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RESEARCH ARTICLE

Lactobacillus acidophilus Supplementation Restores Gut Epithelial Integrities and Barrier Functions in Non-specific Diarrhea

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ABSTRACT

Objectives: *The study was aimed to evaluate the roles of Lactobacillus acidophilus supplementation in maintaining intestinal epithelial integrities, tight junction proteins, and adhesion molecules in non-specific diarrhea.*

Methods: In this study, we used the pre-weaned BL/6 pups (3 weeks of age, same litters) as a model animal. We supplied the non-sterilized and poor-quality water to experimental pups (n=7) to develop non-specific diarrheic symptoms. Then diarrheic pups were supplemented with Lactobacillus acidophilus (LA) for three consecutive weeks. The control group (n=5) was supplied with sterilized water and no LA. The sampling and analysis were performed on day 0, day 7, day 14, and day 21. The expressions of pro-inflammatory cytokines (IL-6, TNF-a) and tight junction proteins (TJPs) of gut mucosa were determined using qRT-PCR. And the serum cytokines level was screened through sandwich ELISA.

Results: The intestinal cytoskeletal integrity becomes disrupted and characterized by lower ZO-1, Occludin, Claudin-1, Claudin-5, and JAM mRNA expressions upon real-time qRT-PCR. However, Claudin-4 was found to be not affected and illustrated with a higher expression like control pups. Interestingly, supplementing Lactobacillus acidophilus was found to maintain gut integrity and effectively reduce diarrheic symptoms. Like the control pups, the Lactobacillus acidophilus supplemented pups exhibited a higher expression of gut epithelial TJPs and adhesion molecules. Moreover, the diseased pups produced significantly increased IL-6, and TNF-α production in blood serum, compared to control BL/6 pups.

Conclusion: We concluded that L. acidophilus supplementation might orchestrate the equilibrium of gut health and immunity against non-specific diarrhea. *J Microbiol Infect Dis 2022; 12(2):54-62.*

Keywords: *mice, gut immunity, tight junctions, and adhesion molecules, Lactobacillus acidophilus supplementatio*

INTRODUCTION

The proper management of laboratory mice is related to sound breeding, to feed, and raising systems with controlled conditions to maintain

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predictable traits and genetics. Mice in laboratory conditions may survive with the appropriate environment and management to conserve predictability and genetic characteristics. The gut mucosal surface, bearing a load of some 1014 commensal, symbiotic microorganisms, is a vital checkpoint for entering and invading food-borne foreign antigens [1]. Those beneficial bacteria supervise gut ecological niches and execute nutritional roles (vitamin K and B complex synthesis). The gut mucosal homeostasis is highly dependent on numerous immunological and non-immunological components, including the inter-epithelial tight junction proteins (TJPs) and adhesion molecules. And the disruption of those TJPs resulted in the increased permeability to macromolecules and subsequent dampening of mucosal immunity against invading or commensal microbes [2-4]. Four major groups of TJPs like occludins, claudins, junctional adhesion molecules (JAM), and tricelluin have been identified in gut epithelium to maintain equilibrium and homeostasis of barrier functions [2]. The interaction of TJ Ps with the actin cytoskeleton (Zonula occluding- ZO) is vital to maintaining the TJ structure and integrities [3-4].

Due to insecure feeding – watering, improper wastage management, and overcrowding of pups in the same cages may increase the susceptibility to bacterial gut attack and concomitant loss of mucosal immunity [5]. Those diarrhea-causing microbes, particularly enterotoxigenic *E. coli* (ETEC), colonize murine lower gut mucosa by fimbrial adhesions. And secrete their enterotoxins that cause fluid loss, diarrhea, disruptions of intestinal barrier integrity, and functional reduction of innate mucosal immunity [2,3,6]. Intestinal microbes help maintain the physiological equilibrium in gut immune homeostasis and convert dietary nutrients to essential metabolites to regulate host immunity [3,7].

Probiotics are known to restore the composition of the gut microbiome maturation of gut epithelium to facilitate favorable functions to the gut ecosystem, thus orchestrating in ameliorating or preventing various systemic inflammations [8]. L. acidophilus has functionally been associated with improved health and promotes the growth and distribution of specific beneficial bacteria with well-defined metabolic functions [9]. L. acidophilus may exert its functionality against the harmful gut microbial attacks, stimulating the innate immune response, enhancing lower gut muscle contraction, hindering cholesterol synthesis, and thus preventing gut inflammations [8, 10]. Several small animal models stated the alterations of gut mucosal immunity and subsequent deterioration of intestinal health due to enteropathogenic *E. coli* (EPEC) and enterotoxigenic *E. coli* (ETEC) infections [7,11,12].

