Strain-Specific Effects of Probiotics on Gut Barrier Integrity following Hemorrhagic Shock

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Probiotic therapy modulates the composition of the intestinal flora and inhibits the inflammatory response. These properties may be of benefit in the preservation of gut barrier integrity after injury or stress. In this study, we examined the effect of two Lactobacillus strains selected for their pathogen exclusion properties on intestinal barrier integrity following hemorrhagic shock. Additionally, the responsiveness of the macrophage cell line RAW 264.7 to combined exposure to Lactobacillus DNA or oligodeoxynucleotides containing CpG motifs (CpG-ODN) and endotoxin was assessed by measuring tumor necrosis factor alpha (TNF-α) release. Rats were administered lactobacilli (5 × 10⁹ CFU) or vehicle for 7 days and were subjected subsequently to hemorrhagic shock by withdrawal of 2.1 ml blood/100 g tissue. Levels of plasma endotoxin, bacterial translocation to distant organs, and filamentous actin (F-actin) in the ileum were determined 24 h later. Rats treated with Lactobacillus rhamnosus showed reduced levels of plasma endotoxin (8 ± 2 pg/ml versus 24 ± 4 pg/ml; P = 0.01), bacterial translocation (2 CFU/gram versus 369 CFU/gram; P < 0.01), and disruption of F-actin distribution following hemorrhagic shock compared with nontreated control rats. In contrast, pretreatment with Lactobacillus fermentum had no substantial effect on gut barrier integrity. Interestingly, DNA preparations from both lactobacilli reduced endotoxin-induced TNF-α release dose dependently, whereas CpG-ODN increased TNF-α release. In conclusion, the pathogen exclusion properties of both Lactobacillus strains and the reduction of endotoxin-induced inflammation by their DNA in vitro are not prerequisites for a beneficial effect of probiotic therapy on gut barrier function following hemorrhagic shock. Although pretreatment with Lactobacillus spp. may be useful to preserve gut barrier integrity following severe hypotension, a thorough assessment of specific strains seems to be essential.

In recent years, considerable research has focused on the modulation of intestinal flora and the inflammatory response by probiotic bacteria. Probiotics are defined as live microorganisms that, when ingested, exert health benefits on the host through microbial actions (19). These live microorganisms are a heterogeneous group of microbes in which a variety of mechanisms (CpG-ODN) and endotoxin was assessed by measuring tumor necrosis factor alpha (TNF-α) release. Additionally, the responsiveness of the macrophage cell line RAW 264.7 to combined exposure to Lactobacillus DNA or oligodeoxynucleotides containing CpG motifs (CpG-ODN) and endotoxin was assessed by measuring tumor necrosis factor alpha (TNF-α) release. Rats were administered lactobacilli (5 × 10⁹ CFU) or vehicle for 7 days and were subjected subsequently to hemorrhagic shock by withdrawal of 2.1 ml blood/100 g tissue. Levels of plasma endotoxin, bacterial translocation to distant organs, and filamentous actin (F-actin) in the ileum were determined 24 h later. Rats treated with Lactobacillus rhamnosus showed reduced levels of plasma endotoxin (8 ± 2 pg/ml versus 24 ± 4 pg/ml; P = 0.01), bacterial translocation (2 CFU/gram versus 369 CFU/gram; P < 0.01), and disruption of F-actin distribution following hemorrhagic shock compared with nontreated control rats. In contrast, pretreatment with Lactobacillus fermentum had no substantial effect on gut barrier integrity. Interestingly, DNA preparations from both lactobacilli reduced endotoxin-induced TNF-α release dose dependently, whereas CpG-ODN increased TNF-α release. In conclusion, the pathogen exclusion properties of both Lactobacillus strains and the reduction of endotoxin-induced inflammation by their DNA in vitro are not prerequisites for a beneficial effect of probiotic therapy on gut barrier function following hemorrhagic shock. Although pretreatment with Lactobacillus spp. may be useful to preserve gut barrier integrity following severe hypotension, a thorough assessment of specific strains seems to be essential.

Inhibition of pathogen adhesion and production of antimicrobial metabolites are believed to be important characteristics of viable probiotic strains, and selection of those strains is often based on these properties (18, 33). Recently, a novel insight was provided by a study from Rachmilewitz et al. showing that probiotic DNA plays a crucial role in the observed protection of probiotic therapy in experimental colitis via a Toll-like receptor 9 (TLR9) signaling pathway (31). These findings suggest that the effects of probiotics on the host immune response may be based on more than the manipulation of intestinal microflora alone and may represent a systemic modulation of inflammatory processes.

