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The Influence of Antibacterial and Antioxidative Probiotic Lactobacilli on Gut Mucosa in a Mouse Model of *Salmonella* Infection

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The purpose of the present study was to test the ability of selected probiotic *Lactobacillus* spp. (with high antimicrobial and antioxidative potential in *in vitro* tests) to compete with invasive *Salmonella enterica* serovar Typhimurium infection and protect the gut mucosa against excessive oxidative stress during inflammatory tissue damage in a mouse model. In total 47 mice were divided into four groups. The control group was treated with either phosphate-buffered saline (PBS, group 1) or alternatively 0.5×10^8 CFU/ml of human intestinal lactobacilli, namely *Lactobacillus fermentum* ME-3 (DSM 14241) and *Lactobacillus acidophilus* 0.5×10^8 CFU /ml (group 4), daily for 15 days. Group 2 and 3 mice were challenged with a clinical isolate of *S.* Typhimurium (0.5×10^5 CFU/ml). The group 3 mice were additionally orally inoculated with lactobacilli for 5 days before and 10 days after the challenge with *S.* Typhimurium. Counts of salmonellae and lactobacilli in blood, intestine, liver and spleen were recorded; the morphological indices of inflammation in the same organs and oxidative stress-indicative biochemical status (lipid peroxidation, total antioxidative activity, redox ratio of glutathione and iron content) of gut mucosa were assessed on the 10th day after oral inoculation. The administration of probiotic lactobacilli of human origin did not increase the total count of lactobacilli in the terminal ileum of mice; however, hyperplasia of lymph nodes was registered in group 4 mice compared with group 1 mice. In the gut the lactobacilli that were antagonistic against *S.* Typhimurium *in vitro* neither decreased the count of salmonellae nor prevented the spread of the infection. In contrast, the antioxidative potential of *L. fermentum* ME-3 influenced gut mucosa by the reduction of iron level (pro-oxidant), lipid peroxidation and increased total antioxidative activity, glutathione redox value. We conclude that properties of probiotic lactobacilli assessed *in vitro* could be expressed differentially during *in vivo* study. The possibility to influence the pro- and antioxidant balance by specific probiotic lactobacilli with antioxidative properties in the course of inflammatory tissue damage could be further explored. *Key words: Salmonella* Typhimurium, mouse model, probiotics, lactobacilli, oxidative stress, pro-oxidant, antioxidant, gut mucosa.

INTRODUCTION

Salmonella spp. are important aetiological agents of various diseases. There are two types of food-borne infections causing serious medical and veterinary problems worldwide: i) intestinal or non-typhoid form (salmonellosis) caused by hundreds of different serovariants of *S. enterica*, including serovar Typhimurium and ii) generalized or typhoid form (enteric fever) caused by *S. enterica* serovar Typhi. Enterocolitis characteristic of non-typhoid forms is by far the most common manifestation of salmonella-derived infections in humans that under certain circumstances are accompanied by septicaemia (1, 2), causing the increase of mortality. The reasons for invasiveness, e.g. extraintestinal disease, are mainly associated with impaired host resistance (1).

Besides the host-specific factors such as phagocytosis and bactericidal activity of tissues and sera, another anti-infectious mechanism, termed colonization resistance, is an important part of innate immunity. Colonization resistance is conferred by indigenous microflora and comprises blocking of the receptors on cells and extracellular matrices, competition for nutritional substrates, production of bacteriocins and continuous stimulation of the immune system (3). Probiotic bacteria are widely used to enhance the colonization resistance. Probiotics are live bacterial preparations developed from normal intestinal microflora that beneficially influence the health of humans (4, 5).

We have found a *Lactobacillus fermentum* strain (DSM 14241, assigned ME-3), expressing high antimicrobial activity against *S.* Typhimurium *in vitro* and possessing substantial antioxidative potential (6). However, it has not been determined whether the administration of probiotic bacteria could enhance colonization resistance and influence the balance of pro- and antioxidants in gut mucosal

cells. The localized inflammation following salmonella invasion generates many activating signals for phagocytes, resulting in killing of the pathogens. In addition to the beneficial effects of inflammation and priming of polymorphonuclear leukocytes for phagocytosis, the production of reactive oxygen species (ROS) can also occur and create collateral tissue injury (7).

