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Colonisation with endogenous *Lactobacillus reuteri* R28 and exogenous *Lactobacillus plantarum* AR17-1 and the effects on intestinal inflammation in mice

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The beneficial effects of probiotics on inflammatory bowel disease (IBD) are well known, although an understanding of colonisation by endogenous and exogenous bacterial strains and the effects on intestinal inflammation remains elusive. In this study, the colonisation of endogenous *Lactobacillus reuteri* R28 and exogenous *Lactobacillus plantarum* AR17-1 was investigated in healthy or PEG-treated mice using a 5(6)-carboxyfluorescein diacetate *N*-succinimidyl ester (cFDA-SE) labelling technique. The effects of these strains on mice with colitis induced by DSS and treated with PEG + DSS were also studied. Endogenous *L. reuteri* R28 and exogenous *L. plantarum* AR17-1 exhibited no significant differences in colonisation in healthy mice, whereas after PEG treatment, colonisation of the intestinal mucosa by *L. reuteri* R28 was greatly enhanced. *L. reuteri* R28 more effectively reduced diarrhoea caused by PEG, and *L. plantarum* AR17-1 more effectively reduced the colitis induced by PEG + DSS and downregulated the expression of the pro-inflammatory cytokines TNF-α, IL-1β, and IL-6. These results suggest that endogenous *L. reuteri* R28 may easily adapt to the intestinal environment, leading to better colonisation, whereas *L. plantarum* AR17-1 has a stronger inhibitory effect on inflammation. This finding is relevant to the selection of probiotics.

1. Introduction

Inflammatory bowel disease (IBD) is a chronic relapsing inflammatory disease represented by two illnesses, Crohn's disease and ulcerative colitis, which can affect the integrity of the intestinal mucosa.[1,2] The disease is characterised by lifelong treatment and incurable idiopathic intestinal inflammatory epithelial injury.[3] Its main manifestation is chronic diarrhoea or sudden colitis, which can lead to colon cancer if not treated in time.[4] In line with accelerated urbanisation and continuous changes in the natural environment, the incidence of IBD has recently increased each year.

A complex interplay of host–microbiota dysbiosis, environmental factors, and the genetic background has been implicated in the pathogenesis of IBD.[5–7] Some studies have shown that when compared with healthy controls, IBD patients have an imbalanced intestinal flora characterised by decreased bacterial diversity and less stable bacterial populations,[8] and concomitant increases in harmful populations, such as *Proteobacteria* and *Actinobacteria*.[9,10] Diarrhoea is a prevalent symptom and sign in patients with IBD. Mild diarrhoea may lead to long-term changes in the gut microbiota composition and has been shown to cause the reproducible extinction of highly abundant taxa and expansion of less prevalent members in mouse microbiotas.[11] However, the effects of the introduction of novel species or the reintroduction of extinct species to intestinal ecological niches is understudied.

At present, IBD treatments are mainly pharmacological and include antibiotics, immunosuppressants, aminosalicylates, and biologic agents.[12] Such treatments usually cause unwanted side effects. Therefore, safe and effective new treatments, including nutritional approaches such as probiotics, are of great interest for the prevention or amelioration of IBD.[13] Several studies have investigated the use of oral probiotics, such as *Lactobacillus* and *Bifidobacterium* strains, in this context. Such probiotics can regulate the relationships among microflora and play a crucial role in maintaining the stability of the gut microbiota.[14,15] *Lactobacillus rhamnosus* LDTM

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7511 has been shown to alleviate inflammation and normalise bacterial dysbiosis in an inflamed gut, and is therefore an effective candidate for IBD treatment.[16] Inoculating the intestines of mice with *Lactobacillus acidophilus* can enhance the host defence against intestinal bacterial infection and bacterial colitis.[17] For *in vivo* animal studies, animal models of IBD are generally induced using dextran sodium sulphate (DSS), which is thought to mimic the disease process and produces symptoms that are similar to those experienced by IBD patients, including mucosal ulceration, diarrhoea, body weight loss, and large intestinal shortening;[18] however, this model often overlooks the early diarrhoea process. Nowadays, many researchers are using DSS models to study the effects of probiotics on IBD. However, changes in the gut microbiota caused by diarrhoea have not been considered, even though these may affect the colonisation and efficacy of probiotics.