Given these modulatory effects on intestinal microflora and the systemic inflammatory response, we hypothesized that probiotic therapy would be beneficial in the preservation of gut barrier integrity following systemic hypotension. It is believed that bacteria and bacterial toxins are able to translocate across the intestinal barrier following severe blood loss (10–12). Subsequently, local activation of inflammatory cells causes the release of inflammatory cytokines, leading to the deterioration of intestinal barrier integrity and increased bacterial translocation (20).

Here, we studied the effect of the administration of Lactobacillus rhamnosus (LMG P-22799) and Lactobacillus fermentum (NumRes2) on intestinal barrier function in a rat model of hemorrhagic shock by measuring the translocation of bacteria...
and endotoxin and the level of structural damage to the intestinal wall (24, 25). Additionally, we assessed the effects of DNA preparations isolated from both strains and of oligodeoxynucleotides containing CpG motifs (CpG-ODN) on tumor necrosis factor alpha (TNF-α) release by the murine macrophage cell line RAW 264.7 in response to endotoxin.

**MATERIALS AND METHODS**

**Pathogen exclusion properties.** Pathogen exclusion was determined using 2-week-postconfluent Caco-2 cells cultured in minimal essential medium supplemented with 1% sodium pyruvate, 10% fetal calf serum (FCS), and 1% penicillin-streptomycin (all purchased from Gibco, Grand Island, NY). Lactobacillus (L. rhamnosus LMG P-22799 and L. fermentum NumRes2) and pathogens (Escherichia coli E374 [enteropathogenic E. coli], Pseudomonas aeruginosa LMG21901, Klebsiella pneumoniae LMG21902, and Shigella flexneri LMG21913S) were resuspended in complete minimal essential medium (free of penicillin-streptomycin) containing 1% FCS and added to Caco-2 cells. Subsequently, the Caco-2 cells were incubated at 37°C for 1 h, washed to remove nonadherent bacteria, and lysed by the addition of 1 ml sterile water. The lysed Caco-2 cells were plated on MRS agar and nutrient agar to determine the numbers of adhered lactobacilli and pathogens, respectively. Pathogen exclusion was calculated as the percentage of normal adhesion of pathogens to Caco-2 cells.

**Oligodeoxynucleotides, genomic DNA, and bacterial toxins.** Purified, immunostimulatory CpG-ODN (5′-TAGCTGGTAAACCTCCGATGTA-3′) plus a phosphorothioate backbone (7) and nonimmunostimulatory non-CpG-ODN (5′-GCCGTAGATCAACCGCGCAAA-3′) (Eurogentec, Seraing, Belgium) were dissolved in sterile, pyrogen-free saline (500 μM). Genomic DNA from both lactobacilli was prepared using a DNA isolation kit (Promega, Madison, WI). Purity of DNA was confirmed by measuring UV absorbance at 260/280 nm using GeneQuant 1 (Pharmacia, LKB Biotech Ltd., Cambridge, England). Lipopolysaccharide (LPS) was removed from DNA preparations using Endotrap 5/1 (Proflos, Regensburg, Germany), after which both preparations contained less than 1 ng LPS per μg DNA.

**Cell culture techniques.** The murine macrophage cell line RAW 264.7 was cultured in RPMI medium supplemented with 10% FCS and 1% penicillin-streptomycin in 250-ml sterile culture flasks. Cells (2 × 10⁶ cells/ml) were washed in RPMI medium without FCS before the experiment, divided in 2 ml sterile vials (Greiner Bio-One, Frickenhausen, Germany), and preincubated for 1 h with 0.5, 1.5, 4.5, and 13.5 μg/ml CpG-ODN, non-CpG-ODN, or DNA preparations from L. rhamnosus LMG P-22799 and L. fermentum NumRes2. Subsequently, cells were washed in RPMI medium, plated at 2 × 10⁵ cells/well (100 μl) in 96-well polystyrene culture plates (Costar, Cambridge, MA), and stimulated with 10 ng/ml LPS (055:B5; Sigma, St. Louis, MO) for 24 h at 37°C. TNF-α was measured in supernatants by sandwich enzyme-linked immunosorbent assay (14).

