Effect of Probiotics *Lactobacillus* and *Bifidobacterium* on Gut-Derived Lipopolysaccharides and Inflammatory Cytokines: An *In Vitro* Study Using a Human Colonic Microbiota Model

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Gut-derived lipopolysaccharides (LPS) are critical to the development and progression of chronic low-grade inflammation and metabolic diseases. In this study, the effects of probiotics *Lactobacillus* and *Bifidobacterium* on gut-derived lipopolysaccharide and inflammatory cytokine concentrations were evaluated using a human colonic microbiota model. *Lactobacillus reuteri, L. rhamnosus, L. plantarum, Bifidobacterium animalis, B. bifidum, B. longum,* and *B. longum* subsp. *infantis* were identified from the literature for their anti-inflammatory potential. Each bacterial culture was administered daily to a human colonic microbiota model during 14 days. Colonic lipopolysaccharides, and Gram-positive and negative bacteria were quantified. RAW 264.7 macrophage cells were stimulated with supernatant from the human colonic microbiota model. Concentrations of TNF-α, IL-1β, and IL-4 cytokines were measured. Lipopolysaccharide concentrations were significantly reduced with the administration of *B. bifidum* (-46.45 ± 5.65%), *L. rhamnosus* (-30.40 ± 5.08%), *B. longum* (-42.50 ± 1.28%), and *B. longum* subsp. *infantis* (-68.85 ± 5.32%) (*p* < 0.05). Cell counts of Gram-negative and positive bacteria were distinctly affected by the probiotic administered. There was a probiotic strain-specific effect on immunomodulatory responses of RAW 264.7 macrophage cells. *B. longum* subsp. *infantis* demonstrated higher capacities to reduce TNF-α concentrations (-69.41 ± 2.78%; *p* < 0.05) and to increase IL-4 concentrations (+16.50 ± 0.59%; *p* < 0.05). Colonic lipopolysaccharides were significantly correlated with TNF-α and IL-1β concentrations (*p* < 0.05).  

These findings suggest that specific probiotic bacteria, such as *B. longum* subsp. *infantis*, might decrease colonic lipopolysaccharide concentrations, which might reduce the proinflammatory tone. This study has noteworthy applications in the field of biotherapeutics for the prevention and/or treatment of inflammatory and metabolic diseases.  

**Key words:** Lactobacilli, bifidobacteria, probiotics, cytokine, lipopolysaccharide, inflammation

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Gut-derived lipopolysaccharides (LPS) are critical to the development and/or progression of a variety of human conditions, such as chronic low-grade inflammation [5], metabolic diseases [5, 6, 8], and liver diseases [1, 34]. LPS are potent immunomodulatory components derived from the cell wall of Gram-negative bacteria [28], which can translocate to the blood circulation [28]. The immune recognition of LPS is mediated through the cluster of differentiation-14 / toll-like receptor-4 receptor complex in epithelial cells of the intestine and immune cell types, predominantly macrophages, B cells, and dendritic cells [12, 28, 43, 45]. An intracellular signaling cascade is subsequently promoted, leading to the secretion of proinflammatory cytokines, such as interleukin (IL)-4, IL-1β, and tumor necrosis factor (TNF)-α [5, 33]. IL-4, IL-1β, and TNF-α have all been linked with the initiation and/or maintenance of metabolic diseases [9].  

In addition, substantial evidence has demonstrated that gut dysbiosis [25, 34, 46] might be responsible for increased endotoxemia [37], which further induces chronic low-grade inflammation [5, 33] and participates in the pathophysiology...
of metabolic [5, 6, 8] and liver [1, 34] diseases. The manipulation of the gut microbiota composition to reduce endotoxemia and prevent and/or treat human diseases has already been performed [6–8, 17, 18, 40, 50]. Studies using antibiotics to selectively eliminate Gram-negative bacteria have reported significant decreases in cecal and plasma LPS concentrations [6, 17, 18]. Strategies based on the use of prebiotics have demonstrated normalized low-grade inflammation and improved glucose tolerance. Those effects were associated with increased gut bifidobacterial content [7, 8]. Interestingly, recent studies have suggested that probiotic supplements might reduce endotoxemia and chronic low-grade inflammation [40, 50]. However, to our knowledge, there have been no previous studies examining the effect of probiotics *Lactobacillus* and *Bifidobacterium* on gut-derived LPS and inflammatory cytokines.