In this study, we focused on *Lactobacillus reuteri* R28 isolated from mouse faeces and *Lactobacillus plantarum* AR17-1 from fermented pickles. We used a 5 (6)-carboxyfluorescein diacetate *N*-succinimidyl ester (cFDA-SE) labelling technique to explore the colonisation and distribution of these species in healthy mice and in mice with diarrhoea induced by polyethylene glycol (PEG). We also investigated the ameliorative effects of these strains on disease severity in mice with DSS-induced colitis with and without PEG administration.

2. Materials and methods

2.1. Bacterial strains and culture conditions

Lactobacillus reuteri R28 isolated from mouse faeces and *Lactobacillus plantarum* AR17-1 isolated from fermented pickles were stored at the Shanghai Engineering Research Centre of Food Microbiology (Shanghai, China). Both strains were anaerobically cultured in de Man, Rogosa and Sharpe (MRS) broth at 37 °C for 20 h, then harvested by centrifugation (8000 rpm for 10 min), washed three times with sterile phosphate-buffered saline (PBS) and finally resuspended in PBS to a concentration of approximately 10^{10} colony-forming units (cfu) per mL.

2.2. Animals

Six-week-old specific-pathogen free male C57BL/6 mice were purchased from Shanghai Jiesijie Experimental Animal Co. Ltd (Shanghai, China). The mice were bred and kept at the animal facility at 22 ± 2 °C and 50% humidity. All mice were housed for at least 1 week before inclusion in the experiments, which performed in accordance with the Guidelines for Care and Use of Laboratory Animals of Shanghai Jiao Tong University and approved by the Animal Ethics Committee of Shanghai Jiao Tong University.

2.3. Preparation and labelling of the fluorescent probe

A fluorescent probe was prepared as previously described.[19] The concentrations of *L. reuteri* R28 and *L. plantarum* AR17-1 were adjusted with PBS to 10^{10} cfu mL^{-1}, after which the bacterial suspension was mixed with an equal volume of cFDA-SE (50 μM) and incubated in a 37 °C water bath in the dark for 20 min. The cell mass was collected by centrifugation at 8000 rpm for 3 min and rinsed with PBS three times to remove unreacted cFDA-SE. Flow cytometry (BD FACSCalibur, Becton, Dickinson and Company, New Jersey, USA) was used to detect the labelled cells. The percentage of fluorescence-labelled live cells was 99.74%.

2.4. Colonisation of *L. reuteri* R28 and *L. plantarum* AR17-1 in the mouse intestine

Sixty-four mice were divided into four groups. The AR17-1 group and R28 group were administered fluorescence-labelled *L. plantarum* AR17-1 or *L. reuteri* R28, respectively, once at a dose of 5×10^8 cfu. The PEG + AR17-1 group and PEG + R28 group were orally administered 15% PEG for 5 days before inoculation, followed by gavage with *L. plantarum* AR17-1 or *L. reuteri* R28, respectively. Control mice were orally administered equal volumes of sterilised PBS solution. On the first, third, fifth and seventh days after administration, four mice in each group were killed by cervical dislocation, and the entire gastrointestinal tracts were removed for fluorescence imaging to measure the fluorescence intensity of lactobacilli in the intestinal tracts.

2.5. PEG + DSS-induced colitis

Six-week-old male C57BL/6 mice were divided into five groups: control, DSS, PEG + DSS, PEG + DSS + AR17-1 and PEG + DSS + R28 (see Fig. 1). The control group was fed a normal diet and given water freely for the whole experimental period (16 days). The DSS group was fed a normal diet and water for the first 12 days and then freely given drinking water containing 3% DSS for the next 4 days. Experimental colitis was induced in the PEG + DSS treatment groups by adding 15% PEG to the drinking water from days 1 to 6, followed by normal water for the next 6 days and then drinking water containing 3% DSS for 4 days. The last two groups were treated as described for the PEG + DSS-treated mice but were administered 5×10^8 cfu of