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**Experimental design.** Probiotics were administered daily by oral gavage 7 days prior to hemorrhagic shock with standard chow. Rats were fasted 18 h before induction of shock: a femoral artery catheter was inserted 45 min before shock, and rats were monitored for 50 min, after which the catheter was removed. Rats were allowed standard chow 6 h after hemorrhagic shock, and rats were sacrificed 24 h later.

**RESULTS**

L. rhamnosus LMG P-22799 and L. fermentum NumRes2 inhibit pathogen adhesion in Caco-2 cells. The effects of both probiotic strains on the inhibition of adhesion of various patho-

FIG. 1. Experimental design. Probiotics were administered daily by oral gavage 7 days prior to hemorrhagic shock with standard chow. Hemorrhage was induced by 20% blood loss; a femoral artery catheter was inserted 45 min before shock, and rats were monitored for 50 min, after which the catheter was removed. Rats were allowed standard chow 6 h after hemorrhagic shock, and rats were sacrificed 24 h later.

leading to a dramatic decrease in systemic blood pressure in all shock groups (from 97 ± 2 mm Hg to 26 ± 1 mm Hg). This decrease in blood pressure is known to cause a systemic hypoperfusion of most of the splanchnic organs. Rats recovered spontaneously after 50 min and were allowed standard chow ad libitum after 6 hours. In the sham-shocked group, the femoral artery was cannulated; however, no blood was withdrawn. At sacrifice (24 h), all rats were anesthetized with sodium pentobarbital (60 mg/kg), tissue was taken aseptically for analysis, and plasma was collected in a heparinized, pyrogen-free glass tube, separated by centrifugation, frozen immediately, and stored (at −20°C) until analysis.

**Endotoxin and bacterial translocation.** Total circulating endotoxin was determined by a Limulus amoeboocyte lysate chromogenic endpoint assay (0.001 to 1 ng/ml) (Hbt, Uden, The Netherlands) according to the manufacturer’s instructions.

Mesenteric lymph nodes (MLN), the mesoderm of the spleen, and a segment (segment IV) of the liver were collected aseptically in 2-ml preweighed thioglycolate broth tubes (Becton Dickinson [BBL], Microbiology Europe, Maylan, France). After being weighed, tissue specimens were homogenized and transferred onto agar plates. All agar plates (Columbia III blood agar base supplemented with 5%, vol/vol, sheep blood [BBL] [duplicate plates], chocolate Polyvitex agar [BioMérieux, Marcy L’Etoile, France], and Schaeder kanamycin-vancomycin agar supplemented with 5% sheep blood [BBL]) were incubated for 48 h in a 5% CO₂-enriched atmosphere or under anaerobic conditions (Shaedler agar plates). After incubation, the numbers of colonies on all aerobic plates were counted and adjusted to the weight of the ground tissue. Colony types were identified to the species level using standard methods. Lactobacillus colonies were typed using 16S rRNA sequencing. In short, colonies were resuspended in water, and the 16S rRNA gene was amplified using primers 5ʼ-CACCGATCGTAGTTGATAYMGCTCAAG-3ʼ and 1510r (5ʼ-GTAACGGTACCTGTAGCACGGA-3ʼ) (Fruitkan, Delft, The Netherlands). Sequencing of the amplicon was done using the ABI PRISM BigDye Terminator cycle sequencing ready reaction kit (Applied Biosystems Inc., Nieuwekerk aan den IJssel, Netherlands) in combination with various primers. The complete sequence region was compared to other 16S rRNA sequences in the GenBank, EMBL, DDBJ, and PDB databases for strain identification.

**Intracellular staining for F-actin.** Frozen sections of ileum (4 μm) were cut and stained for filamentous actin (F-actin). Briefly, slides were fixed in acetone for 10 min and air dried. Slides were stained for 45 min at room temperature with Oregon Green phalloidin that binds specifically to F-actin. After three washes in phosphate-buffered saline, slides were mounted using glycerol–phosphate-buffered saline with 1,4-diazabicyclo[2.2.2]octane and 4’-6-diamidino-2-phenylindole and viewed with an immunofluorescence microscope. In total, three sections per ileum from three rats per group were investigated at ×600 magnification.

**Statistical analysis.** Two-tailed unpaired Student’s t-tests were used to compare groups. The p value was determined by ANOVA and Student’s t-test. Results were considered significant at p < 0.05. A nonparametric Mann-Whitney U test was used for comparisons between treated and nontreated groups.
genic bacterial species to Caco-2 cells were tested in vitro. Both Lactobacillus strains were able to inhibit the adhesion of tested pathogens E. coli, P. aeruginosa, K. pneumoniae, and S. flexneri (Fig. 2) to similar extents.