The most widely used probiotic bacteria belong to *Bifidobacterium* and *Lactobacillus* spp. Those species have been largely investigated for their health promoting properties in human diseases where gut dysbiosis is inferred to play a pathogenic role. They are assumed to re-equilibrate the gut microbiota composition towards health promoting bacterial populations [40, 50]. Among many hypothesized mechanisms of action, Bifidobacteria and Lactobacilli have been extensively studied for their immunomodulatory activities [13–15, 19, 22, 24, 27, 29, 30, 32, 35, 36, 39, 41, 47–49]. Bacterial cells and components interact with a wide variety of cells present in the intestines, such as epithelial, dendritic, and macrophage cells, which further induce the secretion of pro- and anti-inflammatory cytokines [4, 10, 11, 24, 27, 29, 39, 49]. Frequently, *in vitro* studies are performed using bacterial cultures exposed to intestinal cell lines, such as intestinal epithelial cells-6 [4], Caco-2 cells [4], and RAW 264.7 macrophage cells [10, 11]. Those studies are useful to investigate the immunomodulatory properties of bacterial preparations from a pure bacterial culture. However, they neglect the effects induced in the existing gut microbiota.

In this study, we investigated the effect of probiotics *Lactobacillus* and *Bifidobacterium* on colonic LPS and inflammatory cytokine concentrations, using an established model of human colonic microbiota [38] and RAW 264.7 macrophage cell lines.

**Materials and Methods**

**Bacterial Cultures and Growth Conditions**

Probiotic bacteria tested were identified from the literature for their anti-inflammatory potential (Table 1). Bacterial strains included *L. reuteri* NCIMB 701359, *L. rhamnosus* ATCC 53103, *L. plantarum* ATCC 14917, *B. animalis* ATCC 25527, *B. bifidum* ATCC 29521, *B. longum* ATCC 15707, and *B. longum* subsp. *infantis* ATCC 702205.

**Table 1.** List of probiotic strains of *Lactobacillus* and *Bifidobacterium* tested in this study.

<table>
<thead>
<tr>
<th>Bacterial species</th>
<th>Strains screened in this study</th>
<th>Literature on anti-inflammatory potential</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Lactobacillus reuteri</em></td>
<td>NCIMB 701359</td>
<td>[22, 29, 30, 49]</td>
</tr>
<tr>
<td><em>Bifidobacterium bifidum</em></td>
<td>NCIMB 702715</td>
<td>[13, 24]</td>
</tr>
<tr>
<td><em>Lactobacillus rhamnosus</em></td>
<td>ATCC 53103</td>
<td>[15, 19, 36]</td>
</tr>
<tr>
<td><em>Bifidobacterium animalis</em></td>
<td>NCIMB 702242</td>
<td>[27, 39, 47]</td>
</tr>
<tr>
<td><em>Lactobacillus plantarum</em></td>
<td>NCIMB 11974</td>
<td>[32, 41, 48, 49]</td>
</tr>
<tr>
<td><em>Bifidobacterium longum</em></td>
<td>NCIMB 702259</td>
<td>[13, 49]</td>
</tr>
<tr>
<td><em>Bifidobacterium longum</em> subsp. <em>infantis</em></td>
<td>NCIMB 702205</td>
<td>[14, 35]</td>
</tr>
</tbody>
</table>

