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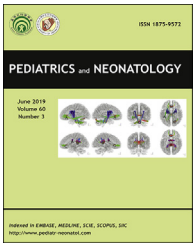

Original Article

SCID/NOD mice model for 5-FU induced intestinal mucositis: Safety and effects of probiotics as therapy

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Background: For chemotherapy patients, intestinal mucositis is a frequent complication. Previously, we evaluated the beneficial effect of oral probiotics in 5-Fluorouracil (5-FU) induced mucositis in BALB/c mice. Here, we used SCID/NOD mice instead to simulate the immunodeficiency of chemotherapy patients: first, to evaluate the safety of probiotic supplementation and second, to determine the probiotic effect in response to 5-FU intestinal mucositis.
Methods: Thirty-six SCID/NOD mice were injected with saline (three control groups) or 5-FU (three experimental groups) intraperitoneally daily for five days. Mice were given either oral saline daily, probiotic suspension of *Lactobacillus casei* variety rhamnosus (Lcr35, Antibiophilus™, France) or *Lactobacillus acidophilus* and *Bifidobacterium bifidum* (LaBi, Infloran™, Italy). Blood, liver, spleen, and lymph node tissue samples were evaluated for probiotic translocation via culture and Q-PCR. Weight change, diarrhea score, jejunal villus height (VH) and crypt depth (CD), and serum cytokine levels of TNF-α, IFNγ, IL-1β, IL-6, IL-4, IL-10, IL-13, and IL-17 were also assessed.
Results: No weight loss was found in the SCID control group. Mean weight loss of 10.63 $\pm$ 0.87% was noted by day five in 5-FU group without probiotics but it was only 6.2 $\pm$ 0.43% if mice were

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given Lcr35 ($p < 0.01$) and 7.1 ± 1.80% ($p < 0.01$) if they were given LaBi. Diarrhea score of 5-FU group without probiotics was 2.0 ± 0.0 by day five, which dropped to 1.33 ± 0.17 ($p < 0.05$) and 1.42 ± 0.24 ($p < 0.05$) with Lcr35 and LaBi, respectively. Average VH significantly decreased and CD significantly increased in SCID mice given 5-FU. With probiotics, average CD improved ($p < 0.05$) while VH lengthened as well. Besides IL-13, all cytokine levels increased in 5-FU SCID mice. Both *Lcr35* and *LaBi* significantly inhibited serum cytokines ($p < 0.05$). No probiotic strains were detected in blood cultures of any mice.

Conclusion: Using SCID/NOD mice as a novel model for 5-FU induced intestinal mucositis, we find that probiotics *Lcr35* and *LaBi* do not lead to bacteremia, can improve diarrhea and body weight, can restore jejunal crypt depth, and significantly inhibit cytokines TNF-α, IL-1β, IFNγ, IL-6, IL-4, IL-10, and IL-17.

1. Introduction

Chemotherapy-induced gastrointestinal mucositis can occur in about 50%—80% of patients, and it has significant implications on the quality of life for cancer patients.[1,2] The most common cytotoxic agents associated with mucositis are the fluoropyrimidines 5-Fluorouracil (5-FU), capecitabine and irinotecan. Despite new agents for management such as keratinocyte growth factor and epidermal growth factor in murine models,[3] supportive care with pain relief, oral and intravenous hydration, and maintaining oral hygiene are still the mainstay of management.[4]

Our previous research shed light on the potential of probiotics to disrupt the dysbiosis of 5-FU induced intestinal mucositis in BALB mice: repairing the jejunum, ameliorating diarrhea and halting body weight loss.[5] To further test for safety of probiotics and more closely simulate the immunodeficiency status of cancer patients, we used SCID/NOD mice model both to explore effects and test for translocation of oral probiotics *Lactobacillus casei* variety *rhamnosus* (*Lcr35*, Antibiophilus™, France) or *Lactobacillus acidophilus* and *Bifidobacterium bifidum* (*LaBi*, Infloran™, Italy) given for 5-FU induced mucositis. We used this novel SCID/NOD mice model to assess 3 areas: mucositis severity in terms of diarrhea score, body weight loss, jejunal mucosa villi and crypt changes; effect on serum cytokine levels post oral probiotics; and probiotic strain organ and blood translocation via Q-PCR and culture.

2. Materials and methods

2.1. 5-FU treatment

5-FU (Fluorouracil-TEVA™, Netherlands) was injected intraperitoneally (IP) at a single dose of 30 mg/kg/day for 5 days to cause mucositis and diarrhea as described in the literature for mice model.[6] Alternatively, IP saline was injected in control groups.

2.2. Probiotics preparation

L. casei variety *rhamnosus* (*Lcr35*, Antibiophilus™, France) and *L. acidophilus* and *B. bifidum* (*LaBi*, Infloran™, Italy) were used in this experiment. Probiotics were diluted in sterile saline and administered by oral gavages. The mice received 100 μL of saline or probiotic suspension containing 1×10^7 CFU daily for 5 days.