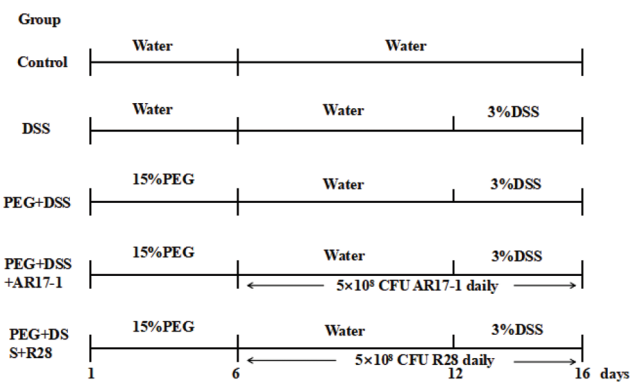

Fig. 1 Feeding schedules for the control, DSS-induced colitis, PEG + DSS-induced colitis, *L. plantarum* AR17-1 intervention and *L. reuteri* R28 intervention groups in C57BL/6 mice.

L. reuteri R28 or *L. plantarum* AR17-1 by oral gavage daily from days 6 to 16 of the experiment.

2.6. Evaluation of the disease activity index

The degree of intestinal inflammation in each group was evaluated daily using disease activity index (DAI) scores. The DAI is a clinical parameter reflecting the stool consistency, weight loss severity and faecal bleeding severity in experimental animals.[20] The scoring criteria were as follows: stool consistency (normal = 0; loose stools = 1 or 2; loss of form/diarrhoea = 3 or 4); weight loss (zero = 0; 1–5% loss = 1; 6–10% loss = 2; 11–15% loss = 3; more than 15% loss = 4) and faecal bleeding (normal colour stool = 0; brown colour = 1; reddish colour = 2; bloody stool = 3; visible blood = 4). Occult blood in the stool was detected using an occult blood kit (Nanjing Jiancheng Co., Ltd, Nanjing, China).

2.7. Assessment of myeloperoxidase activity

As DSS-induced colitis in mice is characterised by colonic shortening, the colon length is often used as a macroscopic marker of inflammation.[21] MPO activity in the colonic mucosa was measured using an MPO assay kit (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) according to the manufacturer's instructions. Colon samples were prepared as follows. After the mice were sacrificed, the colons were weighed and homogenised with physiological saline according to a weight-to-volume ratio of 1 : 19. A 5% tissue homogenate was prepared in a tissue-dispersing machine to determine the MPO activity. One unit of MPO activity was defined as the amount needed to degrade 1.0 μmol of hydrogen peroxide per min at 37 °C.

2.8. RNA extraction and RT-qPCR gene expression analysis

We used Trizol reagent (Invitrogen, Life Technologies, Shanghai, China) to extract the total cellular RNA of colonic tissues, according to the manufacturer's instructions. cDNA synthesis was performed using a PrimeScript RT reagent kit (TaKaRa, Japan). Quantitative real-time PCR reactions were performed using a 2× SYBR Premix Ex Taq (Takara Bio (Dalian) Co., Ltd, Dalian, China) to detect the expression of genes encoding TNF-α, IL-1β and IL-6, according to the manufacturer's instructions. Transcription levels were determined using the $2^{-\Delta\Delta Ct}$ method. The PCR primer pairs (synthesised by Sangon Biological Engineering, Shanghai, China) are shown in Table 2. The conditions were 40 cycles at 95 °C for 2 min, 95 °C for 15 s and 54 °C for 20 s.

Table 1 Disease activity index score parameters

Weight loss (%)	Stool consistency	Faecal bleeding	Scores
0	Normal	Normal colour stool	0
1–5	Formed	Brown colour	1
6–10	Somewhat soft	Reddish colour	2
11–15	Very soft	Bloody stool	3
>15	Watery	Visible blood	4

Table 2 Primer sequences for quantitative real-time polymerase chain reactions

Primer used	Sequence (5′ to 3′)
β-Actin-F	GGCTGTATTCCCCTCCATCG
β-Actin-R	CCAGTTGGTAACAATGCCATGT
TNF-α-F	AGGGTCTGGGCCATAGAACT
TNF-α-R	CCACCACGCTCTTCTGTCTAC
IL-6-F	GAGGATACCACTCCCAACAGACC
IL-6-R	AAGTGCATCATCGTTGTTCATACA
IL-1β-F	CTGAACTCAACTGTGAAATGC
IL-1β-R	TGATGTGCTGCTGCGAGA

F, forward; R, reverse. Primers were synthesised by Shanghai Huada Bioengineering Co., Ltd.

