Lactobacillus plantarum Consumption Increases PepT1-Mediated Amino Acid Absorption by Enhancing Protein Kinase C Activity in Spontaneously Colitic Mice¹⁻³

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Abstract

Although probiotic consumption has generally been shown to have many beneficial effects for the prevention and treatment of inflammatory bowel disease, the effects of Lactobacillus plantarum (LP) on intestinal nutrient absorption, particularly oligopeptide transporter 1 (PepT1)-mediated absorption of dietary protein under inflammatory conditions, has not yet been characterized. In this study, we first investigated the effects of LP consumption on plasma amino acid concentrations and PepT1-mediated absorption of cephalaxin in the small intestine of wild-type (WT) mice and interleukin-10 knockout (IL-10⁻²) mice, a model of spontaneous colitis. We then analyzed expression and distribution of PepT1 and protein kinase C (PKC) activity in the jejunum of these mice. LP consumption (10⁹ colony-forming units/0.5 mL) delivered by gavage once per day for 4 wk increased the total plasma amino acid concentration and the concentration of plasma cephalaxin through enhancement of PepT1-mediated uptake in LP treated IL-10⁻² mice compared with IL-10⁻² mice. Interestingly, IL-10⁻² mice had significantly lower PKC activity and expression of phosphorylated PKC than WT mice. J. Nutr. 140: 2201–2206, 2010.

Introduction

The small intestine is the major site of dietary protein absorption. Under physiological conditions, dietary proteins are degraded into a mixture of free amino acids and small peptides, primarily dipeptides and tripeptides (1), which are efficiently absorbed by the H⁺-coupled oligopeptide cotransporter 1 (PepT1).² PepT1 is primarily found in the brush border membranes of the jejunum and ileum (2). PepT1 has a broad substrate specificity and can transport a number of orally administered peptidomimetic drugs, including β-lactam antibiotics, angiotensin-converting enzyme inhibitors, renin inhibitors, and antiviral prodrugs (3, 4), in addition to proteolytic di- and tripeptides. Thus, PepT1 functions both as a nutrient transporter and a drug transporter. Many studies focused on the regulation of PepT1-mediated transport have demonstrated that the transport activity of PepT1 is affected by phosphorylation of protein kinase C (PKC) (5, 6) in addition to its own substrates, pharmacological agents, proinflammatory cytokines, and hormones, such as insulin and leptin (7). Despite the large number of identified regulatory mechanisms, other endogenous or exogenous regulators undoubtedly also exist. The bacterial flora present in the intestine may represent 1 such regulator (8). However, currently, little is known regarding the effects of orally administered probiotic bacteria on PepT1 expression and activity.

Probiotics are live microorganisms that provide beneficial health effects for the host when consumed in sufficient amounts (9, 10). Probiotics largely have been investigated for prevention and treatment of disease. Encouraging results for probiotic therapy, including treatment with lactic acid bacteria, have been
reported for inflammatory bowel disease, antibiotic-associated diarrhea, pouchitis, and diabetes (11,12). *Lactobacillus plantarum* (LP), a type of lactic acid bacteria, has been reported to be effective in the treatment and prevention of experimental colitis (13,14) and probiotic administration has been shown to reverse imbalances in gut flora in colitis. Furthermore, abnormal expression of PepT1 was also observed in the colons of colitis patients. However, the effect of LP administration on the transport function of PepT1 remains unclear. Our previous studies have shown that LP treatment decreases the expression and transport activity of PepT1 in the colons of interleukin-10 knockout (IL-10-/-) mice, a model of spontaneous colitis. Other studies have shown that probiotics administered over a 4-wk period prevent weight loss in IL-10-/- mice (15,16). Together, these findings indicate that probiotics may play a role in the regulation of intestinal nutrient absorption, particularly PepT1-mediated absorption of amino acids (17).

Based on these studies, we hypothesized that probiotics might control absorption of amino acids by modulating the activity of PepT1. The aim of this study was to test this hypothesis in IL-10-/- mice. First, we assessed whether administration of LP could increase plasma amino acid concentrations and enhance absorption of cephalexin, a PepT1-specific substrate (18), in the small intestine of IL-10-/- mice. To explore the mechanism underlying probiotic effects on PepT1 activity, we investigated the effects of LP consumption on the expression and distribution of PepT1 and the activity of PKC.