2.3. Animal trial

All experiments described were conducted on male SCID/NOD mice obtained from Taiwan's National Laboratory Animal Center and placed under a 12 h light/dark cycle with a temperature of 22 ± 1 °C and a humidity of 55 ± 10%.[6] Animal studies were approved by the Institutional Animal Care and Use Committee (IACUC) of MacKay Memorial Hospital (Taiwan) (IACUC Number: MMH-A-S-102-08). All mice were given ad libitum access to autoclaved food (Laboratory autoclavable rodent diet 5010) and water. The mice were at the age of 6 weeks with weight range of 22—24 g and randomly divided into six groups with six mice in each group ($n = 36$). The mice were injected with saline (three control groups) or 5-FU (three experimental groups) IP daily for 5 days. Mice in each control group and experimental group were daily orally administered saline, probiotic suspension of *Lcr35* or *LaBi*, respectively. Body weight of all mice was recorded daily.

2.4. Diarrhea assessment

Stool passages of all the mice were recorded daily. All mice had normal stool (score: 0) for the 2 days prior to inclusion into the experiment. Diarrhea severity was assessed by using Bowen's score system[7] and was classified into four grades according to the stool consistency: 0, normal stool; 1, slightly wet and soft stool indicating mild diarrhea; 2, wet and unformed stool indicating moderate diarrhea; and 3, watery stool indicating severe diarrhea.

2.5. Histology evaluation of jejunal mucosa

After sacrifice on day 5, a 3-cm ring close to the duodenojejunal flexure of the proximal jejunum was processed and fixed in 10% buffered neutral formalin for 2 h, dehydrated in an ascending series of ethanol concentrations, cleared in xylol, and embedded in paraffin wax. Then, sections of 4-μm thickness were cut and mounted on glass slides and stained with haematoxylin and eosin (HE).[8] A 20x magnification objective was used for image acquisition. Specimens were viewed under the TissueFAXS automatic scanning system, captured by a digital camera and analyzed by HistoQuest software (TissueGnostics, Vienna, Austria).[9] For every jejunal tissue section of each mouse, villus height (VH) and crypt depth (CD) measurements were determined for whole, well-oriented villi and crypts, and these values were averaged.

2.6. Inflammatory cytokines analysis

Blood was collected from the hearts immediately after mice were sacrificed. Blood samples were centrifuged to yield serum. The serum (50 μl) was analyzed by the Bio-Plex (Bio-Rad Laboratories, Inc. USA) ProTM Mouse Cytokine Panel kit (Targets include: IL-4, IL-6, IL-10, IL-13, IL-17, TNF-α, IL-1β, IFNγ) according to manufacturer's instructions. Extracted serum utilized the Bio-Plex-200 system (Luminex Co. Texas, USA).

2.7. Safety of probiotics: translocation and infections

Samples of blood, liver, lymph node and spleen tissues were inoculated in Man, Rogosa and Sharpe (MRS; Oxoid, Basingstoke, UK), Brain Heart Infusion (BHI) (Creative Co. Taiwan) and Bifidobacteria iodoacetate medium-25 (BIM-25) (Creative Co. Taiwan) for isolation of *Lactobacillus* and *Bifidobacterium* groups. Plates were aerobically incubated at 37 °C for 2–5 days depending on culture condition. Bacterial clones were collected for identified strains by genomic sequences. The bacterial colonies were calculated for translocation assay.[10]

2.8. DNA extraction and PCR

Probiotic strains in mesenteric lymph node, spleen, liver and blood samples were detected via PCR with primers as previously reported in the literature. Bacteria, including *Lcr35* and *LaBi* (*B. bifidum* and *L. acidophilus*), were detected by the Maxima SYBR Green/ROX Q-PCR Master Mix (Applied Biosystems, Warrington, UK), as described previously.[5] Pairs of oligonucleotide primers specific to *Lcr35*,[11] *B. bifidum*[12] and *L. acidophilus*[13] were used in Q-PCR analysis to confirm *Lcr35* or *LaBi*.

2.9. Statistical analysis

All parametric data were expressed as the mean ± SE. The statistical significance of differences was analyzed using one-way ANOVA. Data were analyzed with IBM SPSS software (version 21.0; SPSS Institute, Chicago, USA). The results were considered statistically significant if $P < 0.05$.

3. Results

3.1. Diarrhea assessment

Diarrhea scores of all mice were recorded daily and all groups were compared. In the 3 saline control groups, no diarrhea was noted. From day 2 diarrhea started to appear in the 5-FU groups, with a marked increase in severity from days 3–4. Diarrhea score of 5-FU SCID mice without probiotics was 2.0 ± 0.0 by day 5. By day 5, both probiotics groups had significantly reduced diarrhea scores compared to 5-FU alone: 1.33 ± 0.17 ($p < 0.05$) and 1.42 ± 0.24 ($p < 0.05$) for *Lcr35* and *LaBi*, respectively.