Pretreatment with L. rhamnosus LMG P-27299 but not L. fermentum NumRes2 reduces circulating endotoxin levels after hemorrhagic shock. The severities of the hemorrhagic-shock insults reflected by changes in mean arterial pressure, heart rate, and hematocrit were similar for all four hemorrhagic-shock groups (data not shown).

In line with earlier observations (26), circulating endotoxin levels were elevated significantly in nontreated and vehicle-treated rats subjected to hemorrhagic shock (24 ± 4 pg/ml and 21 ± 1 pg/ml, respectively) compared with those of nontreated rats that underwent sham shock (3 ± 1 pg/ml; P < 0.01) (Fig. 3). In contrast, endotoxin levels were reduced after hemorrhagic shock in rats pretreated with L. rhamnosus LMG P-27299 (8 ± 2 pg/ml; P < 0.01) compared with those of nontreated shocked and vehicle-treated rats. Interestingly, pretreatment with L. fermentum NumRes2 did not reduce the level of endotoxemia caused by hemorrhagic shock (21 ± 3 pg/ml) compared with that of nontreated or vehicle-treated rats.

L. rhamnosus LMG P-27299 reduces bacterial translocation to distant organs. Hemorrhagic shock typically causes bacterial translocation to the MLN, spleen, and liver (25, 26). Although pretreatment with L. rhamnosus LMG P-27299 did not significantly reduce bacterial translocation in sham-shocked rats, this probiotic therapy strongly reduced bacterial translocation to distant organs following hemorrhagic shock (total of 2 CFU/gram of tissue) compared with that of control rats (total of 369 CFU/gram; P < 0.01) or rats treated with vehicle (total of 292 CFU/gram; P < 0.01) (Table 1). Overall, the bacteria found most frequently in the cultures of these groups were E. coli, Enterococcus faecalis, and Staphylococcus aureus. Additionally, Proteus spp. and Lactobacillus spp. were sporadically cultured.

In rats pretreated with L. fermentum NumRes2, significantly fewer bacteria were found in the MLN (median, 15 CFU/gram [range, 0 to 69]; P < 0.01) and liver (median, 6 CFU/gram [range, 0 to 13]), but bacterial translocation to the spleen was markedly enhanced (median, 185 CFU/gram [range, 30 to 690]; P < 0.05). Unexpectedly, this increased number of bacteria in the spleen was caused primarily by increased bacterial translocation of Lactobacillus spp., a phenomenon which was not observed with the other groups. Interestingly, with both intervention groups, the cultured lactobacilli were not identical to the supplemented strain, as determined by 16S rRNA gene sequencing (data not shown).

Pretreatment with L. rhamnosus LMG P-27299 preserves the structural integrity of the ileum after hemorrhagic shock. To determine the effects of both Lactobacillus strains on structural components of the intestinal barrier, actin filaments were stained in ileum segments using Oregon Green phalloidin. F-actin was regularly distributed, and a typical fine-meshed pattern was observed throughout villi in control rats subjected to sham surgery (Fig. 4A). No apparent differences were found in sham-shocked rats treated with L. rhamnosus LMG P-27299 (Fig. 4D) or L. fermentum NumRes2 (Fig. 4F). In contrast, hemorrhagic shock caused a significant change of F-actin cytoarchitecture in nontreated and vehicle-treated rats (Fig. 4B and C); F-actin distribution was disrupted, the fine meshwork pattern was decreased, and F-actin disappeared to a large extent at the mucosal site of intestinal epithelial cells. Pretreatment with L. rhamnosus LMG P-27299 markedly attenuated these changes in actin organization caused by hemorrhagic

FIG. 2. L. rhamnosus LMG P-27299 and L. fermentum NumRes2 inhibit the adhesion of pathogens similarly. Lactobacilli and pathogens were added to cultured Caco-2 cells; after a 1-h incubation period, cells were washed, and adherent bacteria were determined by culture techniques. Both L. rhamnosus LMG P-27299 and L. fermentum NumRes2 inhibited the adhesion of E. coli, K. pneumoniae, P. aeruginosa, and S. flexneri, with no significant differences between probiotic strains. Data are presented as percentages of pathogen exclusion compared to a control situation (e.g., adhesion of pathogen without addition of probiotics).