**Materials and Methods**

**Materials.** LP CGMCC No. 1258 was kindly donated by Dr. Xiao-Min Hang, Institute of Bio-medicine, Shanghai Jiao Tong University, Shanghai, China. Cells from a lyophilized LP stock were used to inoculate deMan-Rogosa-Sharpe medium (Oxoid) and were cultured at 37°C overnight under anaerobic conditions generated using anaerobic bags (Anaerocult A, Merck) in anaerobic jars (BBL Becton Dickson). Following the overnight incubation, the cells were washed once in sterile PBS (pH 7.5). The cell concentration was determined by photometric comparison with an established growth curve. Rabbit polyclonal antibodies directed against PepT1 (H-235) were purchased from Santa Cruz Biotechnology. Rabbit polyclonal phospho-panPKC antibodies directed against PepT1 and the activity of PKC.

**Animals.** Female homozygous IL-10 knockout (IL-10-/-) and wild-type (WT) (129/SvEv mice) (Jackson Laboratory) were used for this study. The mice were housed under specific pathogen-free conditions at a constant temperature under a 12-h-light/-dark cycle at Shanghai Jiao Tong University Medical School. Mice were given free access to water and unpurified mouse food (Harlan TekladLaboratory diet no. 8604) (19). Treatment was initiated in the mice at 4 wk of age and continued at 8 wk of age.

**Experimental design.** Mice exhibiting no overall initial differences in body weight were equally randomized into 4 groups of 8; WT (untreated WT mice), WT + LP (WT mice treated with LP), IL-10-/- (untreated IL-10-/- mice), and IL-10-/- + LP (IL-10-/- mice treated with LP). Mice in the probiotic (LP) groups received an oral dose of LP (10^9 colony-forming units/0.5 mL) by gavage once a day in the morning for 4 wk, whereas the other 2 groups consumed PBS alone. Body weights were measured at the end of the treatment period. After uptake studies were completed, blood was collected for analysis of plasma amino acid concentrations and cephalaxin concentrations. Jejunal tissues were harvested for Western-blot analysis, quantitative real-time PCR, immunofluorescence analysis, and PKC activity assays as described below. The experimental design was approved by the Animal Care and Use Committee and the Ethics Committee of Shanghai Jiao Tong University, Shanghai, China.

**Histopathology.** Colon tissues were fixed in 10% neutral phosphate-buffered formalin, routinely processed, sectioned at 6 μm, and stained with hematoxylin and eosin. The histological severity of colitis was graded on a scale of 0–4, as previously described (20).

**PepT1 uptake activity assays.** The transport capacity of PepT1 in the small intestine was evaluated using the in vitro single-pass jejunal perfusion technique, as previously described (21,22). Briefly, an inflow cannula made of silastic tube (1.65-mm o.d., 0.76-mm i.d., 10-cm length) was inserted into the jejunum ~1 cm below the ligament of Treitz and an outflow cannula was set up at a distance of 10 cm. Krebs solution was delivered to the jejunum with a perfusor compact at a flow rate of 2 mL/h (B. Braun Melsungen AG). For peptide transport experiments, 1 mmol/L cephalexin in Krebs buffer was delivered for 2 h after a 20-min stabilization period. Blood samples were collected through the caval vein and plasma was stored at −70°C prior to analysis of plasma amino acid and cephalaxin concentrations.

**Plasma cephalaxin concentrations.** Plasma cephalaxin concentrations were determined by HPLC (Waters) according to the method previously described (22).

**Plasma amino acid concentrations.** Plasma was deproteinized with 2.5% sulfosalicylic acid, incubated for 10 min at 4°C, and clarified by centrifugation at 12,000 × g for 10 min. The supernatant was subjected to amino acid analysis by HPLC using the standard ACCQ-Tag method (Waters). Chromatographic separation was performed on a Supelcosil LC18 column (4.6 mm × 250 mm, 5-μm particle size; Supelco). The UV detector was set at 248 nm to monitor the amino acid peak that came off the column after ~5 min. Mobile phase A consisted of ACCQ-Tag Eluent and mobile phase B consisted of a mixture of ultrapure water and acetonitrile (40:60, v/v). The flow rate was 1.0 mL/min.

