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Immunological, antioxidative, and morphological response in combined treatment of ofloxacin and *Lactobacillus fermentum* ME-3 probiotic in *Salmonella* Typhimurium murine model

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We aimed to elucidate the immunological (cytokines), biochemical (antioxidative), and patho-morphological responses in the gut and liver evoked by the addition of *Lactobacillus fermentum* ME-3 to ofloxacin (OFX) treatment in an experimental infection model of *Salmonella enterica* serovar Typhimurium. After challenge with *S*. Typhimurium and treatment according to different schemes, either with OFX and/or addition of *L. fermentum* ME-3, the mice were killed. Blood, liver, spleen, and small intestine samples were plated to detect *S*. Typhimurium and lactobacilli. Histological slides were prepared from the liver and ileum. The cytokines (IL-10, IFN-γ, and TNF-α), the glutathione peroxidase and reductase, the glutathione ratio, and the lipid peroxides (LPO) in mucosa of the small intestine and liver were estimated. The addition of *L. fermentum* ME-3 to OFX increased the eradication of *S*. Typhimurium from tested sites because of antagonistic and antioxidative properties, reduced the presence of typhoid nodules in the liver, and decreased the values of LPO. The immunological response included the reduction of pro-inflammatory cytokines interferon-γ and tumour necrosis factor-α and the increase in anti-inflammatory cytokine interleukin-10 in the livers of mice without typhoid nodules.

Key words: *Salmonella* Typhimurium; murine model; *Lactobacillus fermentum* ME-3; immunological; biochemical response.

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Salmonella enterica serovar Typhimurium causes systemic infection in an experimental murine model resembling human typhoid fever with bacteraemia and extra-intestinal granulomatous lesions, e.g. typhoid nodules (1–4). Recently, it has been reported that gut inflammation caused by *S*. Typhimurium could change the microbiota composition, disrupt the colonization resistance, and also enhance the multiplication of pathogens (5). In our previous study of experimental *S*. Typhimurium infection, the applied probiotic *Lactobacillus fermentum* ME-3, DSM 14241, as an adjunct to fluoroquinolone treatment increased the eradication of *S*. Typhimurium from the gut and prevented the development of typhoid nodules in

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the liver and spleen (6). Moreover, the oxidative stress-indicative biochemical status was reduced as a result of the antioxidative defence provided by the probiotic administration.

Probiotics are generally defined as live microorganisms which, when consumed in appropriate amounts in the food, confer a health benefit on the host (7). Besides temporal colonization, probiotic strains affect the host by increasing the count of commensal lactic acid bacteria in the gut, granting the competitive exclusion of pathogens (8–10). Probiotics have the potential to modulate the immune response through different receptors on dendritic cells of intestinal mucosa, performing a link between innate and adaptive immune responses (11, 12). Different probiotic bacteria induce strain-specific production of pro- or anti-inflammatory cytokines (13, 14). Diaz-Ropero et al. showed different modulation of cytokines in rodent bone marrow-derived macrophages by two *Lactobacilli* strains isolated from breast milk; that is, *L. fermentum* CECT5716 induced pro-inflammatory cytokines and, in contrast, *Lactobacillus salivarius* CECT5713 activated the production of interleukin (IL)-10 (15). Marcinkiewicz et al. demonstrated in peritoneal mouse macrophages that both *Lactobacillus reuteri* and *Lactobacillus johnsoni* induced the production of anti-inflammatory IL-10 (16). However, the impact of the treatment with the combination of probiotics and antimicrobials on the pro- and anti-inflammatory cytokine status of host during infection has not yet been elucidated.

The aim of the study was to assess the immunological (cytokines), biochemical (antioxidative), and patho-morphological responses in the gut and liver evoked by the addition of *L. fermentum* ME-3 to ofloxacin (OFX) treatment in an experimental infection model of *S. enterica* serovar Typhimurium.

MATERIALS AND METHODS

Bacterial strains and growth conditions

Salmonella Typhimurium is a clinical isolate that was kindly provided by the Estonian Laboratory of Public Health Inspectorate. After incubation in ambient air at 37 °C for 24 h on Xylose-Lysine-Deoxycholate (XLD) media (Oxoid, Basingstoke, Hampshire, England), the suspension in phosphate-buffered saline (PBS) was adjusted according to the McFarland standard to the density of 10^5 cfu/mL.