The aim of the study was to assess if probiotic bacteria with antimicrobial and antioxidative properties defined *in vitro* could suppress experimental *S*. Typhimurium infection in mice and protect the gut mucosa against excessive oxidative stress, by investigating microbiological, morphological and oxidative stress-indicative biochemical markers in a mouse model.

MATERIALS AND METHODS

Study design

In total 47 NIH line conventional male mice (Kuopio, Finland) aged 4–6 weeks were used in the study. The absence of infection was ensured and the mice were divided into four separately housed groups. International regulations for animal experiments were followed (8). Throughout the study the mice were given a commercial diet R-70 (Lactamin, Sweden) and tap water *ad libitum*. Group 1 ($n = 7$) served as a control group and was treated with phosphate-buffered saline (PBS). The experimental infection of *S*. Typhimurium was established in the mice by oral inoculation (group 2, $n = 16$).

Protection against *S*. Typhimurium infection by administration of probiotic lactobacilli was evaluated in group 3 ($n = 14$) in which case milk fermented by probiotic lactobacilli was given for 5 consecutive days before and 10 days after challenging the mice with *S*. Typhimurium. The mice in group 4 ($n = 10$) were fed fermented milk for 15 consecutive days and served as a positive control group.

Bacterial strains and growth conditions

The clinical isolate of *S. enterica* serovar Typhimurium was kindly provided by the National Reference Laboratory of Estonia. It was grown on blood agar for 24 h at $37°C$, the colonies were suspended in PBS and adjusted to the appropriate concentration of 10^5 CFU/ml.

The two lactobacillus strains were isolated from the faecal samples of Estonian children (9). These strains were identified as *L. fermentum* and *L. acidophilus* by API 50 CHL kit (bioMérieux, France) and internal transcribed spacer polymerase chain reaction (ITS-PCR) (10). A high antagonistic activity against *S*. Typhimurium (inhibition zone of 13–15 mm) and a high antioxidative capacity (total antioxidative activity (TAA) value was $24 \pm 4\%$) of the *L. fermentum* ME-3 were observed *in vitro* (6). The *L. acidophilus* strain had minimal *in vitro* antagonistic activity (inhibition zone of 0–2 mm) against *S*. Typhimur-

ium and expressed a low grade antioxidativity (TAA value $8 \pm 3\%$). It was added to favour milk fermentation.

Also, before the experiments in mice, the antagonistic activity of *L. fermentum* and *L. acidophilus* against *S*. Typhimurium was tested in ultrapasteurized milk. Different combinations of *L. fermentum, L. acidophilus* (in pairs or single) for suppression of the growth of *S*. Typhimurium were tested. Seeding to de Man-Rogosa-Sharpe (MRS) and bismuth sulphite agar from serial dilutions was performed after co-incubation for 6 and 12 h.

The lactobacilli strains for mouse experiments were cultivated separately in MRS broth (Oxoid) at $37°C$ for 24 h in a 10% CO_2 environment. The strains were added to separate portions of ultrapasteurized milk and fermentation was carried out at $37°C$ for 48 h in a 10% CO_2 environment. The fermented milk was achieved by mixing equal volumes of both lactobacilli (10^8 CFU/ml) and was divided into daily portions for the whole experiment and maintained at $-20°C$ until administration to mice.

Mice

The mice were infected orally with a single 0.5-ml dose of *S*. Typhimurium suspension (10^5 CFU/ml) using a sterile syringe blunt-ended tube. The 0.5 ml portion of the fermented milk (groups 3 and 4) or the sterile PBS solution (group 1) was administered daily likewise. The deaths of mice were registered and all surviving animals were killed by cervical dislocation on the 10th day after administration of *S*. Typhimurium.