**Quantitative RT-PCR.** Total RNA was extracted from jejunum. RT of the RNA was conducted with 200 U of M-MLV RT RNase H-Deletion Mutant (Promega) at 42°C for 60 min using the LightCycler Real-time PCR system (Roche Molecular Biochemicals). The primer sequences and expected sizes of PCR products were as follows: PepT1, 5'-GAG AAA GGG GAG AAC GGA A-3' (sense) and 5'-CGG TGC CAA AGT CAA GGT-3' (antisense), 214 bp; and b-actin, 5'-AGC GCC AGG AGG TCA TCA CTA TTG-3' (sense) and 5'-ATG CCA GAT GAT TCA ATC CAG-3' (antisense), 91 bp. For PCR, after an initial incubation for 30 s at 95°C, 40 cycles consisting of 5 s at 95°C, 5 s at 60°C, 30 s at 72°C for extension were conducted. Relative differences in mRNA expression were determined using the 2^(-ΔΔCT) method (23). Briefly, the cycle number at which the transcript being analyzed became detectable (CT) was normalized to the cycle number at which the β-actin gene transcript was detected, referred to as ΔCT.

**Western-blot analysis.** Jejunal tissues were homogenized, sonicated, and transferred to ice-cold lysis buffer (50 mmol/L Tris-HCl, pH 7.4; 150 mmol/L NaCl; 1% NP-40/1%gepal CA-630; 0.5% deoxycholate; 0.1% SDS; 5 mmol/L EDTA) containing 1 μL protease inhibitor cocktail in 1 mL buffer for 60 min. Extracted proteins were separated on SDS-PAGE and immunoblotted with the indicated primary antibodies. The blots were then incubated with IRDye 800-conjugated goat anti-rabbit IgG (LI-COR Biosciences). Fluorescence images of bound antibodies were captured using the Odyssey Infrared Imaging System (LI-COR). β-Tubulin was used as an internal loading control.

**Immunofluorescence and confocal microscopy.** Jejunal segments were collected, mounted, and processed according to routine procedures (24). Fixed sections were incubated in prewarmed (95°C) 10 mmol/L sodium citrate buffer for 5 min and blocked in PBS containing 5% bovine-serum albumin.
serum albumin and 5% fetal bovine serum for 30 min. The sections were then incubated with rabbit anti-PepT1 antibody at 4°C overnight followed by incubation with phalloidin-fluorescein isothiocyanate at room temperature for 1 h. Samples were next incubated with Cy3-conjugated secondary IgG antibodies (Jackson ImmunoResearch Laboratories). Nuclei were counterstained with 4–6-diamidino-2-phenylindole. Stained sections were mounted using Vectashield mounting medium (Vector Laboratories). Samples were imaged using an LSM510 laser scanning confocal microscope (Zeiss).

**PKC activity assays.** Jejunal samples were homogenized and lysed in ice-cold lysis buffer (20 mmol/L Tris-HCl, pH 7.5; 0.5 mmol/L EDTA; 2 mmol/L EGTA; 2 mmol/L phenylmethylsulfonyl fluoride; 10 mg/L leupeptin). The PKC activity assay was carried out using the PepTag activity assay kit (Promega) according to the manufacturer’s instructions, as previously described (25). The fluorescence intensity of phosphorylated peptides was analyzed as a reflection of PKC activity (26).

**Statistical analysis.** All statistical analyses were performed using SPSS 16.0. All results are presented as the mean ± SD. Data were analyzed using 2-way ANOVA followed by the Student-Newman-Keuls post hoc test for those interactions deemed significant. Differences were considered significant at P < 0.05.

### Results

**Plasma amino acids.** Of the essential amino acids, concentrations of Ile, Lys, Met, Thr, and Val were lower in IL-10−/− mice than in WT mice (Table 1). However, LP treatment prevented decreases in the concentrations of Ile, Met, Thr, and Val in IL-10−/− mice compared with WT mice. Similarly, of the nonessential amino acids, concentrations of Ala, Arg, Glu, Gly, and Ser were lower in IL-10−/− mice than in WT mice and these decreases in IL-10−/− mice were prevented by LP treatment. Plasma concentrations of His and Leu were lower in IL-10−/− mice than in WT mice and were unaffected by LP administration. The concentrations of Asp, Tyr, Phe, and Pro did not differ between the IL-10−/− and WT groups. Plasma concentrations of Cys were greater in IL-10−/− mice than in WT mice. However, the plasma total amino acid concentrations were not different between IL-10−/− mice treated with LP and those untreated.