Here we hypothesized that non-sterilized water supply might predispose the BL/6 pre-weaned growing pups to be prone to non-specific diarrhea. Those may disturb gut epithelial barrier integrities by altering epithelial tight junction proteins and adhesion molecules.

METHODS

All in-vivo experiments described in this study were performed in the Department of Clinical Microbiology, following the guidelines of the Institutional Animal Care and Use Committees (IACUC) of the University of Nebraska, NE, and were pre-approved by the ECAE (Ethical Committee for Animal Experiments) of the department.

Mice

C57BL/6 (H-2b) mice were screened and separated from CD11c.DTR Transgene (Tg) mice through PCR of blood samples using specific primers (Table-1). The CD11c.DTR Tg negative mice were selected as BL/6 (wild type) mice (Figure 1) and then were appropriately bred and screened for genotyping routinely under the Department of Microbiology animal facilities. The pre-weaned pups (3 weeks of age) were reared in two categorized water supplies; the control and experimental pups were supplied with sterilized and non-sterilized tape water, respectively.

Lactobacillus acidophilus supplementation

Lactic acid-producing bacteria (LAB) ferment carbohydrates (CHO) and produce ATP for cellular functionalities. Lactobacillus acidophilus is used to produce commercial yogurts to boost intestinal homeostasis. We supplemented non-specific diarrheic pups with Lactobacillus acidophilus (Natrol LLC, Chatsworth, USA) @1x106 live LA in 100 µl of 10% (w/v) skimmed milk powder per pups for three consecutive weeks. The control group

received 100 µl of 10% (w/v) skimmed milk powder without LA. The sampling and analysis were performed on day 0, day 7, day 14, and day 21 to screen the gut epithelial integrities and serum proinflammatory cytokines production.

Sampling

Three weeks aged pre-weaned BL/6 pups supplied with non-sterilized water (tap water) have been taken under consideration for the development of intestinal stress and non-specific diarrheic symptoms at day seven (07). We have collected the lower intestinal part of both diarrheic (n=7) and control pups (n=5) following the methods documented elsewhere [13], with minor modifications. In brief, after cutting pieces of the lower intestinal part, defecation and washing were performed clearly with cold PBS. Then those samples were soaked in DTT solutions for 10 mins, and rewashing was carried out with cold PBS again. Finally, the supernatant was discarded twice after centrifugation (13,000 rpm for 5 minutes). Then, the sediment was diluted and homogenized in Easy Blue solution for further steps of RNA extraction.

Gut RNA extraction and cDNA synthesis

Gut tissue was homogenized with Easy Blue®, and RNA extraction was implemented through the classical phenol-chloroform precipitation. Finally, RNA was purified twice with 70% alcohol washing and eluted with RNAse-free DEPC water at a30 µl volume. RNA was reverse transcribed (@1 µg) using High-Capacity cDNA Reverse Transcription Kits (Applied Biosystems, Foster City, CA, USA) before real-time qPCR. (Composition of reverse transcription reaction detailed in Table:2.

Analysis of mRNA gene expressions in the gut

Different mRNA levels in harvested intestinal tissues were measured by real-time qRT-PCR using total RNA. Following the extraction of gut RNA, real-time qPCR using a CFX96™ Real-Time PCR Detection System (Bio-Rad Laboratories, Hercules, CA, USA) was employed. A final volume of 20 µl reaction mixture encompasses 10 µl of 2x SYBR Green-based real-time RT-PCR, 2 µl of cDNA template, and 200 nM of specific primers for the detection of gut epithelial integrities (Table: 3). Double-stranded nucleic acids were denatured at 95°C for 30 s and then subjected to 45 cycles of 95°C for 5 s and 65°C for 15 s. After the reaction cycles, the temperature was increased from 65°C to 95°C at a rate of 0.2°C/10 s, and fluorescence was measured every 5 seconds to construct a melting curve. A control sample devoid of template DNA was run with each assay, and all determinations were performed at least in duplicate to ensure reproducibility. The analysis of the melting curve determined the legitimacy of the amplified product. The expression of tight junction proteins and adhesion molecules was expressed as relative fold expression compared to the control group after normalization to the housekeeping gene gapdh [14]. All data were analyzed using the Bio-Rad CFX Manager, version 2.1 analysis software (Bio-Rad Laboratories).