FIG. 3. Pretreatment with L. rhamnosus LMG P-27299 decreases plasma endotoxin levels in rats subjected to hemorrhagic shock. Plasma endotoxin was measured 24 h after sham and hemorrhagic shock. Endotoxin levels were near the detection level in all sham-treated groups and were markedly elevated by hemorrhagic (Hem.) shock in nontreated or vehicle-treated rats (nontreated, 24 ± 4 pg/ml, and vehicle treated, 21 ± 1 pg/ml). Pretreatment for 7 days with L. rhamnosus LMG P-27299 strongly reduced endotoxin levels compared with those of vehicle-treated controls (8 ± 2 pg/ml (*, P < 0.01), whereas pretreatment with L. fermentum NumRes2 had no effect. Data are presented as means ± SEM.
shock (Fig. 4E), whereas treatment with L. fermentum NumRes2 did not substantially change actin filament organization after hemorrhagic shock (Fig. 4G) compared with that of nontreated or vehicle-treated rats at the villus top.

DNA preparations isolated from L. fermentum NumRes2 and L. rhamnosus LMG P-22799 reduced the response of RAW 264.7 cells to endotoxin. To determine the contribution of probiotic DNA to the differences found in vivo, RAW 264.7

<table>
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<th>Group</th>
<th>No. of rats affected/total no. of rats in group</th>
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<td>0</td>
<td>0</td>
</tr>
<tr>
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<td>0 (0–33)</td>
<td>0</td>
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<td>16 (0–53)</td>
<td>22</td>
</tr>
<tr>
<td>Sham shock + L. fermentum</td>
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<td>0 (0–39)</td>
<td>0</td>
</tr>
<tr>
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<td>300 (114–483)</td>
<td>369</td>
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<tr>
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<td>292</td>
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<tr>
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<td>3/6</td>
<td>2 (0–37)**</td>
<td>2**</td>
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<tr>
<td>Hem. shock + L. fermentum</td>
<td>6/6</td>
<td>15 (0–69)**</td>
<td>238</td>
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</tbody>
</table>

* MLN, spleen, and liver were cultured at sacrifice (24 hours). Rats were treated with L. rhamnosus LMG P-22799, L. fermentum NumRes2, or vehicle prior to sham shock or shock. Each group consisted of six rats. Hem. shock, hemorrhagic shock. *, P < 0.05 compared with value for hemorrhagic-shocked, nontreated group. **, P < 0.05 compared with value for hemorrhagic-shocked, vehicle-treated group.
DISCUSSION

Probiotic therapy is increasingly being used for gastrointestinal diseases and inflammatory conditions to inhibit the inflammatory response and reduce intestinal wall damage with equivocal results (21, 35). The diversity of probiotic strains used in various dosages and regimens may contribute to this ambiguity and makes a comparison of their efficacies in treating disease difficult (37).

In line with studies showing the inhibitory effects of probiotic strains such as _Bifidobacterium_ and _Lactobacillus_ spp. on the adhesion of enteric pathogens, we show that pathogen adhesion to Caco-2 cells was markedly inhibited by both _Lactobacillus_ strains used (i.e., _L. rhamnosus_ LMG P-22799 and _L. fermentum_ NumRes2) (5, 32). Despite these similar pathogen exclusion properties, the strains were found to have distinctly different effects on intestinal barrier integrity loss caused by hemorrhagic shock. The nonlethal hemorrhagic-shock model used typically causes an early disruption of gut barrier structure and function, characterized by translocation of bacteria and detectable endotoxemia (25, 26). Interestingly, we now show for the first time that administration of _L. rhamnosus_ LMG P-22799 markedly reduces endotoxin levels and bacterial translocation following hemorrhagic shock. This finding is supported by studies of models of colitis in which other probiotic strains have an ameliorating effect on endotoxin levels and bacterial translocation (28). The effect was specifically related to the bacterium and cannot be attributed to components of the vehicle used, as has previously been described (8). The finding that pretreatment of sham-shocked rats with _L. rhamnosus_ LMG P-22799 did not reduce bacterial translocation to zero is unexplained and may be attributed to the good adhesion properties of this specific strain, leading to a weak translocation of lactobacilli (19).