**Inflammatory score and body weight.** Histological analysis showed that colitis developed in all IL-10−/− mice analyzed. The inflammatory score was significantly greater in IL-10−/− mice

### Table 1

<table>
<thead>
<tr>
<th>Amino acids</th>
<th>WT</th>
<th>WT + LP</th>
<th>IL-10−/−</th>
<th>IL-10−/− + LP</th>
<th>LP</th>
<th>Genotype</th>
<th>Genotype × LP</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>μmol/L</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ala</td>
<td>348 ± 7a</td>
<td>357 ± 8b</td>
<td>282 ± 11b</td>
<td>339 ± 11b</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Arg</td>
<td>492 ± 8a</td>
<td>504 ± 10a</td>
<td>423 ± 13b</td>
<td>482 ± 9b</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Asp</td>
<td>13.8 ± 1.1</td>
<td>14.7 ± 1.1</td>
<td>11.1 ± 1.0</td>
<td>12.3 ± 1.0</td>
<td>0.24</td>
<td>0.28</td>
<td>0.33</td>
</tr>
<tr>
<td>Cys</td>
<td>210 ± 5a</td>
<td>202 ± 7b</td>
<td>217 ± 6b</td>
<td>2202 ± 7b</td>
<td>&lt;0.01</td>
<td>0.31</td>
<td>0.15</td>
</tr>
<tr>
<td>Glu</td>
<td>24.4 ± 1.7a</td>
<td>23.4 ± 1.4a</td>
<td>16.2 ± 0.7b</td>
<td>22.4 ± 1.1a</td>
<td>&lt;0.01</td>
<td>0.02</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Gly</td>
<td>171 ± 3a</td>
<td>186 ± 6b</td>
<td>122 ± 6b</td>
<td>175 ± 9b</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>His</td>
<td>446 ± 4a</td>
<td>450 ± 7b</td>
<td>353 ± 11b</td>
<td>360 ± 9b</td>
<td>&lt;0.01</td>
<td>0.11</td>
<td>0.57</td>
</tr>
<tr>
<td>Ile</td>
<td>72.0 ± 2.3a</td>
<td>70.6 ± 5.8a</td>
<td>57.0 ± 13.9b</td>
<td>71.5 ± 3.5a</td>
<td>0.02</td>
<td>0.06</td>
<td>0.11</td>
</tr>
<tr>
<td>Leu</td>
<td>112 ± 3a</td>
<td>112 ± 4a</td>
<td>88.6 ± 4.4b</td>
<td>98.0 ± 4.3b</td>
<td>0.03</td>
<td>0.72</td>
<td>0.89</td>
</tr>
<tr>
<td>Lys</td>
<td>217 ± 4a</td>
<td>209 ± 5b</td>
<td>142 ± 8b</td>
<td>198 ± 12b</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
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<tr>
<td>Met</td>
<td>50.5 ± 1.3a</td>
<td>52.1 ± 2.2a</td>
<td>29.5 ± 1.8b</td>
<td>46.8 ± 1.3b</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Phe</td>
<td>56.4 ± 2a</td>
<td>55.4 ± 2.1</td>
<td>48.1 ± 2.2</td>
<td>52.5 ± 2.8</td>
<td>0.65</td>
<td>0.77</td>
<td>0.42</td>
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<tr>
<td>Pro</td>
<td>72.5 ± 2.6</td>
<td>76.2 ± 4.3</td>
<td>62.6 ± 4.6</td>
<td>75.2 ± 3.1</td>
<td>0.80</td>
<td>0.36</td>
<td>0.55</td>
</tr>
<tr>
<td>Ser</td>
<td>166 ± 3a</td>
<td>175 ± 4a</td>
<td>113 ± 6b</td>
<td>154 ± 7a</td>
<td>&lt;0.01</td>
<td>0.01</td>
<td>0.07</td>
</tr>
<tr>
<td>Thr</td>
<td>131 ± 5a</td>
<td>134 ± 6b</td>
<td>93.4 ± 2.6b</td>
<td>85.4 ± 4.5b</td>
<td>&lt;0.01</td>
<td>0.09</td>
<td>0.11</td>
</tr>
<tr>
<td>Tyr</td>
<td>62.5 ± 2.3</td>
<td>60.9 ± 2.4</td>
<td>42.5 ± 3.0</td>
<td>57.6 ± 4.1</td>
<td>0.27</td>
<td>0.15</td>
<td>0.08</td>
</tr>
<tr>
<td>Val</td>
<td>199 ± 4a</td>
<td>188 ± 7a</td>
<td>126 ± 6b</td>
<td>182 ± 9b</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>2.84 × 103 ± 84a</td>
<td>2.87 × 103 ± 157a</td>
<td>2.20 × 102 ± 160b</td>
<td>2.63 × 103 ± 163c</td>
<td>&lt;0.01</td>
<td>0.01</td>
<td>0.02</td>
</tr>
</tbody>
</table>