3.2. Body weight change

During the experiment, all the SCID mice tolerated the treatment well without exhibiting signs of marked adverse effects such as cachexia or bloody stool passage. No mortality was noted. No weight loss was noted in saline SCID control groups, with no significant difference between probiotic and saline groups. By day 5, mean weight loss of 10.63 ± 0.87% was recorded in 5-FU group without oral probiotics; however, mean loss was 6.20 ± 0.43% if mice were given *Lcr35* ($P < 0.01$) and 7.10 ± 1.80% ($P < 0.01$) if given *LaBi* (Fig. 1). All 5-FU groups showed weight loss compared to control, with the 5-FU group without probiotics dropping more than 10% bodyweight by day 5. *Lcr35* had a significantly ($P < 0.05$) stronger protective effect compared to *LaBi*.

3.3. Histological evaluation of jejunal mucosa: villus height and crypt depth

As expected, jejunal mucosa after 5-FU had flattened epithelium, shorter villi, and inflammatory cell infiltration into the lamina propria, which are hallmarks of intestinal mucositis (Fig. 2D). Average VH in saline control mice was 295.89 ± 17.46 μm, which dropped to 196.79 ± 9.61 μm with 5-FU ($p < 0.05$). Although not significant, VH was 235.69 ± 16.81 μm and 205.21 ± 13.48 μm for *Lcr35* and *LaBi*, respectively (Fig. 2G). Similarly, average crypt depth increased to 118.33 ± 10.91 μm in 5-FU groups compared to 62.51 ± 1.97 μm in saline controls. In 5-FU groups, CD was significantly less after probiotics: 72.52 ± 3.93 μm for *Lcr35* and 64.96 ± 3.24 μm for *LaBi* ($p < 0.05$) (Fig. 2H). Although villi height showed improvement after probiotics use, it was crypt depth that showed potential for significant normalization (see Fig. 2H).

3.4. Cytokines analysis

TNF-α, IL-1β, IFNγ, IL-6, (Fig. 3) IL-4, IL -10, and IL-17 (Fig. 4) serum levels increased in 5-FU SCID mice compared to saline controls. Both *Lcr35* and *LaBi* treatments significantly inhibited serum cytokines in the 5-FU SCID mice: TNF-α (274.9 pg/mL (*Lcr35*) and 259.74 pg/mL (*LaBi*) vs. 348.35 pg/mL (saline), $P < 0.001$); IL-1β

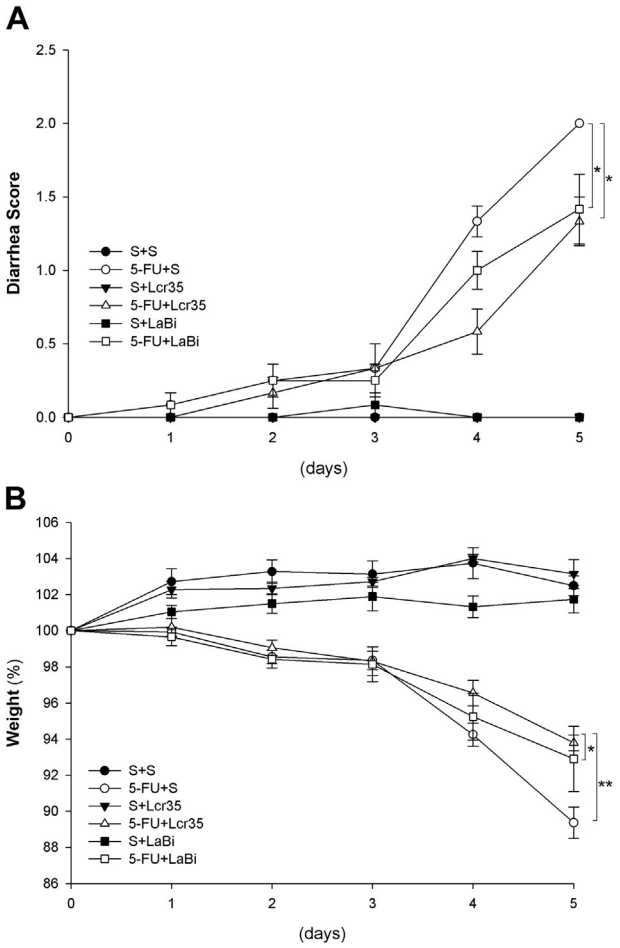

Figure 1 Assessment of SCID mice Intestinal Mucositis Severity. A. Diarrhea Score of SCID/NOD mice after oral probiotics *Lcr35* or *LaBi* with/without 5-FU treatment. The mice were recorded daily and the results of all groups were compared with those in 5-FU + saline groups (S) for 5 days. In the control groups, the mice were injected with saline and administered with saline (●), *Lcr35* (▼), and *LaBi*(■). In the experimental groups, the mice injected with 5-FU and administered with saline (O), *Lcr35* (△), and *LaBi*(□). The severity of the diarrhea was attenuated in the experimental SCID mice treated with probiotics. The * represents significant difference ($P < 0.05$) based on one-way ANOVA. **B. Daily body weight change in percentage of saline or 5-FU injected SCID/NOD mice with/without probiotics *Lcr35* or *LaBi*.** The mice were weighed daily and the results of all groups were compared with those in 5-FU + saline groups (S) for 5 days. In the control groups, the mice were injected with saline and administered with saline (●), *Lcr35* (▼), and *LaBi*(■). In the experimental groups, the mice were injected with 5-FU and administered with saline (O), *Lcr35* (△), and *LaBi*(□). Data of initial bodyweight are expressed as 100% on day 0. The * and ** represents significant difference $P < 0.05$ and $P < 0.01$, respectively based on one-way ANOVA.