The antioxidative and antimicrobial *L. fermentum* ME-3 (DSM14241) is of human origin (17–20). The *L. fermentum* ME-3 strain was cultured on the De Man Rogosa and Sharp (MRS) and incubated in microaerobic conditions in 10% CO_2 at 37 °C for 48 h. The minimum inhibitory concentration (MIC) values of OFX to *S.* Typhimurium and *L. fermentum* ME-3 were 0.19 and 8 μg/mL, respectively. When *S.* Typhimurium and *L. fermentum* ME-3 were tested together, a sixfold decrease in the MIC of OFX from 0.19 to 0.032 μg/mL was observed using both overlay and broth dilution methods (6).

Experimental study

Altogether, 54 NIH 6-week-old male mice were distributed into five experimental groups with twelve mice in each group, except the control group with six animals. Experimental *S.* Typhimurium infection (Gr1, 2, and 3) was induced by a single dose of *S.* Typhimurium (10^5 cfu/mL) intragastrically as described previously (6). To the mice of Gr2, OFX (Hoechst, Germany; 20 mg/kg) (21) was administered intragastrically for 8 days starting 48 h after inoculation with *S.* Typhimurium.

Mice of Gr3 were administered combined treatment of OFX intragastrically and *L. fermentum* ME-3 in 0.5 mL (10^8 cfu/mL) of drinking water. The uninfected mice of Gr4 were administered the same dose of *L. fermentum* ME-3, while Gr5 served as a control with the administration of PBS. The six mice from groups 1–4 were killed on day 5 and six more on day 10 by cervical dislocation. The spleen, liver, and small intestine were removed aseptically.

Microbiological studies

Ten microlitres of heart blood, homogenized tissue of liver, and spleen were plated onto XLD (Oxoid) to detect *S.* Typhimurium and MRS agar (Oxoid) for lactobacilli. The colonies were counted and microorganisms were identified after 24 h of incubation in aerobic (XLD plates) environment and 48 h in 10% CO_2 environment (MRS plates). The identification of lactobacilli was based on colony morphology and biochemical properties (6).

Histological studies

Sections from samples of the liver, spleen, and ileum were fixed with formaldehyde, embedded into paraffin, and stained with haematoxylin–eosin. The pathologist, in a blinded manner using coded slides, estimated alterative and inflammatory changes. The focal collections of inflammatory cells with various degree of

necrosis were defined as typhoid nodules (22). The degree of necrosis was scored on a scale ranging from 1 to 3 (1 – weak, 2 – moderate, and 3 – strong).

Biochemistry and immunology

The liver and mucosa of the small intestine were removed aseptically during autopsy, homogenized in a 1.15% KCl solution (1:10), and the amounts of glutathione (GSSG/GSH ratio) and lipid peroxides (LPO) were tested as described previously (6).

For measuring concentrations of cytokines, interferon (IFN)-γ, IL-10, tumour necrosis factor (TNF)-α from the mucosa of the small intestine and liver, the corresponding cytokine Mouse Immunoassay (R&D Systems, Inc., Minneapolis, MN, USA), based on quantitative sandwich enzyme immunoassay technique, was applied in accordance with the manufacturer's instructions.

Immunohistochemistry for detection of glutathione reductase and glutathione peroxidase in the liver and small intestine

Four-millimetre-thick paraffin sections mounted on SuperFrost slides (Milan, Columbus, OH, USA) were deparaffinized and rehydrated. Peroxidase activity was removed by 0.6% hydrogen peroxide in methanol. Sections were treated with normal 1.5% goat serum for 30 min at room temperature before incubation of the first antibody with glutathione reductase or glutathione peroxidase (Biogenesis, Poole UK; diluted 1:200) for 1 h at room temperature. After washing in PBS, sections were incubated with secondary antibody and VECTASTAIN ABC Reagent (VECTASTAIN; ImmunoVision Technologies, Co, Daly City, VA, USA) for 30 min at room temperature. The sections were incubated in diaminobutynuc acid (DAB) (Vector Laboratories, Burlingance, CA, USA) for 5 min at room temperature. The nuclei of the cells were counterstained with haematoxylin and dehydrated through graded ethanol, cleared in xylene, and mounted with Eukitt (Fluka, Buchs, Switzerland). The glutathione reductase and peroxidase staining was expressed on a subjective scale ranging from 0 to 3 (0 – no staining, 1 – weak, 2 – moderate, and 3 – strong).