The autopsy was performed under sterile conditions using a class II microbiological safety cabinet (Jouan, France). Bacteriological and biochemical investigations were carried out immediately. Heart blood (10 µl) was collected for analysis as well as samples from the liver and terminal ileum. The samples for histological investigation were collected from the ileum, liver and spleen, placed in 10% formaldehyde for fixation and processed further for paraffin embedding.

Bacteriological investigation

Ten µl of blood were seeded into thioglycolate broth (Oxoid) and after 24 h onto bismuth sulphite agar and 5% blood agar to detect *S*. Typhimurium and MRS agar for lactobacilli. The liver samples were weighed, homogenized with sterile glass powder, serially diluted ($10^{-2}–10^{-7}$) in PBS (pH 7.2) and 0.1 ml of each 10-fold dilution was seeded on the aforementioned media. The intestinal contents were serially diluted ($10^{-2}–10^{-9}$) and seeded on bismuth sulphite agar and MRS medium. The incubation was performed at $37°C$ for 24 h either in an aerobic environment (salmonella) or in a 10% CO_2 environment for 48 h (lactobacilli). The particular colonies grown on plates were counted, identified at the genus level and the counts of bacteria were assessed. The detection level of the bacteria

was 2 log CFU/ml for blood, 2 log for liver and 1.7 log CFU/g for intestinal samples, respectively.

Biochemical assays

The samples of mucosa from the ileum were obtained during autopsy and stored at $-80°C$ until homogenization in a 1.15% KCl solution (1:10). All biochemical indices were measured simultaneously.

Total antioxidative activity (TAA) was assessed by applying the lipid peroxidation (LPO) test described elsewhere (6). The TAA was expressed as the inhibition of the peroxidation of the linolenic acid (LA) standard by the sample, measured as a percentage. The high numerical value (%) of TAA indicates the high total antioxidative activity of the sample. Peroxidation of LA standard in isotonic saline served as a control.

LPO (malondialdehyde, MDA plus 4-hydroxynonenal) was measured using a commercial kit (Bioxytech LPO-586; Oxis International, catalogue no. 21012). The assay is based on the reaction of a chromogenic reagent, N-methyl-2-phenylindole, with MDA and hydroxynonenals at $45°C$, yielding a stable chromophore with maximal absorbance at 586 nm. The results were calculated according to the kit formula and the tissue values were given in pmol/mg protein.

Glutathione redox status was tested by measuring total glutathione and oxidized glutathione using the method described by Griffith (11). The glutathione content was quantified by comparison with a standard curve generated using specific amounts of glutathione. The amount of reduced glutathione (GSH) and oxidized glutathione (GSSG) was expressed as µg/ml, and glutathione redox ratio as GSSG/GSH.

A kit (Sigma Diagnostics, procedure no. 565, St Louis, MO, USA) was used for assessment of iron levels and iron-binding capacities. All procedures were performed in triplicate. Iron concentration was calculated using the kit formula. Iron content was expressed as µmol/l. The percentage of saturation of iron-binding capacity (which indicates the percentage of bound iron) was calculated from data measured by using the kit.

Histological investigation

Tissue sections (approx. 5 µm) were stained by haematoxylin and eosin (H&E). Destructive and inflammatory signs, i.e. hyperaemia, necrosis, number of typhoid nodules and hyperplasia of Peyer's patches, were evaluated. Two pathologists using coded slides carried out histopathological evaluation in a blinded manner. The inflammatory changes were graded between 0 and 5, with 0 for no changes and 5 for severe changes (12).

Statistical analysis

The survival rate of the mice from different groups was compared by Fisher's exact test. The group differences in the prevalence and counts of salmonellae and lactobacilli in histopathological and biochemical indices were compared by Mann–Whitney rank sum test. The SigmaStat package for Windows 2.0 (Jandel Corporation, USA) was used. The differences between biochemical indices were calculated using (t-test) statistical software package (Statistics for Windows, StatSoft Inc. and Graph Pad Software, PRISM version 2.0, CA, USA).