1 Data are presented as means ± SD, n = 8. Means in a row with superscripts without a common letter differ, P < 0.05.

### Table 2

<table>
<thead>
<tr>
<th>Item</th>
<th>WT</th>
<th>WT + LP</th>
<th>IL-10−/−</th>
<th>IL-10−/− + LP</th>
<th>P-values</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Inflammation scores</strong></td>
<td>0.3 ± 0.5b</td>
<td>ND</td>
<td>6.8 ± 1.7a</td>
<td>3.0 ± 1.3b</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td><strong>Body weight, g</strong></td>
<td>23.5 ± 1.2a</td>
<td>24.1 ± 1.6a</td>
<td>20.2 ± 1.5b</td>
<td>22.3 ± 1.7a</td>
<td>0.04</td>
</tr>
</tbody>
</table>

1 Data are presented as means ± SD, n = 8. Means in a row with superscripts without a common letter differ, P < 0.05. ND, nondetectable (<0.01).
However, this decrease was completely prevented by LP treatment. Nuclei in blue, and F-actin in green, was broadly distributed across the epithelial apical membrane of Figs. 1 and 2 and IL-10 was observed in IL-10 the jejunum in WT mice. No marked mislocalization of PepT1 protein or mRNA expression in the jejunum between WT mice. Western-blot and quantitative real-time PCR analyses revealed no differences in Expression and distribution of PepT1. PKC activity and phosphorylation. Compared with WT mice, IL-10−/− mice had significantly lower PKC activity and phosphorylation levels in the jejunum detected by Western blotting with the phospho-panPKC antibody (Fig. 3A, B). These changes in PKC activity and phosphorylation levels in IL-10−/− mice compared with WT mice were prevented by LP treatment.

**Discussion**

In this study, we investigated the effects of LP administration on PepT1-mediated cephalexin uptake in the small intestine of the IL-10−/− mouse, a model of spontaneous colitis. Interestingly, compared with WT mice, IL-10−/− mice had significantly lower plasma cephalexin and total plasma amino acid concentrations, which could be completely prevented by LP treatment. Moreover, LP treatment also prevented weight loss and attenuated colitis in IL-10−/− mice, as previously reported (27,28). Together, these observations suggest that LP modulates PepT1-mediated amino acid absorption in the small intestine of IL-10−/− mice, contributing to the improvement of intestinal inflammation.