Serum cytokines determination through ELISA

According to the manufacturer's protocols, the levels of IL-6 and TNF-α in blood serum were determined using sandwich ELISA (BD Bioscience). Capture antibody in coating buffer @ 50 ul/well was kept overnight in 4oc. The next day, plates were washed with ELISA wash buffer (PBS containing 0.05% Tween-PBST), followed by blocking with 100 µL 3% skim milk for 2 hours in RT. The serum samples and standards for recombinant cytokine proteins (IL-6, TNF-α) were added and kept overnight at four °C. After washing with wash buffer, biotinylated antibodies were added and incubated for 2 hours at RT. The plates were washed and incubated with streptavidin-horse radish peroxidase (HRP) for 30 min at 37 °C. Then tetramethylbenzidine (TMB) substrate was added to develop the color, followed by stopping the reaction with 50 µL of stop solution (1 M Phosphoric acid). Finally, the OD values were measured at 450 nm by ELISA reader (Molecular Devices, San Jose, CA, USA). Cytokine concentrations were analyzed with SoftMax Pro3.4.

RESULTS

Screening of C57BL/6 (WT) mice as Tg negative (-) pups from CD11c.DTR litters

PCR has screened the BL/6 pups (Figure 1; band size: 200bp) from the CD11c.DTR pups, where CD11c.DTR-Tg (+) pups were labeled as 700bp-200bp (Figure 1). We bred C57BL/6 pups to produce F1 (generation 1) and kept

the treatment groups as normal mineralized water and non-sterilized tape water to develop non-specific diarrhea.

Gut epithelial integrities of non-specific diarrheic pups

Despite some limitations, murine models are widely used to study various infectious zoonotic diseases. The management system is highly interlinked with the sound health of growing mice. The gut health of pre-weaned pups is considerably related to balanced management regarding the water supply. We found that pre-weaned BL/6 pups kept with non-sterilized water supply led to worsened gut health and diarrheic symptoms (frequently pasty and green colored stools) than pups reared with ad-libitum sterilized drinking water. Our findings showed that untreated typical tape water supply results in non-specific diarrhea and reduced mRNA gene expression of ZO-1, Occludin, Claudin-1, Claudin-5, and JAM upon real-time qRT-PCR (Figure 2). However, the mRNA expression of Claudin-4 was not affected in non-specific diarrheic pups, as revealed by an insignificant pattern. Therefore, the non-specific diarrheic condition is interlinked with the destruction of TJPs and adhesion molecules, and thus intestinal permeability increases and the loss of essential nutrients. And thus, the gut homeostasis becomes reduced, resulting in the loss of gut mucosal immunity.

Induction of proinflammatory cytokines in serum

The induction of pro-inflammatory cytokines (IL-6, TNF-α) is a critical marker of microbial attacks and loss of physiological homeostasis. Our findings showed that the diseased pups produced significantly increased IL-6 and TNF-α production in blood serum than those of control BL/6 pups (Figure 3a,b). Therefore, our findings corroborated the induction of significantly increased IL-6 and TNF-α in the blood serum of diarrheic pups related to the loss of gut immune homeostasis.

Restoration of TJPs and adhesion molecules after LA treatments

Here we demonstrated that the administration of LA could enhance the epithelial TJPs and adhesion molecule integrities. Specifically, compared to control pups, the LA treated non-specific diarrheic pups revealed higher mRNA gene expression of ZO-1, Occludin, Claudin-1, Claudin-4, Claudin-5, and JAM upon real-time qRT-PCR (Figure 4a-f). We observed that the expression of ZO-1 and Occludin started to increase on day seven and then increased significantly according to the times of supplementation (Figure 4b-c). The LA supplementation also exhibited increased mRNA expression of Claudin-1, Claudin-5, and JAM in non-specific diarrheic pups, compared to the non-treated group. Notably, Claudin-1 and Claudin-5 revealed restoration started on day seven as a significant pattern and maintained a similar level on day 14 and day 21 of post-LA supplementation (Figure 4b,f). Claudin-4 shows no significant differences between the supplemented and control groups (Figure 4d). Therefore, our findings echo techoficial effects of LA supplementation in the renovation of epithelial barrier functions in case of diarrheic conditions in growing pre-weaned mice.