RESULTS

Bacteriological studies

In ultrapasteurized milk by co-cultivation of *L. fermentum* ME-3 and *S.* Typhimurium the highest reduction of salmonella count (for 1, 4 log) was assessed after 12 h of co-incubation. *L. fermentum* ME-3 and *L. acidophilus* reduced the salmonella count quite similarly for 1,2 log.

All mice in the control group (group 1) and in the group treated with the probiotic lactobacilli (group 4) survived the experiment. The challenge of mice with *S.* Typhimurium (groups 2 and 3) resulted in development of a generalized infection, as 3 of 30 mice died on the eighth or ninth days. The mortality did not differ significantly (2/16 vs 1/14; $p > 0.05$) in the group challenged with *S.* Typhimurium (group 2) and mice treated with probiotic lactobacilli (group 3). We excluded the indices of dead animals from further analysis of results. The prevalence of salmonellae in the heart blood of animals in group 2 and in group 3 mice did not differ significantly (3/14 vs 3/13, respectively). Trend for reduction of the prevalence of salmonellae in the liver from the group 3 mice was found as compared with the mice of group 2 (8/13 vs 12/14, respectively). In the terminal ileum no significant differences in the prevalence and number of salmonellae were detected between the mice treated and untreated with probiotic lactobacilli (Table I).

Lactobacilli were present in the terminal ileum of all investigated animals. A somewhat increased count and smaller variation in the number of lactobacilli was detected in group 3 mice as compared with the group 2 mice. Unexpectedly, in ileum of mice after 15 days of probiotic lactobacilli administration (group 4) the total count of lactobacilli was reduced ($p < 0.05$) as compared with the control group (group 1).

Morphological studies

In the intestinal mucosa of *S.* Typhimurium-challenged mice (group 2) moderate hyperaemia and hyperplasia of solitary follicles were found (Fig. 1) as compared with group 1 control animals (Table II). Of 14 mice in group 2, multiple typhoid nodules and necrosis were seen in the liver (Fig. 2) and spleen (11 and 9 typhoid nodules and 10 and 9 necroses,

Table I

The prevalence and count of lactobacilli and salmonellae in the ileum of lactobacilli-treated mice with and without Salmonella Typhimurium challenge

Microbes	Group 1 $n = 7$	Group 2[a] $n = 14$	Group 3[a] $n = 13$	Group 4 $n = 10$
Lactobacilli				
Number of animals (%) harbouring	7 (100)	14 (100)	13 (100)	10 (100)
Range/median in log 10 CFU/g	8.7–10.5/9.6*	6.8–10.7/9.2	8.3–10.0/9.3	8.3–9.6/8.8*
Salmonella Typhimurium				
Number of animals (%) harbouring	0	7 (50)	6 (46)	0
Range/median in log 10 CFU/g	0	0–7.1/1.0	0–9.9/0	0

Group 1, control; group 2, *Salmonella*-infected mice; group 3, challenged with *Salmonella* and pre- and post-treated with probiotic lactobacilli; group 4, treated with probiotic lactobacilli.
[a]Two mice from group 2 and one mouse from group 3 were excluded as they did not survive the experiment.
*$p = 0.036$.

respectively). In group 3 mice the hyperplasia of intestinal lymph patches was less expressed than in group 2 animals and no intestinal typhoid nodules were discovered. In group 4 animals the only morphological change in comparison with the controls (group 1) was mild hyperplasia of Peyer's patches in the intestinal mucosa (Fig. 3) and hyperaemia in liver.

Biochemical studies

Both lipid peroxidation and glutathione redox ratio were significantly higher in mice challenged with *S*. Typhimurium (group 2) as compared with the control animals (group 1) and the mice treated only with lactobacilli (group 4)

(Table III). Also, the iron indices were elevated in mice challenged with *S*. Typhimurium (group 2) versus control animals (group 1).

Treatment of the mice with lactobacilli before and during salmonella infection (group 3) had a positive influence on parameters indicative of excessive oxidative stress. A reduction of LPO, iron content, iron-binding protein saturation and an increase in the values of the gut mucosal TAA was found in group 3 as compared with the same indices of salmonella-challenged mice in group 2.