2.9. Statistical analysis

All data are expressed as means ± standard deviations (SD). SPSS version 22.0 (SPSS Inc., Chicago, IL, USA) was used for the statistical analysis. Statistical comparison of two groups was performed using Student's *t*-test, or Wilcoxon–Mann–Whitney test (when normality test fails). A one-way ANOVA was used to identify significant differences between groups, with the level of statistically significance set at $p < 0.05$. The graphics were plotted using GraphPad Prism 16.0.

3. Results

3.1. Time-course imaging of bacteria in mice given different treatments

As shown in Fig. 2, in the healthy mice, *L. plantarum* AR17-1 was mainly concentrated in the small intestine during the first

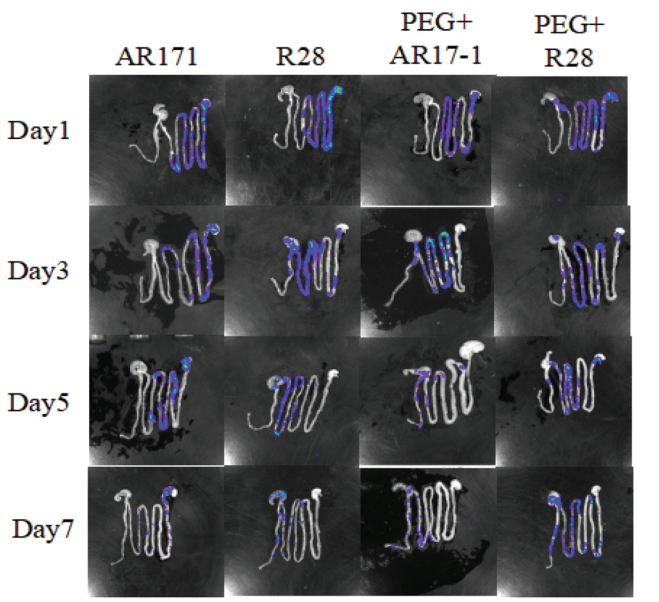

Fig. 2 Images of mouse intestines in the *L. plantarum* AR17-1, *L. reuteri* R28, PEG + *L. plantarum* AR17-1 and PEG + *L. reuteri* R28 groups at different time points after the administration of bacteria.

3 days, and then moved to the colon over time. The fluorescence intensity slowly weakened. *L. reuteri* R28 transited slightly more rapidly than *L. plantarum* AR17-1 and was widely distributed in the gastrointestinal tract and retained longer in the colon and cecum, even on the seventh day of colonisation. However, there were no significant differences in the colonisation sites between *L. reuteri* R28 and *L. plantarum* AR17-1 (Fig. 2).

After PEG treatment, *L. plantarum* AR17-1 transited faster than in PEG-untreated mice. It arrived quickly in the colon, after which the fluorescence intensity decreased rapidly before disappearing. In contrast, *L. reuteri* R28 remained in the gastrointestinal tract for longer and was more widely distributed. Strong fluorescence intensity of *L. reuteri* R28 could be observed in the small intestine, cecum and colon even on the seventh day, and the intensity was stronger than that observed in the tracts of the healthy mice. By day 7, almost no fluorescence was observed in the tracts of the mice treated with *L. plantarum* AR17-1.

These results suggest that endogenous *L. reuteri* R28 has better a colonisation ability than exogenous *L. plantarum* AR17-1 in the mouse intestine, especially after the administration of PEG. This may be because PEG causes mild osmotic diarrhoea, which reduces the relative abundances of gut microbes and weakens their competitive abilities.[22] *L. reuteri* R28 obtained from mice faeces may be able to quickly adapt to the gut environment and have a competitive advantage over exogenous *L. plantarum* AR17-1, which would benefit its colonisation ability in PEG-treated mice.