Table 1. specific primers for CD11c.DTR-transgene negative (WT) mice genotyping.

Primers name	Primer sequence
DTR-Tg (WF)	CAA ATG TTG CTT GTC TGG TG
DTR-Tg (WR)	GTC AGT CGA GTG CAC AGT TT
DTR-Tg (DCF-DTR1)	GGG ACC ATG AAG CTG CTG CCG
DTR-Tg (DCR-DTR2)	TCA GTG GGA ATT AGT CAT GCC

Table 2. Composition of reverse transcription reaction for mouse guts tissue reagents.

Reagents	Volume
RNA template	10 µl
10x reaction buffer	2 µl
10x RT primer	2 µl
25 dNTP (100mM)	0.8 µl
RNase inhibitor	0.5 µl
RTase enzyme (high capacity)	1 µl
Deionized DW	4.3 µl

Effects of LA on serum pro-inflammatory cytokines production

Our results disclose of regulatory effects of LA on serum pro-inflammatory cytokines (IL-6 and TNF-α) production. Remarkably, the LA supplemented pups showed a significantly decreased production of both IL-6 and TNF-α in serum compared to control pups on days 14 and 21 (Figure 5a, b). However, no differences

were found between those of LA supplemented and control mice on day seven. Therefore, our data suggest a regulatory role of LA supplementation on serum pro-inflammatory cytokines induction in the aspect of non-specific diarrheic conditions. Thus, LA supplementation results in the boost of gut mucosal immunity and reduction inflammation and helps in maintaining the gut homeostasis.

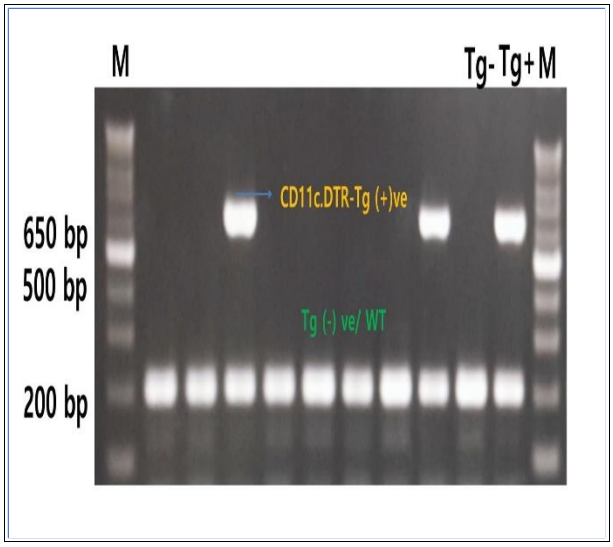

Figure 1. Screening of CD11c.DTR-transgene negative pups for the experiment.
Agarose gel electrophoresis shows the specific band size of DNA to separate the CD11c.DTR-Tg negative (WT) mice from those of CD11c.DTR-Tg positive mice (Figure 1). Upon PCR, the CD11c.DTR-Tg negative mice (200 bp) and CD11c.dTR-Tg positive mice (650bp-200bp) were selected from the same litter size.

DISCUSSION

Under physiological homeostasis, the gut epithelium absorbs nutrients and plays a crucial defense mechanism against intestinal microbes to orchestrate optimum mucosal immunity [7]. Due to microbial attacks and stresses, the gut integrities become decreased and may result in inappropriate barrier functions, destruction of tight junction proteins and adhesion molecules, and intestinal hyper-permeability [6, 7, 13]. Tight junction proteins and adhesion molecules form the continuous intercellular barrier between intestinal epithelium to prevent microbial attacks and maintain homeostasis in gut mucosa [15,16]. The epithelial cytoskeleton maintains gut barrier integrities via occludin, claudins, ZO-1 proteins, and junction adhesion molecules (JAM). These TJPs and JAMs are mainly located apically on the lateral membrane of the gut mucosal surface [19,15,16].

Growth and differentiation of pathogenic bacteria in the intestine may result in the production of different enterotoxins, disruption of gut epithelial integrities, and induce diarrhea. Those toxigenic bacteria disrupt the intestinal integrities by reducing TJPs like Zo-1, Occludin, Claudin-1, Claudin-4, Claudin-5, JAM, etc. [2,5,6]. Intestinal enteric bacteria induce membrane destruction by delocalization of ZO-1, reduction of occluding, and disruption of claudin and JAM [6,11,15,16].