The mice treated solely with lactobacilli showed changes in oxidative stress indices: somewhat elevated LPO and reduced TAA values, yet the smallest glutathione redox ratio as compared to the control group (Table III).
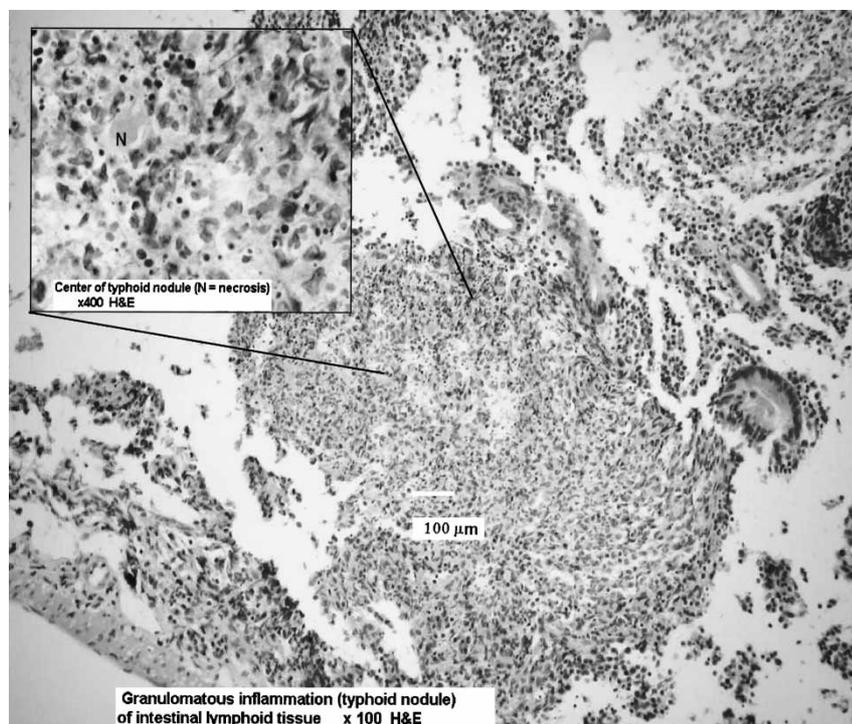

Fig. 1. Histological sample (H&E, ×100) of the terminal ileum of a mouse challenged with *Salmonella* Typhimurium (group 2). Necrotic typhoid nodule, surrounded by mononuclear cell infiltrate in mucosa (inset; ×400). Columnar epithelium is mostly necrotic and detached.

Table II

Histopathological evaluation of intestine and liver from lactobacilli-treated mice with and without Salmonella Typhimurium challenge

| Sample origin	Score values (range/median)			
	Group 1 $n=7$	Group 2 $n=14$	Group 3 $n=13$	Group 4 $n=10$
Intestine				
Hyperaemia	0–1/1[5,6]	1–3/2[5]	1–3/1[6]	0–1/0.5
Hyperplasia of lymph patches	0–1/0[1,5]	1–2/1.5[1,2]	0–1/1[2]	0–2/1[5]
Typhoid nodules	0[5]	0–4/1[5,1,2]	0[1]	0[2]
Liver				
Hyperaemia	0–1/0[1,2]	1–3/2[1]	1–3/2[2,5]	0–3/0.5[5]
Typhoid nodules	0[1,5]	0–5/3[5]	0–4/3[1,6]	0[6]

Group 1, control; group 2, *Salmonella*-infected mice; group 3, challenged with *Salmonella* and pre- and post-treated with probiotic lactobacilli; group 4, treated with probiotic lactobacilli.
[1,2] $p < 0.01$; [5,6] $p < 0.05$.