Statistical analysis

Statistical analysis was performed using R 2.6.2 (a Language and Environment; http://www.r-project.org) and the statistical program Sigma Stat for Windows 2.0 (Jandel Corporation, San Rafael, CA, USA). The prevalence of liver typhoid nodules was compared using the Fisher's exact test in *S.* Typhimurium-challenged groups. For comparing the differences in continuous variables, the Student's *t*-test or Mann–Whitney test – and in *Salmonella*-challenged groups, the analysis of variance (ANOVA) with Bonferroni correction test – were applied. A linear logistic regression model was applied to find the relationship between the presence of typhoid nodules and values of cytokines in the small intestine and liver, and to calculate odds ratios (ORs) between the presence of typhoid nodules in the liver of *S.* Typhimurium-challenged groups and the administration of *L. fermentum* ME-3.

RESULTS

Detection of viable *Salmonella* Typhimurium and typhoid nodules in infected and treated animals (Table 1)

All infected animals survived. The viable *S.* Typhimurium was found in more than half (7/12; 58%) of any tested site of infected mice

Table 1. Detection of viable *Salmonella* Typhimurium in the tested organs and typhoid nodules in the liver of infected mice on days 5 and 10

Experimental group	Number of mice with											
	Viable *S.* Typhimurium in									Typhoid nodules in the liver		
	Blood		Liver		Spleen		Gut		Total in any tested site	D5	D10	Total (%)
	D5	D10	D5	D10	D5	D10	D5	D10				
Gr1 (ST), n = 12	1	0	0	0	0	3	2	4	7[1] (58%)	2	6	8[2] (67%)
Gr2 (ST + OFX), n = 12	0	1	0	1	0	1	3	1	4 (33%)	3	5	8[2] (67%)
Gr3 (ST + OFX + ME-3), n = 12	0	0	0	0	0	0	1	0	1[1] (8%)	0	2	2[2] (17%)

[1]p = 0.014; significant differences between Gr1 and Gr3.
[2]p = 0.038; significant differences between Gr1 and Gr2 vs G3.
D, day of autopsy; OFX, ofloxacin.

from Gr1, accompanied with bacteraemia in one mouse. OFX (Gr2) treatment reduced the number of mice with viable salmonellas to 4/12 and the combined treatment (Gr3) to 1/12 ($p = 0.014$ Gr3 vs Gr1).

Typhoid nodules in the liver were found equally in 8/12 of mice in both Gr1 and Gr2, while the addition of *L. fermentum* ME-3 to OFX reduced the prevalence of mice with typhoid nodules to 2/12 ($p = 0.038$ Gr2 vs Gr3).

After administration of *L. fermentum* ME-3 to *S.* Typhimurium-infected and OFX-treated mice (Gr3), a reduction in the number of typhoid nodules in the liver was observed [OR: 0.08; 95% confidence interval (CI): 0.02–0.40; $p = 0.002$]. The relationship remained significant even after adjusting for OFX administration (OR: 0.06; 95% CI: 0.01–0.33; $p = 0.0014$).

Profile of cytokines in the small intestine and liver on days 5 and 10 of *Lactobacillus fermentum* ME-3-administered mice compared with control group

In non-infected mice, the administration of *L. fermentum* ME-3 (Gr4) reduced the amount of the pro-inflammatory cytokine TNF-α in the small intestine (23.3 pg/mg tissue; 20–100) and in the liver (46.8 pg/mg tissue; 7.4–41) on day 5 ($p = 0.015$ and 0.002) and increased the anti-inflammatory cytokine IL-10 ($p = 0.004$ and 0.004, respectively) compared with control group mice in the small intestine (127 pg/mg tissue; 892–180) on day 5 and in the liver on day 5 (252 pg/mg tissue; 212–325) and day 10 (224 pg/mg tissue; 164–324; Fig. 1A,B). In the liver, the high values of IL-10 remained until day 10 ($p = 0.014$), whereas in the small intestine, it progressively decreased at day 10. The increase in IFN-γ was detected on days 5 and 10 ($p = 0.001$ and $p < 0.001$) in the liver, although no changes were found in the gut.