(116.07 pg/mL (*Lcr35*, $P < 0.01$) and 124.76 pg/mL (*LaBi*, $P < 0.05$) vs. 169.14 pg/mL (saline)); IL-6 (20.32 pg/mL (*Lcr35*) and 21.31 pg/mL (*LaBi*) vs. 26.51 pg/mL (saline), $P < 0.05$); IL-4 (14.59 pg/mL (*Lcr35*, $P < 0.01$) and 13.23 pg/mL (*LaBi*, $P < 0.001$) vs. 18.16 pg/mL (saline)); IL -10 (63.66 pg/mL (*Lcr35*) and 60.70 pg/mL (*LaBi*) vs. 80.23 pg/mL (saline), $P < 0.001$); IL-17 (12.88 pg/mL (*Lcr35*, $P < 0.05$) and 10.52 pg/mL (*LaBi*, $P < 0.001$) vs. 15.37 pg/mL (saline)).

3.5. Probiotic safety and translocation

No evidence of *Lcr35* or *LaBi* bacteremia was found in blood culture samples in SCID mice (Table 1). Almost all blood samples showed no evidence of probiotic strain via Q-PCR detection. However, for both probiotic groups, mesenteric lymph node samples frequently showed evidence of translocation via culture and Q-PCR. Also, *LaBi* was detected in spleen and liver samples via Q-PCR relatively more frequently than *Lcr35*.

4. Discussion

In SCID/NOD mice lacking T and B cells, probiotics *Lcr35* and *LaBi* were shown to improve symptoms of chemotherapy-induced mucositis, affect mucosa histologically, and alter serum cytokine levels. Given this severely immunodeficient model, we looked for evidence of possible strain translocation via blood culture and Q-PCR in organ samples to evaluate the safety of administering probiotics.

4.1. Weight loss

The SCID control mice given *LaBi* showed 3% body weight increase by end of day 5, although it was not significant when compared to saline or *Lcr35* control. All SCID mice in 5-FU groups, regardless of which probiotic strains were given, dropped 8—11% in body weight at the end of day 5, which was significantly different from the saline groups. This result is similar to other results in the literature.[2,7] After 3 days of chemotherapy induction, the rate of weight loss began to increase. This trend was also observed in our previous study with BALB mice given 5-FU[5]: *Lcr35* and *LaBi* had a protective effect against weight loss.

4.2. Diarrhea score

As expected, diarrhea scores increased when SCID mice were given 5-FU injections. In the chemotherapy mice, oral administration of *LaBi* and *Lcr35* both significantly improved the diarrhea scores. In our previous BALB mice study, *LaBi* also had a stronger effect in improving the diarrhea score compared to *Lcr35*.[5]

4.3. Histology of jejunal mucosa: villus height and crypt depth

In this study, shortening of average jejunal villus height and lengthening of crypt depth were seen when SCID mice were given 5-FU ($p < 0.05$). Adding *Lcr35* and *LaBi* restored some villus height but this change was not significant. *Lcr35* appears to have a greater effect on VH than *LaBi* in SCID mice with mucositis. It is interesting to note that in our previous study with BALB mice, villus height was restored significantly, and *LaBi*, not *Lcr35*, had the stronger healing effect. Further,