DISCUSSION

S. Typhimurium-induced enterocolitis, complicated by the generalization of infection, is the most common cause of death from food-borne illnesses (2, 13). Due to emerging antibiotic resistance of salmonellae (1, 14) probiotics have been suggested as an adjunct or alternative to antibacterial therapy of gastro-enteritis to prevent the severe course of salmonellosis. Lactobacilli are widely used as probiotics to enhance colonization resistance and provide protection against salmonella infection (15–18). We have applied an experimental mouse model to study the putative protective effect of antibacterial and antioxidative lactobacilli of human origin (*L. fermentum* and *L. acidophilus*) against

invasion by *S.* Typhimurium. *L. fermentum* ME-3 produces substantial amounts of lactic acid and ethanol, with smaller amounts of acetic and succinic acids (19) and relatively low amounts of hydroxygen peroxide (6). Our previous *in vitro* studies have shown good suppressive activity of the strain *L. fermentum* ME-3 against *S.* Typhimurium (6). This was also confirmed by co-cultivation experiments in ultrapasteurized milk. Under our experimental conditions the orally inoculated clinical strain of *S.* Typhimurium disseminated in the blood and was detected in different organs of mice by the 10th day of infection, yet the intestinal mucosa was only modestly damaged. The morphological changes in various organs of mice, particularly the presence of multiple

100 µm

Typhoid nodule in liver. x400, H&E
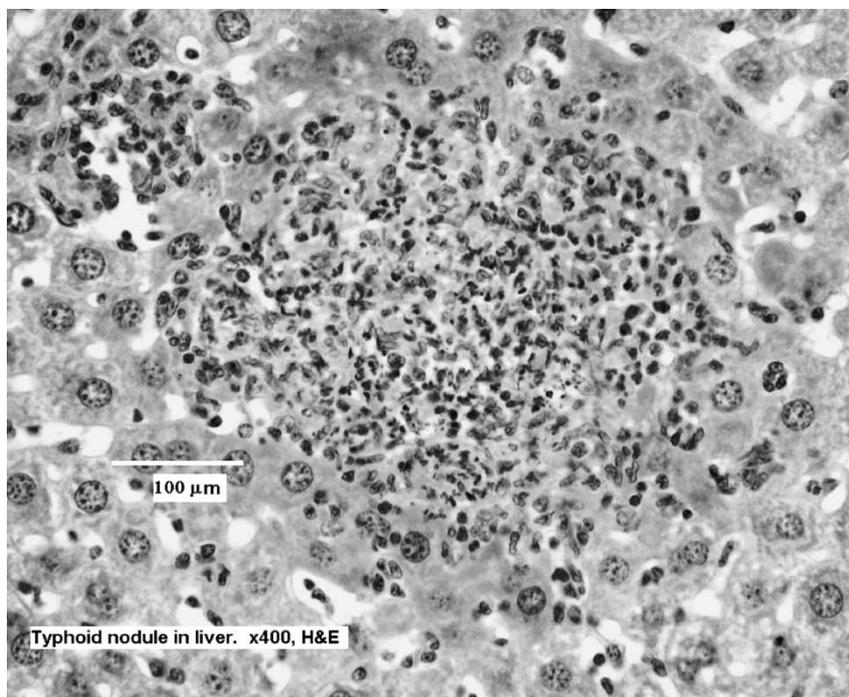
Fig. 2. Histological sample (H&E, ×400) of the liver from a mouse challenged with *Salmonella* Typhimurium (group 2). Typhoid nodule.
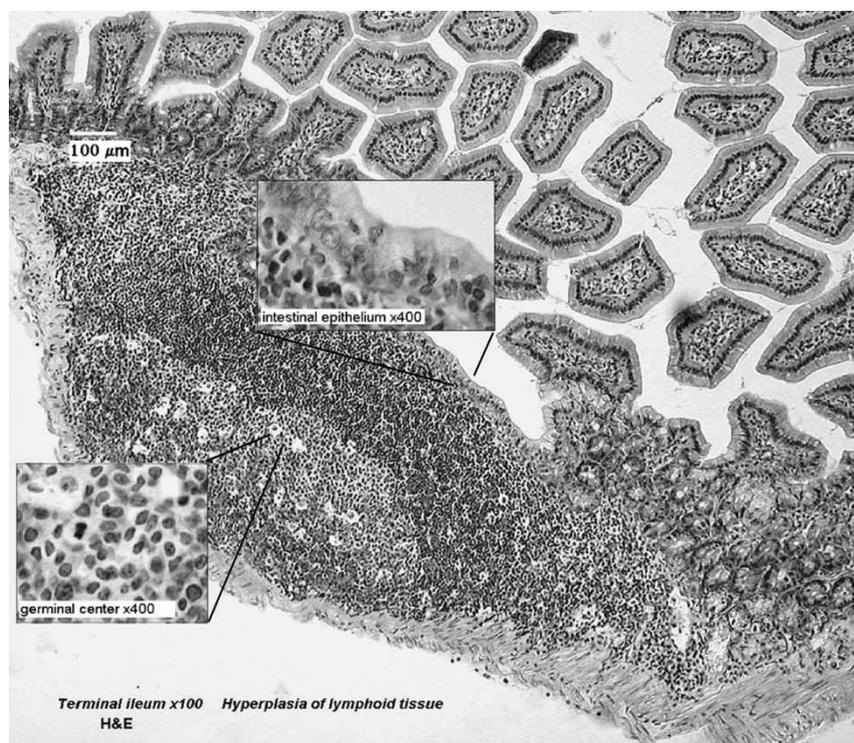
Fig. 3. Histological sample (H&E, ×100) of the terminal ileum of a mouse challenged with probiotic lactobacilli (group 4). Hyperplasia of solitary lymphatic follicule with well pronounced germinal centre can be seen (×400). Mucosa with intestinal villi is preserved and intact (×400).