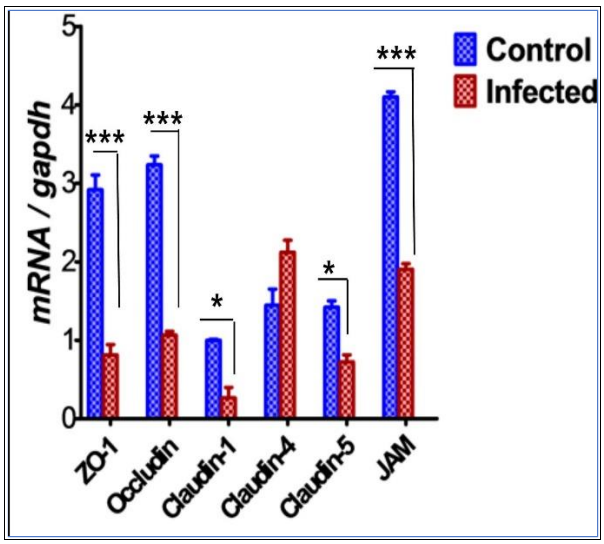

Figure 2. Disruption of gut epithelial TJPs in non-specific diarrhea.
The intestinal cellular integrities were disrupted due to non-specific diarrheic conditions. On day 7, the mRNA expression of TJPs including ZO-1, occludin, claudin-1, claudin-4, claudin-5, and JAM were determined by real-time qRT-PCR. Data represent the average ± SEM of the levels derived from the experimental mice (n=5-6). **p*<0.05, ***p*<0.01 and ****p*<0.001 compared between the indicated groups.

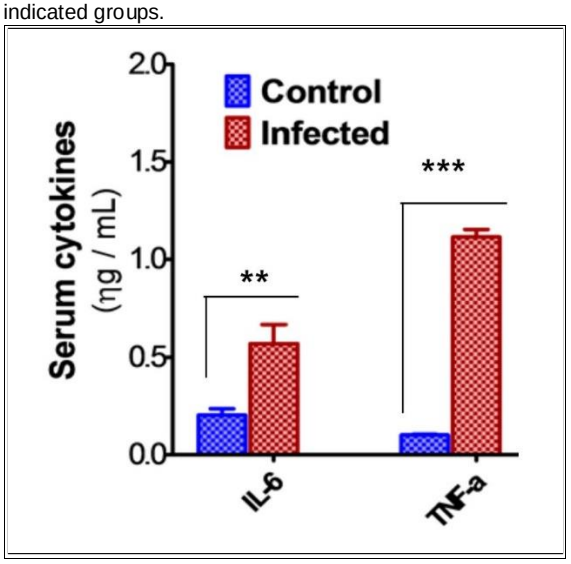

Figure 3. Induction of serum pro-inflammatory cytokines in non-specific diarrheic condition.
On day 7, the serum pro-inflammatory cytokines (IL-6 and TNF-α) of control and infected mice were determined

through Sandwich ELISA (Figure 3). Data represent the average ± SEM of the levels derived from the experimental mice (n=5-6). *p<0.05, **p<0.01 and ***p<0.001 compared between the indicated groups.