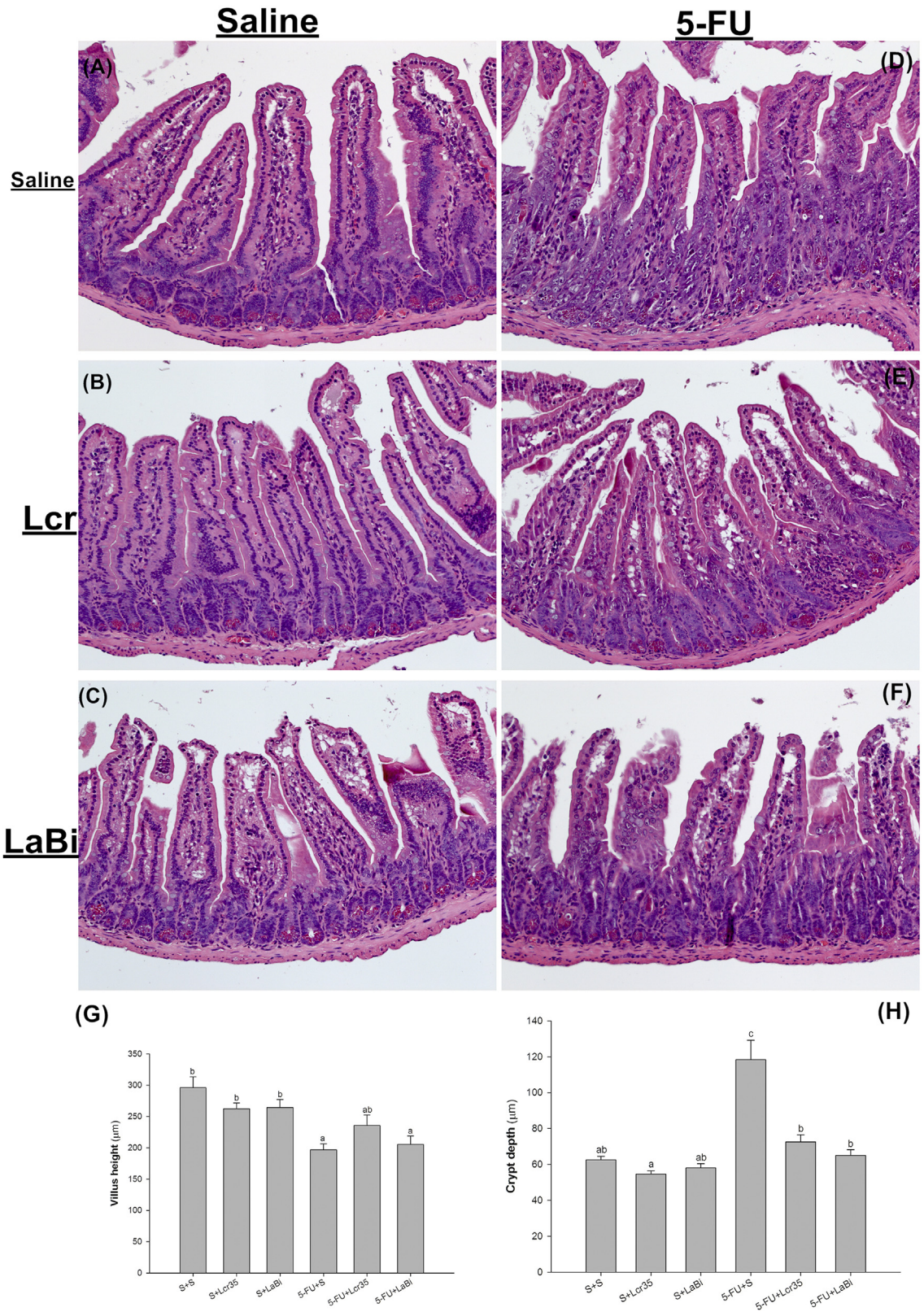

Figure 2 Representative histology of jejunal villus height(VH) and crypt depth(CD) with HE stain in mice on day 5 with saline or 5-FU with/without probiotics(*Lcr35* or *LaBi*). (A, B, C, D, E, F) The image acquisition phase was done with a 20x magnification objective. **(G,H) Average jejunal villus height and crypt depth measurements.** Values are represented as mean ±SEM and were analyzed using one-way ANOVA.