typhoid nodules in the liver and spleen, were not similar to severe enterocolitis, but largely resembled the lesions of typhoid fever patients, as pointed out by some other authors (10, 20).

The 15-day administration of lactobacillus strains did not reduce significantly the early mortality of mice challenged with *S*. Typhimurium (2/16 in group 2 vs 1/14 in group 3). Moreover, our finding is in contrast with several experimental studies showing the protective effect of different probiotic lactobacilli (*L. casei*, *L. reuteri*, *L. rhamnosus* GG, *L. acidophilus*) against *S*. Typhimurium infection in mice (15–17). Most of these studies explain the positive effect by enhancement of colonization resistance, due to competitive exclusion of salmonellas from the gut lumen via various mechanisms, relying on the anti-adhesive or antagonistic activity of probiotic strains. Hudault et al. (17),

investigating *S*. Typhimurium and *L. casei* GG in a mouse model, suggested that in lactobacilli-treated animals the presence of antimicrobial substances or lactic acid decreased the number of *S*. Typhimurium. In contrast, Silva et al. (18) found that the protection against *S*. Typhimurium in mice was not due to the reduction of the intestinal populations of the pathogenic bacteria after treatment with bifidobacteria.

In our study the absence of a clear protective effect of probiotic lactobacilli against the generalization of *S*. Typhimurium infection could be explained as follows. First, the administration of probiotic lactobacilli of human origin over a 15-day period did not result in an increase in the total number of lactobacilli in the mouse gut, as the introduction of a new strain originating from different host species does not allow its rapid colonization. In a further

Table III

Oxidative stress-related indices in the gut mucosa of lactobacilli-treated mice with and without Salmonella Typhimurium challenge

Indices	Group 1 $n=7$	Group 2 $n=14$	Group 3 $n=13$	Group 4 $n=10$
LPO (pmol/mg)	$109\pm6.7^{1/2/3}$	$297\pm58^{1/4/5}$	$224\pm46^{2/5}$	$157\pm41^{3/4}$
TAA (%)	$38\pm0.5^{1/2}$	41 ± 12^{5}	$51\pm5^{1/\ 3/5}$	$30\pm2^{2/3}$
GSSG/GSH	$0.12\pm0.02^{1/2}$	0.44 ± 0.375^{1}	$0.34\pm0.15^{2/3}$	0.1 ± 0.03^{3}
Fe (µmol/l)	10 ± 6^{5}	$23\pm12^{5/6}$	15 ± 10^{6}	11 ± 5
Saturation with Fe (%)	15 ± 6^{5}	$29\pm14^{5/6}$	20 ± 10^{6}	18 ± 8