_L. fermentum_ NumRes2 did not affect plasma endotoxin levels and total bacterial translocation following hemorrhagic shock, which reflects the variety in actions between _Lactobacillus_ strains and demonstrates that the comparison of various strains remains complex (1, 36). An interesting and unexpected finding was that, in rats treated with _L. fermentum_ NumRes2, bacterial translocation to the spleen was markedly increased, and almost all translocated bacteria were _Lactobacillus_ spp. All of these bacteria appeared to be derived from the host, as 16S rRNA gene sequence analyses did not reveal homology to the supplemented strain. In a clinical setting, translocation of lactobacilli occurs rarely and is often regarded as harmless, although bacteremia with lactobacilli may be detrimental and requires specific antimicrobial treatment (34). The underlying cause of this increased translocation of lactobacilli remains unexplained; however, such an undesired side effect needs to be avoided.

The effects of _L. rhamnosus_ LMG P-22799 and _L. fermentum_ NumRes2 on F-actin distribution were in line with the effects on endotoxin translocation. F-actin is part of the cytoarchitecture of cells and is anchored to tight junctions via zonula occludens (ZO) proteins (15). Tight-junction expression is markedly affected by proinflammatory cytokines such as TNF-α (17). In previous studies, we showed that hemorrhagic shock causes rapid disruption of ZO-1, paralleled by an increased release of inflammatory cytokines (TNF-α and interleukin 6 [IL-6]) (24, 25). The current finding that F-actin distribution is affected even 24 h after hemorrhagic shock reveals that early disruption of ZO-1 has prolonged consequences for actin filament organization in intestinal cells. The fact that _L. rhamnosus_ LMG P-22799 prevented the disorganization of ac-
tin filaments in intestinal cells is in line with in vitro studies showing that Lactobacillus acidophilus strain LB protects against E. coli-induced alterations of F-actin expression in Caco-2 cells (23).

Based on a study from Rachmilewitz et al. (31) indicating that the protective effects of probiotics in colitis are mediated by the binding of their DNA to TLR9, we investigated the immunomodulatory properties of DNA isolated from both Lactobacillus strains. Bacterial DNA or unmethylated CpG motifs are taken up in lysosomes, followed by recruitment of TLR9 to these uptake sites, which leads to an inflammatory response via signaling molecules such as Myd88 (22). DNA preparations from both Lactobacillus strains used were not immunogenic (data not shown), which is supported by previous reports on the reactivity of DNA from probiotic bacteria in the VSL no. 3 compound (21). In line with others, we confirmed a strong exacerbating effect of preincubation with CpG-ODN on E. coli-induced inflammation, a previously unrecognized property. It has been shown previously that probiotic DNA inhibits IL-8 secretion by HT-29 cells to pathogenic bacterial DNA in a competitive manner (21). Next, probiotics also have been shown to inhibit NF-κB in colonic epithelial cells and to suppress IL-8 secretion in intestinal epithelia (3, 30). The current data indicate that, next to potential competition with bacterial DNA, probiotic DNA induces tolerance to a subsequent trigger with another TLR ligand dose dependently. The capacity of probiotic DNA to inhibit endotoxin-induced inflammation may underlie the effect on gut barrier function loss in vivo, since the magnitude of the inflammatory response is directly related to the extent of intestinal damage (2, 24, 25). The optimal dosages of DNA preparations of both strains to inhibit endotoxin-induced inflammation are probably different. We administered a single (commonly accepted) dose of both strains that may have been optimal for the L. rhamnosus strain but that may not have been favorable for the L. fermentum strain. A (slight) difference in effect on the inflammatory response in combination with the potent pathogen exclusion properties of both strains may have been the cause for the differences in the effects of both strains on bacterial translocation following hemorrhagic shock. Further studies are necessary to identify the exact mode(s) of action of probiotics.

In conclusion, the current study shows that two Lactobacillus strains with similar pathogen exclusion properties have distinctive, different effects on intestinal barrier integrity loss following hemorrhagic shock. Whereas L. rhamnosus LGP-22799 reduced gut barrier integrity loss caused by systemic hyperfusion, L. fermentum NumRes2 had no substantial effect and even resulted in the increased translocation of Lactobacillus spp. Furthermore, DNA isolated from both Lactobacillus strains suppresses endotoxin-induced inflammation dose dependently, which was previously unrecognized. Our data indicate that certain probiotic strains may be useful in a clinical setting to preserve gut barrier integrity following severe blood loss. However, potentially useful probiotic strains need to be thoroughly studied using in vitro and in vivo approaches to select suitable strains before applying such therapies in various disease states.

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