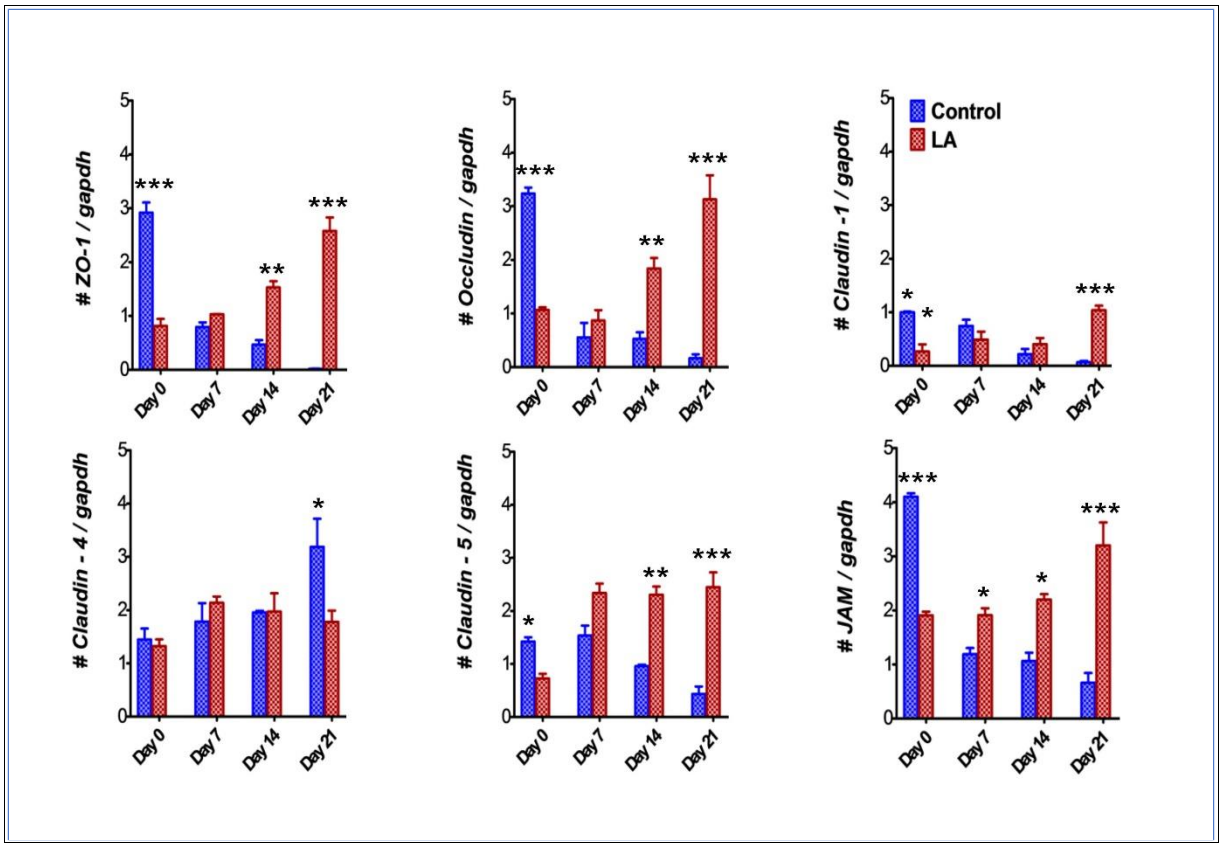

Figure 4. Restoration of gut epithelial TJPs following *L. acidophilus* supplementation.
Following *Lactobacillus acidophilus* supplementation, the mRNA expression of TJPs including ZO-1 (Figure 4a), occluding (Figure 4b), claudin-1 (Figure 4c), claudin-4 (Figure 4d), claudin-5 (Figure 4e), and JAM (Figure 4f) were determined by real-time qRT-PCR on day 7, 14 and 21. Data represent the average ± SEM of the levels derived from the experimental mice (n=3-5). *p<0.05, **p<0.01 and ***p<0.001 compared between the indicated groups.

Table 3. Specific primers for the expression of different TJPs and adhesion molecules in real-time qRT-PCR.

	Primer Sequence (5'-3')	Position cDNA	Gene bank ID
Occludin	F: GCT GTG ATG TGT GTG AGC TG	2054–2074	NM_008756
	R: GAC GGT CTA CCT GGA GGA AC	2105–2125	
ZO-1	F: AGG ACA CCA AAG CAT GTG AG	6227-6246	NM_009386.2
	R: GGC ATT CCT GCT GGT TAC A	6295-6313	
Claudin-1	F: TCT ACG AGG GAC TGT GGA TG	350–369	NM_016674.4
	R: TCA GAT TCA GCA AGG AGT CG	414–433	
Claudin-4	F: GGG AAT CTC CTT GGC AGT CC	216-235	NM_009903.2
	R: CGA TGT TGC TGC CGA TGA AG	291-310	
Claudin-5	F: GTG GAA CGC TCA GAT TTC AT	1054-1073	NM_013805.4
	R: TGG ACA TTA AGG CAG CAT CT	1131-1150	
JAM	F: ACC CTC CCT CCT TTC CTT AC	1136–1182	NM_172647.2
	R: CTA GGA CTC TTG CCC AAT CC	1238–1257	
gapdh	F: AAC GAC CCC TTC ATT GAC	186-203	NM_001289726.1
	R: TCC ACG ACA TAC TCA GCA C	358-376	