Group 1, control; group 2, *Salmonella*-infected mice; group 3, challenged with *Salmonella* and pre- and post-treated with probiotic lactobacilli; group 4, treated with probiotic lactobacilli.
[1;2;3;4] $p<0.01$; [5;6] $p<0.05$.

mouse experiment the increase of *L. fermentum* ME-3 count was detected only after longer administration, i.e. 28 days (unpublished data). In contrast, during our previous human volunteer trial the consumption of goat milk fermented by *L. fermentum* ME-3 resulted in successful passage of living lactobacilli and an increase of faecal lactobacilli counts in all investigated persons (21). Wong et al. (22) found that in mice the activation of the applied probiotic strains is reduced as microbes of human origin produced only 25% of the expected organic acids. *In vitro* the suppression of salmonellas by lactobacilli is largely dependent on the production of lactic and acetic acid (19).

Second, it is possible that due to rapid invasion of the clinical *S.* Typhimurium isolate the antimicrobial substances produced by the probiotic lactobacilli did not suppress salmonellas in our mouse model. It has been shown previously that just a few hours after inoculation the highly virulent invasive *S.* Typhimurium enters the membranous epithelial cells (M cells) overlying lymphoid follicles, e.g. Peyer's patches in the terminal ileum (2). Up to now, only some cell culture (23, 24) and germ-free animal studies (25) have shown the ability of defined lactobacilli to suppress the invasive pathogens. Therefore, we suggest that the *Lactobacillus* sp. is probably not conferring the colonization resistance of gut against the invasion of *S.* Typhimurium infection.

However, the activation of the adaptive immunological mechanisms by the non-indigenous lactobacilli could be suggested. In our study the hyperplasia of the lymph patches of the small intestine after administration of probiotic lactobacilli to mice (both in salmonella-challenged and lactobacilli-treated animals) indicates the activation of the adaptive immunological mechanisms by the non-indigenous lactobacilli. In some other studies (16–18) the enhancement of the appropriate immunological response has been suggested as one of the mechanisms for the reduction of late mortality (at 14 days) of mice treated with particular probiotic bacteria in an *S.* Typhimurium infection model. At late stages of salmonella infection increased macrophage phagocytotic activity has been noticed (26). The defensive function of macrophages was not impaired by antioxidative lactobacilli, as in liver and spleen the potential of macrophages to form typhoid granulomas for localization of infection persisted in mice also treated with probiotic lactobacilli. However, further studies are required to investigate the possible immune activation by *L. fermentum* ME-3 and its role against mouse typhoid following application of the probiotic strain for prolonged periods, i.e. more than 10 days.

Despite the failure to suppress *S.* Typhimurium infection, the biochemical assays showed that treatment with *L. fermentum* ME-3 with high antioxidative properties suppressed the excessive oxidative stress reactions that are harmful for intestinal epithelial cells. Reduction of high-grade oxidative stress indices (lipid peroxidation, glutathione redox ratio) and an increase of TAA in the gut mucosa of mice challenged with *S.* Typhimurium and treated with probiotic milk were documented. Similarly to human cells, lactobacilli have a mechanism for elimination of excess oxygen radicals and hydrogen peroxide by superoxide dismutase and/or by the glutathione system (27). The lowest glutathione redox ratio as well as the lowest TAA observed in the group of mice treated solely with the probiotic lactobacilli as compared with control animals may be explained by the beneficial influence of *L. fermentum* ME-3.

We conclude that the administration of probiotic lactobacilli of human origin did not increase the colonization resistance against *S.* Typhimurium infection in mouse gut and prevent the generalization of the infection. However, we have demonstrated the ability of specific probiotic lactobacilli to reduce pro-oxidant levels and oxidative stress indices, thus improving gut mucosal antioxidative activity. The possibility of influencing the pro- and antioxidant balance by specific probiotic lactobacilli with antioxidative properties in the course of inflammatory tissue damage could be further explored.

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