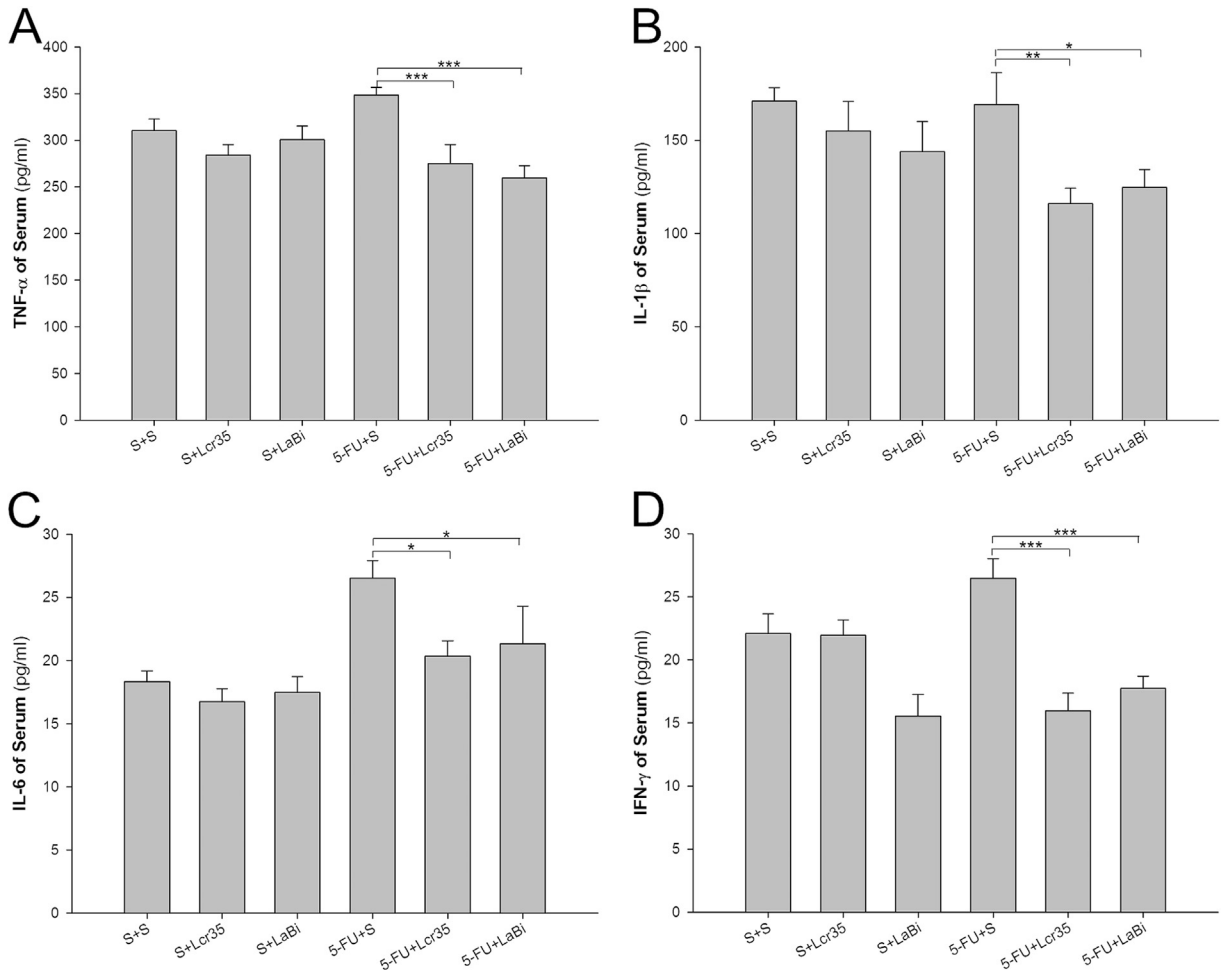

Figure 3 Serum levels of TNF-α, IL-1β, IL-6, and IFNγ of 5-FU injected SCID/NOD mice with/without probiotics *Lcr35* or *LaBi*. The statistical analysis was performed by One-way ANOVA. (*: $P < 0.05$, **: $P < 0.01$, ***: $P < 0.001$).

average VH in BALB mice controls were above 500 μm while that of SCID mice before 5-FU administration was around 300 μm. It is possible that increased T lymphocyte activity, coupled with increased mucin secretion in BALB mice, enables stronger protection and healing of mucosal villi.[5,8] However, since villi lengthening by probiotics in this experiment was not significant, we can not extrapolate this finding further, for which future study is needed.

Average jejunal crypt depth increased significantly when the SCID mice were given 5-FU, and both *Lcr35* and *Labi* had significantly returned the crypts to near control levels. As reviewed previously, CD in animal models with mucositis has varied and inconsistent results in the literature.[5] This is further complicated by the fact that T cell regulators and effectors modulate mucosa integrity, and thus villus height and crypt depth in normal mice compared to SCID mice.[14] A next step in our research may be duplicating the study with reconstituted T cells SCID mice and reexamining jejunal mucosa changes.

4.4. Cytokines analysis

Serum cytokine levels increased after 5-FU injection and were variously affected by probiotic administration in SCID/NOD mice, suggesting that innate immunity plays a role in the pathogenesis of intestinal mucositis. TNF-α, IL-1β, and IL-6, often referred to as pro-inflammatory cytokines, can promote inflammation when tissue injury or infection occurs.[15] The pathogenesis of common inflammatory diseases have involved these cytokines, in not only mucositis[16–18] but also inflammatory bowel disease, rheumatoid arthritis, and even sepsis.[19,20] In our experiment, TNF-α, IL-1β, and IL-6 serum levels were significantly inhibited after probiotic strains were given to 5-FU induced SCID mice.

In SCID mice, TNF-α most likely originates from NK cells and activated macrophages since T lymphocytes are lacking.[21] Several animal and human studies[21–23] have shown improvement in severity and frequency of mucositis following administration of TNF inhibitors, and here we demonstrate an inhibitory effect with *Lcr35* and *LaBi*. As when Lima et al. used pentoxifylline and thalidomide to inhibit cytokine synthesis for protection against oral 5-FU mucositis, we find that inhibition of TNF-α, even in SCID mice, is also involved in diarrhea improvement and mitigating weight loss.[23] In another cyclosporine mice study, etanercept, a TNF-α inhibitor, did not improve weight loss and diarrhea, despite decreasing gut epithelium apoptosis.[24] A likely explanation for this phenomenon is that there exists other parallel cytokine pathways to induce

Table 1 Culture and Q-PCR for individual probiotic strains *Lcr35* and *LaBi* in mesenteric lymph node, spleen, liver, and blood samples.

Culture for bacteria	Mesenteric lymph node	Spleen	Liver	Blood
S + S	3/6	0/6	0/6	0/6
S + *Lcr35*	2/6	1/6	0/6	0/6
S + *LaBi*	1/6	0/6	0/6	0/6
5-FU + S	3/6	1/6	0/6	0/6
5-FU + *Lcr35*	2/6	1/6	1/6	0/6
5-FU + *LaBi*	4/6	1/6	1/6	0/6
PCR for *Lcr35*	Mesenteric lymph node	Spleen	Liver	Blood
S + S	1/6	0/6	0/6	1/6
S + *Lcr35*	1/6	0/6	0/6	1/6
5-FU + S	1/6	0/6	0/6	0/6
5-FU + *Lcr35*	3/6	1/6	0/6	0/6
PCR for *LaBi*	Mesenteric lymph node	Spleen	Liver	Blood
S + S	4/6	1/6	2/6	0/6
S + *LaBi*	5/6	2/6	2/6	1/6
5-FU + S	4/6	3/6	2/6	1/6
5-FU + *LaBi*	6/6	4/6	3/6	1/6

mucositis or that there are multiple initiators of the inflammatory cascade response.