Fig. 1. Schematic representation of the experimental design: Administration of probiotics *Lactobacillus* and *Bifidobacterium* in a human colonic microbiota model and quantification of colonic lipopolysaccharides, and Gram-negative/positive bacterial cell counts and inflammatory cytokines. A human colonic microbiota model was set up. After inoculation with fresh human feces, a model stabilization period of 14 days was performed. From day 0, *Lactobacillus* or *Bifidobacterium* probiotic strains were administered daily to the human colonic microbiota model. A control fermentation system was performed without administration of probiotic bacteria. Statistical analysis was analyzed to determine the effect of time vs. day 0. On days 0, 7, and 14, samples were extracted from the colonic model for quantification of colonic LPS and Gram-negative/positive bacterial concentrations. RAW 264.7 macrophage cells were stimulated with bacterial supernatant extracted from the colonic model. Positive and negative controls consisted of RAW 264.7 cells exposed to, respectively, 2 μg/ml LPS, and DMEM supplemented with 10% FBS. Concentrations of TNF-α, IL-1β, and IL-4 cytokines were measured.
The Human Colonic Microbiota Model
A semi-continuous human colonic microbiota fermentation system was operated, as described previously [38]. The system was operated with a retention time of 144 h. Following the fermentation system start-up, a stabilization period of 14 days was performed to allow the bacterial communities to stabilize in order to be representative of the gut microbiota. On day 0, administration of pure cultures of Bifidobacterium or Lactobacillus strains was initiated, where 5 × 10⁷ CFU was administered daily to the human colonic microbiota model during 14 days. A control fermentation system was performed without daily administration of probiotics. A schematic representation of the experimental procedure is given in Fig. 1.

Quantification of Gram-Positive and Gram-Negative Bacteria
Gram-negative and positive bacteria were enumerated by plate counting. On days 0, 7, and 14, samples were extracted from the human proximal colonic microbiota model and serially diluted in physiological solution (8.5 g/l NaCl). Bacterial cell viability was determined on selective agar triplicate plates at 3 different dilutions. McConkey agar (Becton Dickinson, Mississauga, Canada) was used for the quantification of Gram-negative bacteria. Phenyl ethyl alcohol agar (Becton Dickinson) was used for the quantification of Gram-positive bacteria. The agar media were supplemented with 0.05% (w/v) cysteine (Fisher Scientific) and incubated in anaerobic jars with anaerobe atmosphere-generating bags (Oxoid, Nepean, Canada) for 48 h at 37°C.

Quantification of Inflammatory Cytokines Secreted
On days 0, 7, and 14, samples were removed from the human colonic microbiota model and centrifuged for 20 min at 2,000 × g and 4°C. The supernatant, containing the free LPS, was collected. RAW 264.7 cells adjusted to a density of 5 × 10⁶ cells/ml were exposed for 24 h to the bacterial supernatant. The positive control consisted of RAW 264.7 macrophage cells stimulated with 2 μg/ml LPS from Escherichia coli (Sigma-Aldrich, Oakville, Canada). The negative control consisted of RAW 264.7 macrophage cells exposed to DMEM supplemented with 10% FBS. After 24 h of stimulation, the culture supernatant was collected and stored at −20°C until cytokines analysis. The induction of TNF-α, IL-1β, and IL-4 cytokines was assayed using commercial mouse ELISA kits (eBioscience, San Diego, USA). Kit instructions were followed to pursue the solid-phase enzyme-linked immunosassay procedure.

Statistical Analysis
Values are reported as the mean ± SD of triplicates. All data were analyzed using Prism software (Prism, Version 5.0). The D’Agostino and Pearson normality test was performed to assess the Gaussian distribution of the data. Bartlett’s test was performed to assess the homogeneity of variances. Statistical analysis was analyzed using one-way ANOVA followed by Bonferroni post-hoc tests or Kruskal-Wallis test followed by Dunn’s post hoc tests. Correlations were performed using Spearman’s rank correlation. Values of p < 0.05 were considered significant.