3.2. Effects of *L. reuteri* R28 and *L. plantarum* AR17-1 on PEG-treated mice with DDS-induced colitis

To effectively evaluate the development of colonic inflammatory symptoms and the effects of *L. reuteri* R28 and *L. plantarum* AR17-1 intervention, the body weight losses, DAI scores, colon lengths, and MPO activity were determined in the PEG-treated mice with DDS-induced colitis.

Fig. 3A shows the changes in the DAI scores of the five groups of mice over time. In contrast to the control group, the score for the PEG-treated mice (drinking water containing 15% PEG) gradually increased from 0 to 5.4 over the first 5 days and decreased gradually thereafter when the mice were returned to normal drinking water. Following the *L. reuteri* R28 and *L. plantarum* AR17-1 intervention in the PEG + DSS group, the DAI decreased significantly on day 6, and this effect lasted until day 12 (treatment for 1 week). The DAI score for the mice given *L. reuteri* R28 had returned to normal on day 12, and the score for the *L. plantarum* AR17-1 group was 0.81, which was approaching that of the healthy mice. The DAI score of the group without bacterial treatment was 2.01. These results demonstrate that *L. reuteri* R28 and *L. plantarum* AR17-1 interventions can significantly reduce diarrhoea caused by PEG,

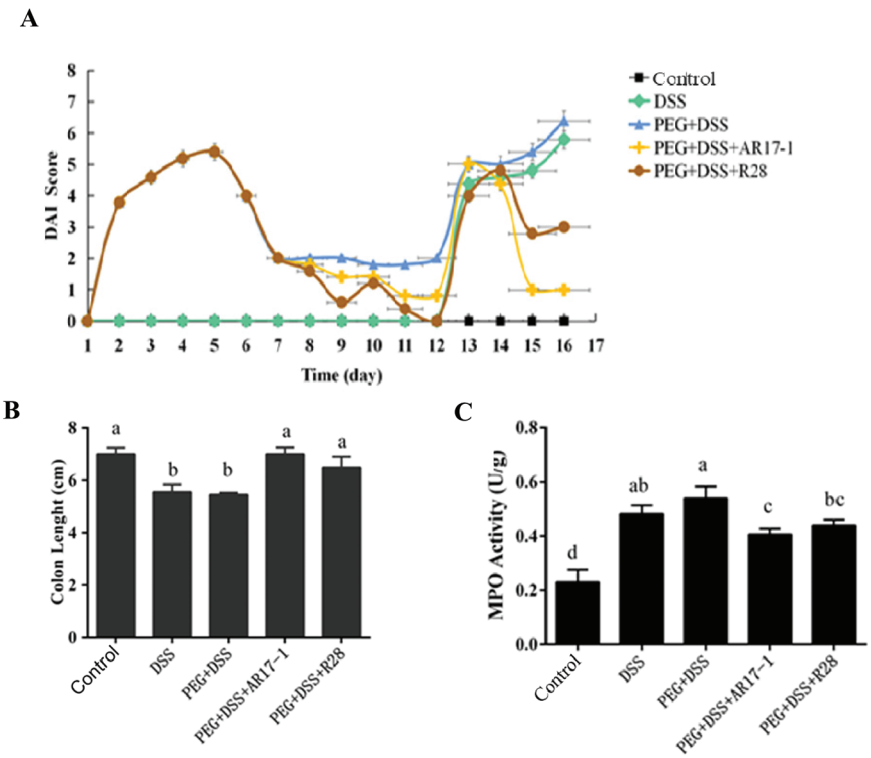

Fig. 3 Effects of *L. plantarum* AR17-1 and *L. reuteri* R28 on mice with PEG-DDS-induced colitis. (A) Disease activity index (DAI) according to the criteria proposed in Table 1; (B) colon length and (C) myeloperoxidase (MPO) activity. The data are expressed as means ± standard deviations (n = 5 per group). Statistical significance among the groups was evaluated using a one-way ANOVA. Bars with different letters indicate significant differences ($p < 0.05$).