IL-1β plays a crucial role in the activation of the NF-κB pathway, even working with TNF for a synergistic effect in kickstarting inflammatory response of endothelial adhesion molecules.[25] Blocking the action of IL-1 via IL-1Ra in vivo has been shown to reduce mice mortality, body weight loss, and diarrhea severity.[26]

In a SCID mice model, IL-6 is mostly likely secreted by endothelial cells, fibroblasts, and keratinocytes.[27,28] From IBD studies, IL-6, like IL-1β, was shown to also activate the NF-κB pathway, with serum levels corresponding to disease severity.[29] From mice transplantation experiments, it is known that TNF induces IL-6 secretion, but at the same time, IL-6 actively inhibits TNF production as well.[30] This negative feedback loop between IL-6 and TNF provides a mechanism to suppress the activation of the pro-inflammatory cascade, highlighting the complex role of IL-6 in being both a pro- and anti-inflammatory cytokine. From the literature, it is unclear if this negative feedback loop occurs in the pathogenesis and healing of intestinal mucositis. Perhaps an extended experiment where serum cytokine levels are monitored over a longer period of time can confirm this dual role of IL-6.

In this experiment, we found that serum IL-4, IL-10, and IL-17, commonly classified as anti-inflammatory cytokines, were all significantly inhibited by probiotics in 5-FU SCID mice. In our results, IL-13 decreased with 5-FU injection and then showed a non-significant drop in serum levels with oral probiotics (Fig. 4). If the function of this subclass of cytokines is to suppress the pro-inflammatory response and down-regulate the inflammatory reaction,[31] why do serum levels decrease when 5-FU mice are given probiotics? To the best of our knowledge, there is no published evidence that IL-4, IL-10, and IL-17 play an anti-inflammatory role in chemotherapy-induced mucositis. One possible reason is that a strong anti-inflammatory response has been known to lead to over-inhibition of the immune system, increasing the risk of systemic infection.[31,32] Another is simply that SCID mice, lacking T and B lymphocytes, cannot sufficiently overexpress anti-inflammatory cytokines. Clearly, overlapping pathways and myriad of immunoregulatory elements coordinate the host immune response.

IL-4 acts on a variety of tissues, with receptors found in endothelial, epithelial to even brain and liver cells in vitro,[33] and it is originally secreted from Th2 type helper T cells, mast cells, and basophils.[34,35] IL-4 has many pathways of regulating anti-inflammation: suppressing IL-1β, a major pro-inflammatory cytokine, and increasing IL-1 receptor antagonist (IL-1ra) expression to block IL-1α and IL-1β.[36,37] However, the nature of cytokine activity varies widely, depending on the model of intestinal inflammation. Soares et al. recently showed that 5-FU mucositis was ameliorated in IL-4$^{-/-}$ mice, suggesting that IL-4 is pro-inflammatory in nature in the mice model.[38] Our data further support the idea that IL-4 is a pro-inflammatory cytokine in mice with chemotherapy-induced intestinal mucositis, and further research into its role is needed.

4.5. Safety and translocation

In the pediatric oncology population, which at baseline is relatively more immunodeficient compared to adults, the absolute safety of using probiotics must be determined first before it can be considered as a possible treatment for chemotherapy-induced mucositis. In our previous study,[5] we found no evidence of strains in blood, liver, and spleen samples of BALB mice. To establish even stronger evidence for probiotic safety, we chose to use SCID/NOD mice as an example of a severe immunodeficient environment and test whether translocation will occur. Even under this condition, no bacteremia was noted. Likewise, liver and spleen samples via culture were minimal. However, mesenteric lymph node translocation was more frequently observed, in both saline and 5-FU groups, via culture or Q-PCR. For 5-FU SCID mice, this is to be expected as damage to the epithelium allows the bacteria access to the lamina propria and lymphatic drainage. Even with an intact epithelium, dendritic cells with protrusions between enterocytes can make contact with bacteria at the apical surface, allowing phagocytosis via Peyer's patches that transport the strains to the mesenteric lymph nodes.[10]

Especially in the spleen and liver samples, strains detected via PCR were observed more frequently in the *Labi* group, compared to *Lcr35*. It is interesting to note that Lcr35 translocation was rarely observed in our SCID samples, whether chemotherapy or saline groups. Could *Lcr35* be a less invasive strain compared to *LaBi*? Or is there some synergistic effect with the combination of *L. acidophilus* and *B. bifidum*? The key may lie in the immune interaction of the individual strains which needs further clarification.

