrera-Silva, M. Shibayama, A. Betanzos, P. Nava, E. A. Galinski and M. Schnoor, Homoectoine Protects Against Colitis by Preventing a Claudin Switch in Epithelial Tight Junctions, *Dig. Dis. Sci.*, 2019, **64**, 409–420.
69 N. Pakravan, F. Kermanian and E. Mahmoudi, Filtered Kombucha tea ameliorates the leaky gut syndrome in young and old mice model of colitis, *Iran. J. Basic Med. Sci.*, 2019, **22**, 1158–1165.
70 S. Zhang, W. Xu, H. Wang, M. Cao, M. Li, J. Zhao, Y. Hu, Y. Wang, S. Li, Y. Xie, G. Chen, R. Liu, Y. Cheng, Z. Xu, K. Zou, S. Gong and L. Geng, Inhibition of CREB-mediated ZO-1 and activation of NF-κB-induced IL-6 by colonic epithelial MCT4 destroys intestinal barrier function, *Cell Proliferation*, 2019, **52**, e12673.

71 S. Al-Ghadban, S. Kaissi, F. R. Homaidan, H. Y. Naim and M. E. El-Sabban, Cross-talk between intestinal epithelial cells and immune cells in inflammatory bowel disease, *Sci. Rep.*, 2016, **6**, 29783.

72 M. Yang, W. Jia, D. Wang, F. Han, W. Niu, H. Zhang, D. Q. Shih and X. Zhang, Effects and Mechanism of Constitutive TL1A Expression on Intestinal Mucosal Barrier in DSS-Induced Colitis, *Dig. Dis. Sci.*, 2019, **64**, 1844–1856.