although *L. reuteri* R28 is more effective. To further explore the effects of *L. plantarum* AR17-1 and *L. reuteri* R28, 3% DSS was administered to mice (excepting the control group) *via* the drinking water for the final 4 days of the experiment. As shown in Fig. 3A, the DAI scores of both the PEG + DSS group and the DSS group increased over the last 4 days, while the DAI score of the PEG-treated group was higher than that of the DSS only group. With the administration of *L. plantarum* AR17-1 and *L. reuteri* R28, DAI decreased on the day 14 (*i.e.*, the first day of *L. reuteri* R28 and *L. plantarum* AR17-1 administration in the PEG + DSS-induced colitis model) and remained at this lower level throughout the remainder of the experiment. The DAI of the *L. plantarum* AR17-1 group was only 1.0, while that of the *L. reuteri* R28 group was 3.0 on the day 16 of the experiment. Taken together, these results indicate that PEG treatment can lead to an increase in the DAI score, which is aggravated after DSS induction. Oral administration with *L. plantarum* AR17-1 and *L. reuteri* R28 did reduce the DAI score in the PEG + DSS group. Compared to *L. reuteri* R28, *L. plantarum* AR17-1 had a better preventive effect on acute colitis induced by PEG + DSS during the course of the experiment.

As noted above, DSS-induced colitis in mice can result in colonic shortening.[21] Fig. 3B shows that when compared with the control group, the DSS-treated group and PEG + DDS-treated group exhibited significantly decreased colon lengths (0 *vs.* 5.57 ± 0.55 and 5.47 ± 0.43 cm, respectively). The administration of *L. plantarum* AR17-1 effectively prevented colon shortening caused by PEG + DSS (7.04 ± 0.42 cm *vs.* 5.47 ± 0.43 cm; $p < 0.05$). The colons of mice in the *L. plantarum* AR17-1 group were of a similar length to those of the healthy mice (6.9 ± 0.27 cm).

Fig. 3C shows that the MPO activity levels in the DSS group (0.48 ± 0.03 U g^{-1}) and PEG + DSS group (0.54 ± 0.04 U g^{-1}) were significantly higher ($p < 0.05$) than that of the healthy group (0.23 ± 0.05 U g^{-1}), indicating more severe inflammation. The PEG + DSS group was most affected, indicating that the colons of the DSS-treated mice had significant neutrophil accumulation and that combined PEG + DSS treatment aggravated inflammation. In contrast to the DSS and PEG + DSS groups, the *L. reuteri* R28 and *L. plantarum* AR17-1 groups exhibited significantly decreased MPO activity (0.44 ± 0.02 and 0.41 ± 0.02 U g^{-1}, respectively; $p < 0.05$). A decrease in MPO activity implies a decrease in the number and distribution of neutrophils in the tissues (*i.e.*, decreased neutrophil infiltration and inflammatory symptoms).[23] Evidently, the *L. reuteri* R28 and *L. plantarum* AR17-1 interventions alleviated the PEG + DSS-induced MPO activity to a significant degree, indicating that both strains can effectively relieve colitis in mice. *L. plantarum* AR17-1 was more effective, but this difference was not significant ($p > 0.05$).

3.3. Effect of *L. reuteri* R28 and *L. plantarum* AR17-1 on inflammatory cytokine expression

TNF-α is the main cytokine in the pathogenesis of IBD, and its coordinating role in colonic inflammation has been verified by the efficacy of anti-TNFα therapy for IBD.[12] TNF-α plays a pro-inflammatory role by increasing the production of IL-1β and IL-6 and the expression of adhesion molecules and procoagulant factors. IL-6 is a pleiotropic cytokine that exerts its pro-inflammatory effects *via* the soluble IL-6 receptor (sIL-6R).[24] As shown in Fig. 4, when compared with the control group, the levels of TNF-α, IL-1β and IL-6 in the DSS-treated group and the PEG + DSS-treated group had increased significantly, but there was no significant difference between these latter two treatment groups. The administration of *L. reuteri* R28 and *L. plantarum* AR17-1 notably inhibited the upregulation of IL-1β, TNF-α and IL-6 observed in the PEG + DSS group, although the difference between the bacteria-treated groups and the control group remained significant ($p < 0.05$). However, *L. plantarum* AR17-1 treatment reduced the expression of TNF-α to a normal level (Fig. 4A). The inhibitory effect of *L. plantarum* AR17-1 on inflammation was better than that of *L. reuteri* R28, and significant differences in IL-1β and IL-6 expression were observed between these groups. These analyses of colonic tissue samples corroborated the alleviation of intestinal inflammation symptoms *via* the ingestion of probiotics. The results suggest that the mechanisms of action of these two strains may be similar, but that *L. plantarum* AR17-1 has a stronger anti-inflammatory effect on intestinal tissues.