Profile of cytokines in the small intestine and liver on days 5 and 10 of *Salmonella* Typhimurium-challenged mice

After inoculation with *S.* Typhimurium (Gr1), the values of the pro-inflammatory cytokines IFN-γ and TNF-α in the small intestine were the highest when compared with the tested groups (Table 2).

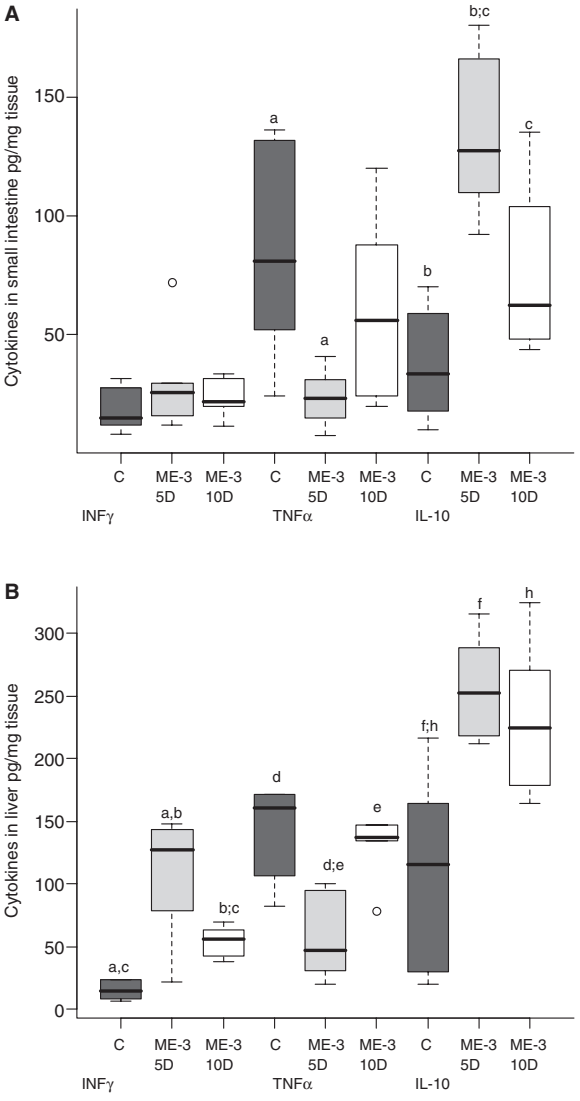

Fig. 1. Production of cytokines (medians and ranges) in the small intestine (A – a: $p = 0.015$; b: $p = 0.004$; c: $p = 0.017$) and in the liver (B – a: $p = 0.001$; b: $p = 0.026$; c: $p < 0.001$; d: $p = 0.002$; e: $p = 0.002$; f: $p = 0.004$; g: $p = 0.014$) on day 5 (D5) and day 10 (D10) in the control group Gr5 (C) and *Lactobacillus fermentum* ME-3-administered Gr4 (ME-3).

To find the impact of the combined treatment on the profile of cytokines, the Gr3 was set as a reference to all *S.* Typhimurium-challenged groups (Table 2). The addition of *L. fermentum* to OFX treatment reduced the values of the tested pro-inflammatory cytokines: IFN-γ in the liver on both days ($p = 0.04$ and $p = 0.002$, respectively), and TNF-α on both days ($p = 0.03$ and 0.04 in the liver; $p = 0.02$ and

Table 2. Production of cytokines (median; range) in the small intestine and liver on days 5 and 10 in *Salmonella* Typhimurium-challenged mice

