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 (5×10^9 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 *Lactobacillus* spp. and *Bifidobacterium* spp. are frequently used (4, 6, 13, 38). Probiotics have been investigated intensively with inflammatory bowel disease and pouchitis, in which it was shown previously that such a therapy has a beneficial effect on gut barrier integrity and can ameliorate inflammatory responses (27–30). Although some of these studies show a beneficial effect of probiotic therapy on inflammation and bacterial translocation, there is limited documentation of the efficacy of these agents in properly designed controlled trials, which complicates the practical application of probiotics in a clinical setting (16, 35, 37). Furthermore, the underlying mode of action is not yet fully understood, and it remains to be determined whether the described effects are limited to inflammatory bowel disease or can also be applied to other disease states in which gut barrier integrity is compromised.

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

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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). Lactobacilli (*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* LMG21935) 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'-TGACTGTGACGTTTCGAGATGA-3') plus a phosphorothioate backbone (7) and nonimmunostimulatory nonCpG-ODN (5'-GCTTGATGACTCAGCCGGA-3') (Eurogentec, Seraing, Belgium) were dissolved in sterile, pyrogen-free saline (500 μ M). 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 I (Pharmacia, LKB Biochrom Ltd., Cambridge, England). Lipopolysaccharide (LPS) was removed from DNA preparations using Endotrap 5/1 (Profos, Regensburg, Germany), after which both preparations contained less than 1 pg LPS per μ g 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 (2×10^6 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 μ g/ml CpG-ODN, nonCpG-ODN, or DNA preparations from *L. rhamnosus* LMG P-22799 and *L. fermentum* NumRes2. Subsequently, cells were washed in RPMI medium, plated at 2×10^5 cells/well (100 μ l) in 96-well polystyrene culture plates (Costar, Cambridge, MA), and stimulated with 10 ng/ml LPS (O55:B5; Sigma, St. Louis, MO) for 5 h at 37°C. After incubation, the plates were centrifuged for 3 min at 1,500 rpm, and supernatants were collected and stored at -20°C. TNF- α was measured in supernatants by sandwich enzyme-linked immunosorbent assay (14).

Hemorrhagic-shock experiment. This study was performed by following the guidelines of the Animal Care Committee of the University of Maastricht, and that committee approved the protocol. Healthy male Sprague-Dawley rats, weighing 319 to 403 g (average, 364 g), were purchased from Charles River (Maastricht, The Netherlands), housed under controlled conditions of temperature and humidity, and fed water and chow ad libitum.

Seven days prior to the hemorrhagic-shock experiment, the rats received *L. rhamnosus* LMG P-22799 or *L. fermentum* NumRes2 (5×10^9 CFU) daily via oral gavage and standard chow and water ad libitum (Fig. 1). Nontreated rats received only standard chow and water prior to the experiments before subjection to sham shock or hemorrhagic shock, and vehicle-treated rats received 75% maltodextrin, 15% monosodium glutamate, and 10% sodium L-ascorbate 7 days prior to hemorrhagic shock. In total, eight groups of six rats per group were included: (i) controls (not fed with lactobacilli and not subjected to hemorrhagic shock), (ii) nontreated rats subjected to sham shock, (iii) sham-shocked rats fed with *L. rhamnosus* LMG P-22799, (iv) sham-shocked rats fed with *L. fermentum* NumRes2, (v) nontreated hemorrhagic-shock-treated rats, (vi) vehicle-treated shocked rats, (vii) *L. rhamnosus* LMG P-22799-treated shock rats, and (viii) *L. fermentum* NumRes2-treated shocked rats.

A nonlethal hemorrhagic-shock model was used as previously described (25, 26). In short, rats were anesthetized with sodium pentobarbital (40 mg/kg, intraperitoneally); the femoral artery was dissected and cannulated with polyethylene tubing containing heparinized saline (10 IU/ml). Mean arterial pressure and heart rate were recorded during a 50-minute observation period. Hemorrhagic shock was induced by withdrawal of 2.1 ml blood per 100 g of body weight,

FIG. 1. 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.

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* amoebocyte 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 midsection 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 Schaedler 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 8f (5'-CACGGATCCAGAGTTTGATYMTGGCTCAG-3') and 1510r (5'-GTGAAGCTTACGGYTACCTGTGTACGACTT-3') (Biolegio BV, Malden, The Netherlands). Sequencing of the amplicon was done using the ABI PRISM BigDye Terminator cycle sequencing ready reaction kit (Applied Biosystems Inc., Nieuwerkerk aan den IJssel, Netherlands) in combination with various primers. The complete sequenced region was compared to other 16S rRNA sequences in the GenBank, EMBL, DDBJ, and PDB databases for strain identification.

Immunofluorescence for F-actin. Frozen sections of ileum (4 μ m) 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 a $\times 600$ magnification.

Statistical analysis. Bacterial translocation data are represented as medians and ranges; other data are represented as means $\pm$ standard errors of the means (SEM). A nonparametric Mann-Whitney U test was used for comparisons between treated and nontreated groups.

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. 2. *L. rhamnosus* LMG P-22799 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-22799 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).