RESULTS
Effects of Probiotics Lactobacillus and Bifidobacterium on Colonic Lipopolysaccharides Concentrations
Colonic LPS concentrations in the human colonic microbiota model were determined before and after 7 and 14 days of daily administration of probiotics Lactobacillus and Bifidobacterium (Table 2). L. reuteri, B. bifidum, L. rhamnosus, B. longum, and B. longum subsp. infantis reduced LPS concentrations in a time-dependent manner. Reductions in LPS concentrations on day 14 averaged 23.81 ± 3.18% with L. reuteri (1.80 × 10⁴ ± 2.92 × 10⁴ EU/ml on day 0 vs. 1.37 × 10⁴ ± 2.55 × 10⁴ EU/ml on day 14), 46.45 ± 5.65% with B. bifidum (1.80 × 10⁴ ± 1.42 × 10⁴ EU/ml on day 0 vs. 9.63 × 10³ ± 1.06 × 10⁴ EU/ml on day 14), 30.40 ± 5.08% with L. rhamnosus (1.92 × 10³ ± 1.59 × 10⁴ EU/ml on day 0 vs. 1.33 × 10³ ± 3.50 × 10⁴ EU/ml on day 14), 42.50 ± 1.28% with B. longum (1.86 × 10³ ± 1.87 × 10⁴ EU/ml on day 0 vs. 1.07 × 10³ ± 1.29 × 10⁴ EU/ml on day 14), and 68.85 ± 5.32% with B. longum subsp. infantis (2.09 × 10³ ± 2.03 × 10⁴ EU/ml on day 0 vs. 6.50 × 10² ± 5.30 × 10⁴ EU/ml on day 14). The effect was significant with B. bifidum, L. rhamnosus, B. longum, and B. longum subsp. infantis (p < 0.05). Conversely, L. plantarum administration significantly increased LPS concentrations by 28.84 ± 4.44% on day 7 (1.79 × 10⁴ ± 8.46 × 10⁴ EU/ml on day 0 vs. 2.31 × 10⁵ ± 3.18 × 10⁵ EU/ml on day 7) (p < 0.05). The effect was not maintained after 14 days of daily L. plantarum administration.
**Table 2. Effects of probiotics *Lactobacillus* and *Bifidobacterium* on colonic lipopolysaccharides concentrations in a human colonic microbiota model.**

<table>
<thead>
<tr>
<th>Bacteria administered to the colonic model</th>
<th>Lipopolysaccharides concentration (EU/ml)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Day 0</td>
</tr>
<tr>
<td>Control</td>
<td>$2.02 \times 10^4 \pm 2.53 \times 10^3$</td>
</tr>
<tr>
<td><em>L. reuteri</em></td>
<td>$1.80 \times 10^4 \pm 2.92 \times 10^3$</td>
</tr>
<tr>
<td><em>B. bifidum</em></td>
<td>$1.80 \times 10^4 \pm 1.42 \times 10^3$</td>
</tr>
<tr>
<td><em>L. rhamnosus</em></td>
<td>$1.92 \times 10^4 \pm 1.59 \times 10^3$</td>
</tr>
<tr>
<td><em>B. animalis</em></td>
<td>$1.95 \times 10^3 \pm 7.82 \times 10^2$</td>
</tr>
<tr>
<td><em>L. plantarum</em></td>
<td>$1.79 \times 10^4 \pm 9.46 \times 10^2$</td>
</tr>
<tr>
<td><em>B. longum</em></td>
<td>$1.86 \times 10^4 \pm 1.87 \times 10^3$</td>
</tr>
<tr>
<td><em>B. longum</em> subsp. <em>infantis</em></td>
<td>$2.09 \times 10^4 \pm 2.03 \times 10^3$</td>
</tr>
</tbody>
</table>

*Lactobacillus* and *Bifidobacterium* probiotic strains were administered daily to a human colonic microbiota model. A control fermentation system was performed without probiotic administration. On days 0, 7, and 14, colonic LPS were quantified (EU/ml). Data represent the mean ± SD (n = 3). * Indicates statistically significant difference ($p < 0.05$) obtained by a Kruskal-Wallis test followed by Dunn’s post hoc tests.