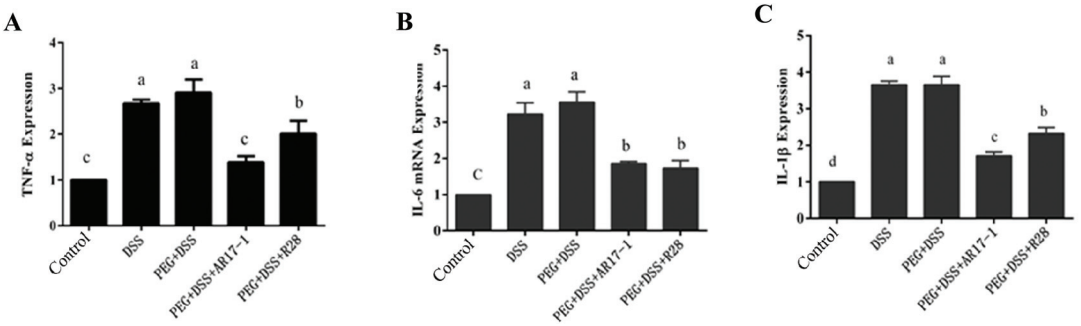

Fig. 4 Expression levels of inflammatory cytokines. The expression of (A) TNF-β (B) IL-6, and (C) IL-1β mRNA in the colon was quantified using quantitative real-time PCR. The data are expressed as means ± standard deviations (n = 3 per group). Means with different letters differ significantly ($p < 0.05$).

4. Discussion

Our results indicate that endogenous *L. reuteri* R28 (isolated from mouse faeces) and exogenous *L. plantarum* AR17-1 (isolated from fermented pickles) exhibited no significant differences in colonisation in healthy mice. After PEG treatment, however, there were significant differences in the colonisation range and intensity between the two strains (Fig. 2). cFDA-SE was used to label *L. reuteri* R28 and *L. plantarum* AR17-1 and explore their distribution and colonisation in the mouse intestines because fluorescence imaging allows the effects to be observed directly and quickly.[25] In PEG-treated groups, *L. reuteri* R28 colonisation was superior to that of the *L. plantarum* AR17-1 treatment group and normal mice (Fig. 2), perhaps because PEG treatment significantly reshaped the microbiota and reduced the relative abundance and competitiveness of gut microbes,[22] thus allowing the wide distribution and long-term colonisation of *L. reuteri* R28. PEG treatment can cause lasting changes to the microbiota and host that affect the introduction of novel species or the reintroduction of species to ecological niches.[11] The presence of a certain number of probiotics in the intestines of mice can affect the gut microbial metabolism. However, there is no conclusive evidence that exogenous probiotics adhere to the intestinal mucosa.[26] However, probiotics can become well colonised in the intestines of germ-free mice. The limited colonisation of probiotics in the gut mucosa of different mice may result from the resistance of the microbiome to colonisation by the supplemented strains.[27] Similarly, we found that exogenous *L. plantarum* AR17-1 quickly disappeared from the mouse intestine, and its colonisation pattern was similar to that of *L. casei*.[19] Interestingly, endogenous *L. reuteri* R28 was also unable to colonise the mouse intestine for a long time. With PEG treatment, the speed of transit was increased, but the colonisation ability of exogenous strains was not enhanced. However, the colonisation quantity, duration and range of endogenous *L. reuteri* R28 were greatly increased. PEG treatment may destroy the balance of gut microbes in the mice, making it easier for endogenous strains to adapt to their own environment and overcome microflora-mediated colonisation resistance. The experimental results suggest that this good colonisation ability lays a foundation for the follow-up treatment of enteritis.