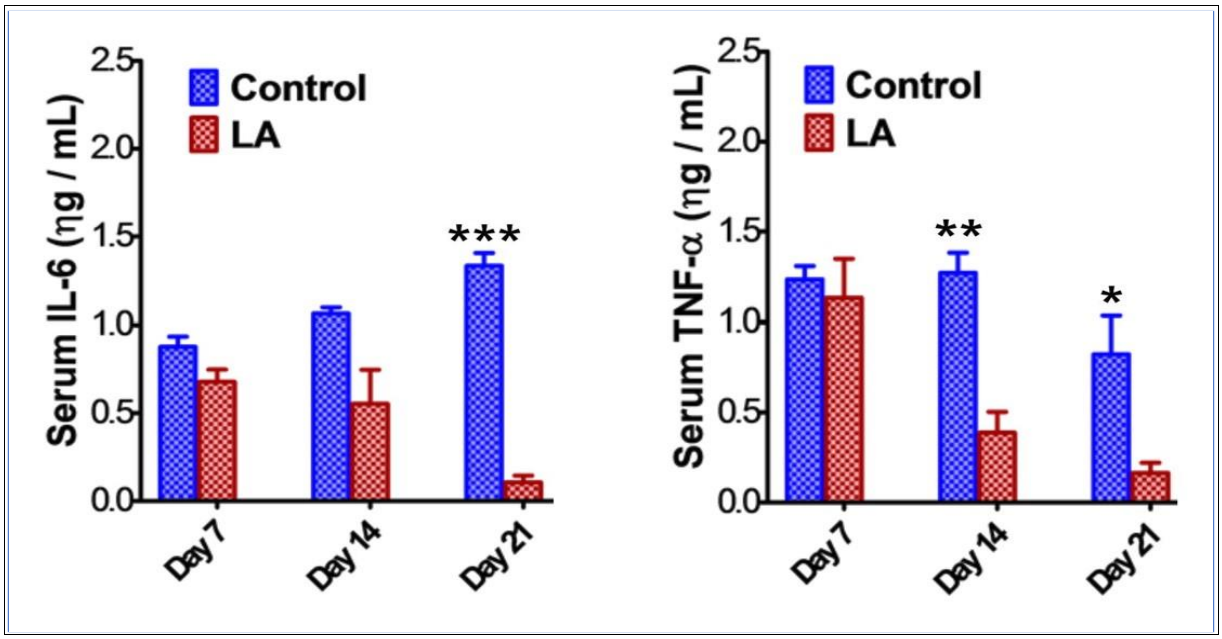

Figure 5. Supplementation of LA results in reduced serum IL-6 and TNF-α production.
Following *Lactobacillus acidophilus* supplementation for three consecutive weeks, the serum IL-6 and TNF-α were determined on days 7, 14, and 21; using Sandwich ELISA (Figure5). Data represent the average ± SEM of the levels derived from the experimental mice (n=3-5). **p*<0.05, ***p*<0.01 and ****p*<0.001 compared between the indicated groups.

Probiotics proved to ferment non-digestible food, inducing ATP to cellular functions, exerting anti-pathogenicity and anti-inflammatory activities [5, 17]. Probiotics bacteria, mainly L. acidophilus, have been extensively studied and described as promoting innate and adaptive immune responses, suppressing the mononuclear cellular trafficking, and maintaining gut mucosal homeostasis [6-8]. The intestinal epithelium epitomizes a physical barrier by balancing the TJPs and adhesion molecules responsible for gut permeability to nutrients and beneficial molecules, thus protecting the host immune equilibrium [6,8,11]. Numerous studies reconnoitered the influence of different beneficial lactic acid bacteria (LAB) on TJPs expression in pathological circumstances. L. acidophilus helps gut immunity and barrier function [18, 19].