**Effects of Probiotics *Lactobacillus* and *Bifidobacterium* on Colonic Gram-Negative and -Positive Bacterial Cell Counts**

Cell counts of Gram-negative bacteria (LPS-producing bacteria) (Fig. 2A) and Gram-positive bacteria (non-LPS producing bacteria) (Fig. 2B) in the human colonic microbiota model were determined before and after 7 and 14 days of daily administration of probiotics *Lactobacillus* and *Bifidobacterium*. There was a significant increase in the Gram-negative bacterial cell count on day 7 with *L. reuteri* (+21.78 ± 1.56%; 6.12 ± 0.07 log CFU/ml on day 0 vs. 7.46 ± 0.12 log CFU/ml on day 7; $p < 0.05$) and *L. plantarum* (+7.71 ± 1.06%; 6.18 ± 0.05 log CFU/ml on day 0 vs. 6.66 ± 0.09 log CFU/ml on day 7; $p < 0.05$). The effect was not significant on day 14. Conversely, there were significant reductions on day 14 in Gram-negative bacteria with *B. longum* (-7.28 ± 1.88%; 6.11 ± 0.06 log CFU/ml on day 0 vs. 5.67 ± 0.06 log CFU/ml on day 14; $p < 0.05$) and *B. longum* subsp. *infantis* (-9.55 ± 1.51%; 6.14 ± 0.06 log CFU/ml on day 0 vs. 5.55 ± 0.05 log CFU/ml on day 14; $p < 0.05$). *L. reuteri* administration was associated with a significant increase in total Gram-positive bacteria on day 7 (+18.37 ± 0.01%; 6.56 ± 0.07 log CFU/ml on day 0 vs. 7.36 ± 0.01 log CFU/ml on day 7; $p < 0.05$). *B. animalis* administration induced a significant reduction in Gram-positive bacteria on day 14 (-18.37 ± 0.01%; 6.56 ± 0.11 log CFU/ml on day 0 vs. 5.36 ± 0.07 log CFU/ml on day 14; $p < 0.05$). Colonic LPS concentrations were not correlated with Gram-negative and -positive bacterial cell counts.

**Effects of Probiotics *Lactobacillus* and *Bifidobacterium* on TNF-α, IL-1β, and IL-4 Cytokine Concentrations**

To investigate the immunomodulatory effects of probiotics *Lactobacillus* and *Bifidobacterium*, RAW 264.7 macrophage cells were stimulated with bacterial supernatant (containing most of the free LPS) sampled from the colonic model before and after 7 and 14 days of daily probiotics administration. The TNF-α (Fig. 3A), IL-1β (Fig. 3B), and IL-4 (Fig. 3C) concentrations secreted by positive control (2 μg/ml LPS) were significantly increased compared with the negative control (DMEM supplemented with 10% FBS) ($p < 0.05$).
There were major reductions in TNF-α concentration with *B. animalis* (−67.11 ± 0.56%; 1174.33 ± 8.87 µg/ml on day 0 vs. 386.20 ± 6.98 µg/ml on day 14; *p* < 0.05), *L. plantarum* (−68.01 ± 1.88%; 1133.09 ± 62.29 µg/ml on day 0 vs. 362.49 ± 2.51 µg/ml on day 14; *p* < 0.05), *B. longum* (−65.14 ± 1.45%; 1083.17 ± 30.48 µg/ml on day 0 vs. 377.62 ± 5.69 µg/ml on day 14; *p* < 0.05), and *B. longum* subsp. *infantis* (−69.41 ± 2.78%; 1142.80 ± 88.43 µg/ml on day 0 vs. 349.55 ± 5.32 µg/ml on day 14; *p* < 0.05). As for IL-1β concentrations, reductions were most extreme on day 14 with *L. reuteri* (−25.40 ± 2.55%; 637.13 ± 11.56 µg/ml on day 0 vs. 475.33 ± 17.58 µg/ml on day 14; *p* < 0.05), *B. bifidum* (−26.96 ± 2.52%; 650.17 ± 21.75 µg/ml on day 0 vs. 474.87 ± 10.29 µg/ml on day 14; *p* < 0.05), and *L. rhamnosus* (−21.48 ± 2.39%; 647.10 ± 5.52 µg/ml on day 0 vs. 508.11 ± 18.50 µg/ml on day 14; *p* < 0.05). There was a time-dependent increase in IL-4 concentration with the administration of *L. reuteri* (+30.73 ± 7.07%; 214.57 ± 1.65 µg/ml on day 0 vs. 284.55 ± 35.55 µg/ml on day 14; *p* < 0.05), *L. plantarum* (+46.12 ± 3.88%; 207.58 ± 8.90 µg/ml on day 0 vs. 303.32 ± 4.95 µg/ml on day 14; *p* < 0.05) and *B. longum* subsp. *infantis* (+16.50 ± 0.59%; 233.05 ± 2.11 µg/ml on day 0 vs. 271.50 ± 4.38 µg/ml on day 14; *p* < 0.05).