Patients with IBD exhibit reduced microbial diversity when compared to healthy subjects, and it is clear that ecological disorders contribute to the pathogenesis of IBD.[8–12] Probiotics and prebiotics have been widely reported to confer beneficial effects on IBD.[13,14] Probiotics are thought to result in the amelioration or prevention of IBD by restoring the composition of the gut microbiome and inducing the beneficial functioning of gut microbial communities.[28] Because the exogenously administered probiotics seem to pass into the faeces without long-term colonisation, probiotics must be ingested continually to obtain an ongoing effect.[25] Prebiotics cannot be digested in the small intestine but pass into the colon, where they are selectively utilised by endogenous probiotics and stimulate their growth.[25] However, intestinal microbes are vulnerable to diarrhoea and other factors, which can cause long-term alteration of the gut microbiota and affect the functions of probiotics. Colitis induced by DSS is one of the most commonly used models to explore the efficacy of probiotics in the treatment of IBD. PEG treatment can cause mild osmotic diarrhoea and reduce the relative abundance of gut microbes,[11,22] but the combination of the two models is rarely studied. Therefore, we chose to investigate a PEG + DSS model. PEG treatment clearly aggravated the severity of DSS-induced colitis. Endogenous *L. reuteri* R28 relieved mild osmotic diarrhoea induced by PEG more effectively than exogenous *L. plantarum* AR17-1, which more effectively reduced the colitis induced by PEG + DSS. A reduction in pro-inflammatory cytokines can effectively inhibit inflammation, and TNF-α, IL-1β and IL-6 play crucial roles in the pathogenesis of IBD.[29] *VSL#3* and other probiotics were reported to significantly reduce the expression of such inflammation-related factors.[30] We observed that *L. plantarum* AR17-1 was more effective than *L. reuteri* R28 for reducing inflammatory cytokine expression. *L. reuteri* R28 exhibited better colonisation ability in the mouse intestine and a better effect on mild osmotic diarrhoea, but it was less effective than *L. plantarum* AR17-1 at alleviating the symptoms of mice with DSS-induced colitis. Possibly, *L. reuteri* R28 isolated from mice faeces had adapted to the long-term evolution of the intestinal tract, and therefore its effect was milder than other probiotics and closely related to the commensal bacteria. Several beneficial effects of *L. reuteri* have been reported. *L. reuteri* may compete with other bacteria, and this competition reduces the bacterial load and decreases related symptoms.[31] *L. reuteri* benefit the host by producing antimicrobial molecules that inhibit colonisation by pathogenic microbes and remodel the commensal microbiota, thus strengthening the intestinal barrier and stimulating a mild, transitory inflammatory response.[32] We found that when compared with the PEG + DSS group, *L. reuteri* R28 downregulated the expression of pro-inflammatory cytokines but was not as effective as *L. plantarum* AR17-1. However, the underlying mechanisms remain unclear.

5. Conclusions

This study explored the colonisation, distribution and effects on colonic inflammatory symptoms of endogenous *L. reuteri* R28 (isolated from mouse faeces) and exogenous *L. plantarum* AR17-1 (isolated from fermented pickles). *L. reuteri* R28 exhibited a better colonisation ability than *L. plantarum* AR17-1 in C57 BL/6 mice, especially after treatment with PEG. *L. plantarum* AR17-1 exhibited a better preventive effect on acute colitis induced by PEG + DSS, as indicated by decreases in the body weight loss, DAI, colon shortening and MPO activity. Both *L. plantarum* AR17-1 and *L. reuteri* R28 regulated the expression of pro-inflammatory factors in mice with colitis induced by PEG + DSS and enhanced the intestinal barrier function. However, the preventive effect of *L. plantarum* AR17-1

was stronger than that of *L. reuteri* R28. The complexities of microbial fitness in the gut and the regulation of enteritis *via* the use of probiotics remain unresolved. Further research is warranted to clarify the correlations revealed by this study.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

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