From what we know, SCID/NOD mice is a novel model for 5-FU induced intestinal mucositis and probiotic therapy. However, our study has some limitations. First is the small sample size of mice models used in this experiment. Second

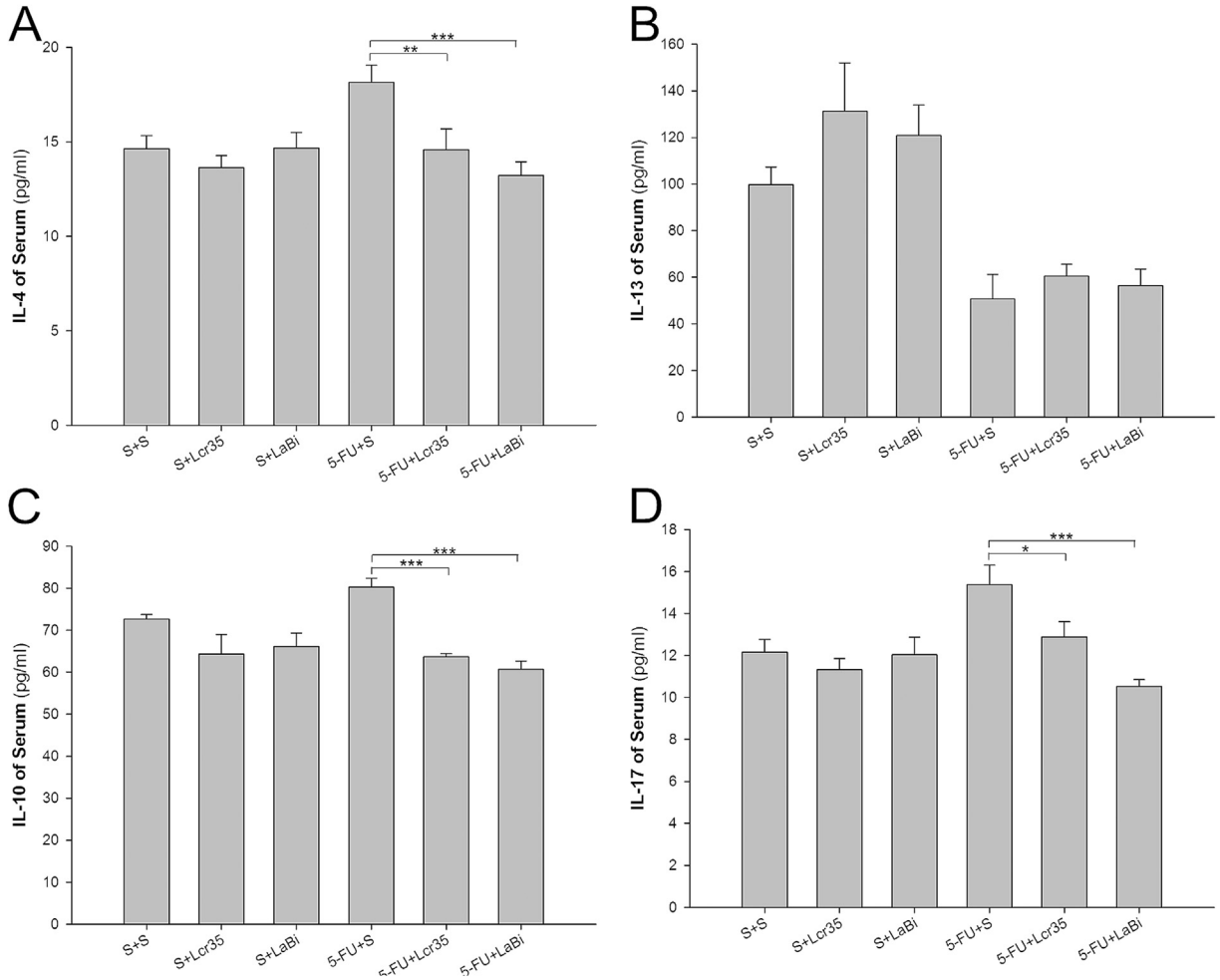

Figure 4 Serum levels of IL-4, IL-10, IL-13, and IL-17 of 5-FU injected SCID/NOD mice with/without probiotics *Lcr35* or *LaBi*. The statistical analysis was performed by One-way ANOVA. (*: $P < 0.05$, **: $P < 0.01$, ***: $P < 0.001$).

is that we did not test individual strains for their independent effects. We chose these strains because our previous research showed their abilities in maintaining tight junction integrity.[39,40] Third, the duration of the experiment should be extended in future studies to evaluate long-term serum cytokine response, rather than only the acute changes due to 5-FU injection. Further study should also focus on longer duration of probiotics use and diarrhea severity. Finally, if a BALB/c cohort were included, the differences between immunodeficient and wild type mice would be more directly comparable, and our results more comprehensive.

5. Conclusion

Using SCID/NOD mice as an immunodeficient model for 5-FU chemotherapy-induced mucositis, we found that oral administration of probiotics *Lcr35* or *LaBi* was a potentially safe therapeutic option with no evidence of bacteremia in blood cultures, that it could significantly inhibit serum cytokines TNF-α, IL-1β, IFNγ, IL-6, IL-4, IL-10, and IL-17, and that it could improve diarrhea and halt body weight loss with jejunal mucosa repair. To the best of our knowledge, using SCID/NOD mice as model for 5-FU induced intestinal mucositis and probiotic therapy has not been evaluated previously.

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Conflict of interest

All authors have no conflict of interest to disclose.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.pedneo.2018.07.007.