Correlations Between Colonic Lipopolysaccharides Concentrations and Secretion of Inflammatory Cytokines
There was a strong positive correlation between colonic LPS concentrations and TNF-α (*r* = 0.447, *P* = 0.002; Fig. 4A) and IL-1β (*r* = 0.504, *P* = 0.002; Fig. 4B) concentrations. Conversely, colonic LPS concentrations were negatively...
correlated with IL-4 concentrations, but the effect was not significant (r = -0.160, P = 0.170; Fig. 4C).

**DISCUSSION**

Compelling evidence demonstrates that gut microbiota dysbiosis may promote systemic chronic low-grade inflammation [5, 33], and initiate metabolic [5, 6, 8] and liver [1, 34] diseases through a mechanism that involves increased exposure to Gram-negative bacteria-derived LPS. Current therapies to improve the inflammatory status have been developed with the aim to reduce colonic LPS concentrations have been achieved through a mechanism that involves increased exposure to Gram-negative bacteria-derived LPS. Current therapies to improve the inflammatory status [6–8, 17, 18, 50]. However, to our knowledge, there have been no previous studies examining the effect of probiotics *Lactobacillus* and *Bifidobacterium* on gut-derived LPS and inflammatory cytokines. This was the objective of this study.

The effects of *L. reuteri* NCIMB 701359, *L. rhamnosus* ATCC 53103, *L. plantarum* ATCC 14917, *B. animalis* ATCC 25527, *B. bifidum* ATCC 29521, *B. longum* ATCC 15707, and *B. longum* subsp. *infantis* ATCC 15697 on colonic LPS, and TNF-α, IL-1β, and IL-4 inflammatory cytokine concentrations were determined using a human colonic microbiota model [38] and RAW 264.7 macrophage cells. Although the model of human colonic microbiota used in this study reflects differences in individual gut microbiotas and diseases, it allows the experimentation of a complex culture of the gut microbiota under well-standardized conditions. Results demonstrated that the administration of *B. bifidum*, *L. rhamnosus*, *B. longum*, and *B. longum* subsp. *infantis* to the human proximal colonic microbiota model significantly reduced LPS concentrations in a time-dependent manner. No data on intestinal or cecal LPS have been reported in probiotic studies, which impedes further comparison. Nevertheless, preclinical studies have already shown that *Bifidobacterium* and *Lactobacillus* bacteria can decrease plasma LPS concentrations [40, 50]. Other studies using antibiotics have reported significant decreases in cecal and plasma LPS concentrations [6, 17, 18]. Strategies based on the use of probiotics have shown reduced endotoxemia, improved glucose tolerance, and normalized low-grade inflammation [7, 8]. Since colonic bacterial LPS are derived from Gram-negative bacteria, colonic Gram-negative and -positive bacterial cell counts were monitored. Reductions in Gram-negative bacterial cell counts were significant following administration of *B. animalis*, *B. longum*, and *B. longum* subsp. *infantis*. Surprisingly, neither the Gram-negative nor -positive bacterial cell count was correlated with LPS concentrations. We hypothesize that uncultivable bacteria, which are not quantified using plate counting, might account for the discrepancy. Molecular techniques, such as real-time polymerase chain reaction should be used to quantify Gram-negative/positive bacterial cell counts in future studies [3].