It has been shown to progress the resistance to pathogenic microbes and reduces the burden of enteric bacteria causing traveler's diarrhea in humans [5, 20]. Thus, L. acidophilus also exerts protective roles on enteric pathogens by modulation of TJPs and subsequent barrier function improvement. Multiple probiotics and prebiotics have been reported to modulate the expression of proteins constituting the TJs, and therefore we also noted the beneficial effects of supplementing LA in this study. The gut mucus layer, epithelium, and underlying lamina propria construct the barrier function of the intestine [3,7]. Gut epithelium plays a central role in immune protective activities through the cytoskeletal firmness of TJPs and adhesion molecules. Specific probiotics regulate the epithelial barrier functions and TJPs, and the loss of integrities is associated with enteric microbial infections [7,15,16]. Our findings indicate that the loss of ZO-1, occludin, claudin-1, claudin-4, claudin-5, and adhesion molecules (JAM) can be renovated with the Lactobacillus acidophilus supplementation. Similarly, Lactobacillus acidophilus was found to restore the phosphorylation of ZO-1 and occluding in enteroinvasive Escherichia coli infection [16, 21]. In another study on enteropathogenic Escherichia coli infected T84 cells, the LAB was found to inhibit the paracellular permeability by refurbishing TJPs [8,22]. Different types of in-vitro studies also echoed that Lactobacillus acidophilus prevents the destruction and loss of ZO-1 on cell-cell contacts while incubating monolayers with aspirin treatment. Another mouse model of colitis demonstrated a loss of occludin, ZO-1, claudin-1, claudin-5, and LAB administration entirely preserved gut barrier function by

preventing DSS-induced disruption of TJPs subsequent apoptosis [15,16,19].

Pro- and anti-inflammatory cytokines are crucial modulators of gut immunity in microbial attacks and inflammation. In a normal and balanced state, the gut mucosal immunity is highly maintained under regulating and balancing pro and anti-inflammatory cytokines streaming [23]. The multi-functional cytokine IL-6 regulates gut immune response and hematopoiesis and may play a pivotal role in host defense mechanisms. In intestinal microbial attacks, TNF-α starts to execute an immune response to microbes and activates neutrophils by altering the integrity of gut endothelium [23,24]. The enteric inflammatory process and diarrheic conditions are interlinked with the streaming of pro and anti-inflammatory cytokines production. DCs, macrophages, and T cells produce the anti-inflammatory cytokine (IL-10) and B cells and then impede the roles of pro-inflammatory cytokines (IL-6, TNF-α), different chemokines, and chemokine receptors responsible for gut inflammation [15, 19, 24-26]. Pro-inflammatory cytokine IL-6 helps the clonal expansion of IgA from B cells, stimulates IgG Iand gM production, and prevents IgE secretion [27]. An elevated level of pro-inflammatory cytokines like TNF-α and IL-6 was observed in the inflamed gut animals with dextran sulfate sodium (DSS) induced colitis [28, 29]. Our findings echo the enhancement of serum IL-6 and TNF-α production in this study due to diarrheic conditions in mice. As assessed by Sandwich ELISA, we observed IL-6 and TNF-α elevation in the serum of diarrheic mice, suggesting the destruction of gut barrier function, disruption of TJPs, and reduction of the mucosal immune response previously assessed described in acute DSS-colitis models [27-29].

We also found a decreasing pattern of serum IL-6 and TNF-α is associated with LA supplementation. Probiotics have been reported to interact with early dendritic cells (DCs) migration, activation of Th1, Th2, and regulatory T cells (Tregs) in the gut mucosa, and successive modulation of adaptive immune responses. Besides, the regulatory roles of probiotic bacteria on pro-inflammatory cytokines (IL-6 and TNF-α) corroborated elsewhere in the case of gut mucosal immunity [23-25]. In the human subject, oral supplementation of LAB increased anti-inflammatory cytokines (IL-10) and reduced secretion of pro-inflammatory cytokines IFN-γ, TNF-α, and IL-6 [15,25,26].

Hence, our findings verified that oral administration of L. acidophilus augments gut barrier functions, readjusts the disrupted TJPs and adhesion molecules, and enhances the specific gut mucosal and systemic immune responses in mice. Thus, L. acidophilus supplementation could be considered an effective probiotic strain to prevent gut mucosal disorders related to non-specific diarrhea. To justify our observations, further studies should be performed to scrutinize diarrhea-associated pathogens and their virulence factors. Besides our promising findings, we had some limitations in our study. We could not address the mechanistic pathways and cellular trafficking underneath the restoration of gut equilibrium following L. acidophilus in non-specific diarrhea. Moreover, we need further steps to reveal whether our results could echo the similar mechanism in microbes-induced specific diarrhea.

CONCLUSION

The non-specific diarrhea is highly responsive to the induction of serum pro-inflammatory cytokines (IL-6 and TNF-α). The alteration of cytoskeletal TJPs and adhesion molecules leads to the loss of gut epithelial barrier functions. And the supplementation of L. acidophilus helps restore the epithelial tight junction integrities, reduces serum IL-6 and TNF-α, and enhances gut mucosal immunity.

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