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-22799 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-22799 (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-22799 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-22799 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*,

FIG. 3. Pretreatment with *L. rhamnosus* LMG P-22799 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-22799 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 $\pm$ SEM.

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-22799 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-22799 (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-22799 markedly attenuated these changes in actin organization caused by hemorrhagic

TABLE 1. Total bacterial translocation to distant organs

Group	No. of rats affected/total no. of rats in group	Median no. of CFU/g of tissue (range)			Total
		MLN	Spleen	Liver	
Control	0/6	0	0	0	0
Sham shock, nontreated	2/6	0 (0–33)	0 (0–17)	0 (0–95)	0
Sham shock + <i>L. rhamnosus</i>	3/6	16 (0–53)	0 (0–15)	0 (0–11)	22
Sham shock + <i>L. fermentum</i>	1/6	0 (0–39)	0	0	0
Hem. shock, nontreated	6/6	300 (114–483)	12 (0–998)	34 (0–249)	369
Hem. shock + vehicle	6/6	109 (74–262)	33 (14–832)	27 (7–161)	292
Hem. shock + <i>L. rhamnosus</i>	3/6	2 (0–37)*/**	0 (0–11)**	0 (0–6)***	2**/**
Hem. shock + <i>L. fermentum</i>	6/6	15 (0–69)*/**	185 (30–690)	6 (0–13)***	238

^a 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.

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

FIG. 4. Pretreatment with *L. rhamnosus* LMG P-22799 prevents disruption of F-actin filament organization in ileum segments by hemorrhagic shock. Immunolocalization of F-actin (green) at a $\times 600$ magnification showed a regular organization throughout the cell in terminal ilea of nontreated and treated rats subjected to sham shock (A, D, and F). Hemorrhagic shock markedly disrupted the filament organization of F-actin in fasted animals and those that were pretreated with vehicle (B and C). Moreover, tissue was disrupted and disorganized, illustrated by an irregular distribution of nuclei (blue). In contrast, pretreatment for 7 days with *L. rhamnosus* LMG P-22799 prevented disruption of cytoarchitecture by hemorrhagic shock (E), whereas *L. fermentum* NumRes2 had no substantial effect (G). The histology shown is representative of all tissue samples studied (see Materials and Methods).

FIG. 5. DNA from *L. rhamnosus* LMG P-22799 and *L. fermentum* NumRes2 suppresses endotoxin-induced TNF- α in RAW 264.7 cells. RAW 264.7 cells were incubated with CpG-ODN (open circles), non-CpG-ODN (filled squares), or DNA preparations from *L. rhamnosus* LMG P-22799 (filled circles) or *L. fermentum* NumRes2 (stars) at 0.5, 1.5, 4.5, and 13.5 μ g/ml before exposure to 10 ng/ml endotoxin. Pre-stimulation with CpG-ODN increased TNF- α concentrations dose dependently in the supernatant with a maximum at 13.5 μ g/ml DNA (18 ± 1.4 ng/ml). Compared with preincubation with medium (3.7 ± 0.5 ng/ml), preincubation with DNA from *L. rhamnosus* LMG P-22799 reduced TNF- α levels maximally at 13.5 μ g/ml (2.1 ± 0.8 ng/ml; *, $P < 0.001$), and preincubation with DNA from *L. fermentum* NumRes2 reduced TNF- α levels maximally at 4.5 μ g/ml (2.5 ± 0.5 ng/ml; †, $P < 0.001$). There were no significant differences between DNA preparations from both lactobacillus strains. Data are presented as means $\pm$ SEM on a log scale (y axis).

cells were preexposed to DNA preparations isolated from both probiotic strains and subsequently challenged with endotoxin. Oligodeoxynucleotides containing unmethylated CpG motifs (CpG-ODN) and nonCpG-ODN were included as controls. Stimulation of RAW cells with 10 ng/ml endotoxin without prior exposure to DNA or immunostimulatory ODNs resulted in detectable TNF- α (3.7 ± 0.1 ng/ml) (Fig. 5). Cells preincubated with CpG-ODN showed a dose-dependent increase in TNF- α release, reaching a maximum TNF- α response at a dose of 13.5 μ g/ml (18 ± 1.4 ng/ml). In contrast, preexposure of macrophages to DNA from *L. fermentum* NumRes2 and *L. rhamnosus* LMG P-22799 did not increase the responsiveness of macrophages to endotoxin but rather reduced TNF- α release. DNA from *L. fermentum* NumRes2 maximally reduced TNF- α release at a dose of 4.5 μ g/ml (2.5 ± 0.5 ng/ml; $P < 0.001$) and DNA from *L. rhamnosus* LMG P-22799 at 13.5 μ g/ml (2.1 ± 0.8 ng/ml; $P < 0.001$). No significant differences were found between DNA preparations of both *Lactobacillus* strains.

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 the responsiveness of macrophages to endotoxin (9), whereas *Lactobacillus* DNA preparations markedly inhibited endotoxin-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- κ B 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* LMG P-22799 reduced gut barrier integrity loss caused by systemic hypoperfusion, *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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