Immunomodulatory properties of probiotics *Lactobacillus* and *Bifidobacterium* were assessed using RAW 264.7 macrophage cells. Macrophages are known to play an important role in the regulation of innate inflammatory responses to intestinal bacteria, particularly to the bacterial toxin LPS [16]. Macrophage recognition of bacterial conserved elements is mediated predominantly by toll-like receptors and nucleotide-binding oligomerization domain molecules [28, 43]. This further triggers the production of inflammatory cytokines and chemokines [5, 33, 43]. In addition, macrophages, along with dendritic cells, present foreign antigens to cells of the acquired immune system [12, 43, 45]. Thus, both the innate and acquired immune systems are integrated for efficient inflammatory responses to microorganisms. It is believed that the activation of innate immune signaling pathways by toll-like receptors may help induce and/or maintain chronic inflammation due to continuous exposure to LPS [23]. Previous *in vitro* studies have investigated the inflammatory responses of RAW 264.7 macrophage cells to pure bacterial cultures [10, 11]. Those studies were useful in determining the direct effects of a bacterial strain on macrophage activation. However, they disregarded the effects of a mixed culture induced in the gut microbiota ecosystem. Soluble components, predominantly LPS, released from bacterial cells into the gut environment are responsible for the majority of inflammatory cytokines secreted [4, 11, 14, 26]. Lamina propria-resident macrophages have been shown to suppress or have anergic responses to LPS stimuli [42, 44]. However, studies involving the inflamed mucosa have not observed this feature, probably due to the recruitment of blood monocytes-derived macrophages [20, 21]. In addition, it was recently suggested that the inflammatory profile elicited by murine RAW 264.7 macrophage cells depends primarily on the initial dosage of LPS challenge [31]. In the present study, murine macrophage cells were stimulated with bacterial supernatant, which contained the free LPS, derived from our human colonic microbiota model. This novel approach, which allows to investigate probiotic immunomodulatory activities in the existing gut microbiota, has already demonstrated accuracy and reproducibility [2]. Cells density and LPS concentration (positive control) were based on published publications [10, 11]. TNF-α, IL-1β, and IL-4 inflammatory cytokines were selected for quantification because they are promoted following the immune recognition of gut-derived LPS [5, 33]. In addition, they have been linked with inflammatory and metabolic diseases [9]. Results demonstrated that the exposure of macrophage cells to bacterial supernatant and LPS solution stimulated major secretions of TNF-α, IL-1β, and IL-4 cytokines compared with the negative control. In addition, probiotics *Lactobacillus* and *Bifidobacterium* induced
significant biological reductions in the TNF-\(\alpha\) concentration. The reductions were extreme with \textit{B. animalis}, \textit{L. plantarum}, \textit{B. longum}, and \textit{B. longum} subsp. \textit{infantis}. Reductions in the IL-1\(\beta\) concentration were extreme with \textit{L. reuteri}, \textit{L. rhamnosus}, and \textit{B. bifidum}. The IL-4 concentration was significantly increased with \textit{L. reuteri}, \textit{L. plantarum}, and \textit{B. longum} subsp. \textit{infantis}. Overall, \textit{B. longum} subsp. \textit{infantis} demonstrated a higher capacity to reduce colonic LPS concentrations, to decrease TNF-\(\alpha\) proinflammatory cytokines, and to increase IL-4 anti-inflammatory cytokine secretions. Interestingly, the colonic LPS concentrations were positively and significantly correlated with the TNF-\(\alpha\) and IL-1\(\beta\) concentrations. We suggest that specific probiotic strains of \textit{Lactobacillus} and \textit{Bifidobacterium} can decrease colonic LPS concentrations, which might further reduce the proinflammatory tone.

This is the first \textit{in vitro} study to investigate the effects of probiotics \textit{Lactobacillus} and \textit{Bifidobacterium} on colonic LPS and inflammatory cytokine concentrations using a human colonic microbiota model. The findings revealed that specific probiotic strains, such as \textit{B. longum} subsp. \textit{infantis}, can decrease colonic LPS concentrations, which might further reduce the secretion of inflammatory cytokines in macrophage cells. This study has noteworthy applications in the field of biotherapeutics for the prevention and/or treatment of inflammatory and metabolic diseases.

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