Mechanisms involved in the regulation of PepT1 include changes in mRNA and protein expression levels, alterations in transport activity, and modification of protein recruitment to the plasma membrane (29). For example, Vavricka et al. (30,31) found that high concentrations of interferon-γ and tumor necrosis factor-α increased PepT1-mediated dipeptide uptake in a dose- and time-dependent manner through increased expression of total and apical membrane-localized PepT1 in Caco-2 BBe cells. In addition, long-term treatment with leptin, a hormone secreted by both adipocytes and the stomach, caused a significant increase in dipeptide uptake in ob/ob mice and this effect was associated with increases in expression of the PepT1 mRNA and protein (32). Furthermore, bacterial infections caused by endotoxin administration in rats caused a decrease in PepT1 activity through downregulation of PepT1 mRNA and protein expression in the intestinal tract (33). Interestingly, increased cephalexin uptake was not associated with a concomitant increase in PepT1 mRNA or protein expression in the present study, although a slight decrease in PepT1 protein and mRNA expression levels was observed in IL-10−/− mice compared with WT mice. This result is consistent with results from Neudeck et al. (34), who found that treatment with Lactobacillus casei led to an increase in PepT1 activity in Caco-2 cells by a mechanism distinct from increased gene expression. Alterations in post-translational trafficking or modification of PepT1 represent potential mechanisms that could explain the changes in PepT1 activity caused by LP treatment. Altered intracellular trafficking could lead to increased membrane association of PepT1 and, therefore, increased activity. Increased translocation from a preformed cytoplasmic pool would result in an increased amount of protein available for transport of cephalexin. Thus, phenotypically, increased cephalexin transport could occur in the absence of changes in gene expression. Similar effects of insulin and leptin on PepT1-mediated transport have been documented (35,36). For example, Nielsen et al. (37) found that
treatment of the basolateral membrane with insulin increased Gly-Sar uptake in Caco-2 cells. Addition of leptin to the apical membrane of Caco-2 cells or mouse jejunum was shown to increase cephalixin and Gly-Sar transport through increases in membrane PepT1 and reduction of intracellular quantities of the protein (21). Stimulation of peptide transport by insulin or leptin is thought to involve increased trafficking of the cytoplasmic pool of PepT1 to the apical membrane. However, results from immunofluorescence confocal microscopy analysis did not reveal a significant redistribution of PepT1 among any of the groups analyzed in this study.

Another potential post-translational modification of PepT1 is phosphorylation. Interestingly, PepT1 contains potential sites for PKC-dependent phosphorylation but not for PKA-dependent phosphorylation (5,38). Many studies have shown that the transport activity of PepT1 is regulated by PKC (6,39). Therefore, we also investigated PKC activity in the small intestine of LP-treated animals. Results from these experiments showed that compared with WT mice, IL-10−/− mice had lower PKC activity and phospho-PKC expression, which were prevented by 4 wk of LP administration. These results suggest that the enhanced transport activity of PepT1 in LP-treated IL-10−/− mice may be due to increased PKC activity.

Although the total concentrations of plasma amino acids were greater in LP-treated IL-10−/− mice than in IL-10−/− mice and amino acids derived from dietary protein are transported into the enterocytes primarily in the form of di- and tripeptides by PepT1, amino acids can also be transported into the bloodstream in their free forms by a variety of different amino acid transporters (40,41). Thus, changes in plasma amino acid concentrations could also be due to effects of LP on other transporters. However, in the present study, the cephalixin uptake experiment confirmed that PepT1 transport activity was indeed enhanced by LP treatment, at least partly explaining the observed increase in plasma amino acid concentrations.

The bacteria/intestinal epithelium interaction is highly complex and the mechanisms that regulate probiotic-mediated changes in epithelial function are only beginning to be understood. For example, treatment with the probiotic *L. casei* enhanced PepT1-mediated Gly-Sar uptake in Caco-2 cells through direct contact of *L. casei* with PepT1 in the apical membrane (34). Similar interactions of *L. casei* with intestinal epithelial cells in mice may also be involved in modulation of cellular metabolic activities (42). Attaching and effacing enteropathogenic *Escherichia coli* induces PepT1 expression and transport activity in human colonic HT29-CL19A cells, which do not express detectable levels of PepT1 under normal conditions, by intimately attaching to host cell membranes (43). Probiotics have also been reported to exert beneficial effects through modulation of gut flora. Thus, determination of whether the effect of LP on modulation of PepT1 activity is indirect or direct requires further investigation in the complex in vivo intestinal environment.

In conclusion, the present study showed that LP administration improves the plasma concentrations of amino acids and cephalixin through enhancement of PepT1-mediated uptake in IL-10−/− mice after 4 wk of treatment. Enhanced transport activity of PepT1 is likely due to enhanced PKC activity as opposed to increased expression or redistribution of PepT1. Our results suggest that LP-induced promotion of amino acid absorption may lead to improvement of inflammatory bowel disease in these animals.

**Acknowledgments**

H-L. Qin, H-Q. Chen, and X-M. Hang were responsible for the experimental design; H-Q. Chen, Y-K. Zhou, T-Y. Shen, and M. Zhang conducted the experiments; M. Zhang and Z-X. Chu analyzed the data; H-Q. Chen and H-L. Qin wrote the manuscript; and H-L. Qin had primary responsibility for the final content. All authors read and approved the final manuscript.

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