Group	Day of autopsy	IFN-γ (pg/mg tissue)		TNF-α (pg/mg tissue)		IL-10 (pg/mg tissue)	
		Small intestine	Liver	Small intestine	Liver	Small intestine	Liver
Gr1 (ST), n = 6	5	73 (56–78)	157 (88–298)[1]	83 (27–148)[1]	130 (95–272)[1]	81 (72–126)	129 (66–202)[1]
	10	78 (68–101)[2]	124 (67–238)[2]	156 (136–198)[2]	161 (106–194)[2]	74 (32–181)	165 (144–224)[2]
Gr2 (ST + OFX), n = 6	5	56 (16–86)	224 (42–533)[1]	52.5 (15–116)	127 (42–196)[1]	95 (59–121)	192 (111–210)
	10	55 (31–70)	92 (8–377)	69 (32–166)	120 (74–256)	82 (1–108)	235 (122–300)
Gr3 (ST + OFX + ME-3), n = 6	5	53 (36–86)	60 (22–129)[1]	33.5 (13–53)[1]	52 (1–159)[1]	89 (72–140)	223 (126–315)[1]
	10	46 (30–69)[2]	19 (2–24)[2]	39 (28–200)[2]	120 (78–138)[2]	68 (28–116)	265 (160–422)[2]

[1]Significant differences ($p < 0.05$) between Gr3 (ST + OFX + ME-3) and test groups on day 5.
[2]Significant differences ($p < 0.05$) between Gr3 (ST + OFX + ME-3) and test groups on day 10.
D, day of autopsy; OFX, ofloxacin; IFN, interferon; TNF, tumour necrosis factor; IL, interleukin.

0.004 in the small intestine) when compared with Gr1. The values of anti-inflammatory IL-10 in the liver increased on both experimental days when compared with *S*. Typhimurium-challenged Gr1 ($p = 0.01$ and 0.005, respectively).

The additive effect of the probiotic to OFX was confirmed by the decrease in the values of IFN-γ and TNF-α on day 5 in the liver [224 pg/mg tissue (42–533) vs 60 pg/mg tissue (22–129) $p = 0.0007$, and 127 (42–196) vs 52 (11–159) $p = 0.02$, respectively] when compared with Gr2.

The presence of typhoid nodules in the liver (Table 3) was associated with high values of pro-inflammatory IFN-γ in the liver on both tested days ($p = 0.002$ and $p = 0.039$, respectively). The absence of typhoid nodules was associated with high IL-10 values in the liver on day 10 ($p = 0.001$). Moreover, the degree of necrosis of typhoid nodules in the liver was associated with the increase in TNF-α in the small intestine ($R^2 = 0.18$, $p = 0.002$) in *S*. Typhimurium-challenged mice (data not shown).

Furthermore, the total number of lactobacilli in the small intestine of *L. fermentum* ME-3-administered groups (Gr3 and Gr4) was negatively correlated ($r = -0.422$; $p = 0.039$) with the values of IFN-γ and positively correlated with IL-10 ($r = 0.551$; $p = 0.005$).

Biochemical indices of oxidative stress in the gut and liver

With respect to the indices of glutathione reductase and peroxidase, no statistically significant differences between the groups were found (data not shown). In the liver (Fig. 2), the addition of *L. fermentum* ME-3 to the OFX treatment (Gr3) reduced the high LPO values when compared with Gr2 mice ($p = 0.004$). The ratio of glutathione GSSG/GSH was reduced in the small intestine when compared with *S*. Typhimurium-challenged mice ($p = 0.046$; Fig. 3).

DISCUSSION

In the present study, the impact of the combined treatment of *S*. Typhimurium infection with an antimicrobial preparation and a probiotic was elucidated using in parallel bacteriological, biochemical, morphological, and immunological approaches. The administration of the probiotic strain *L. fermentum* ME-3 as an adjunct to

Table 3. The median values and ranges of cytokines (pg/mg tissue) in the liver in the presence/absence of liver typhoid nodules on day 5 and day 10 in *Salmonella* Typhimurium-challenged mice

Indices	Typhoid nodules		No typhoid nodules		p-values
	D5 (n = 5)	D10 (n = 13)	D5 (n = 13)	D10 (n = 5)	
Interferon-γ	272 (223–533)*	113 (8–377)**	113 (22–30)*	26 (2–63)**	0.002* 0.039**
TNF-α	138 (95–196)	74 (10–256)	116 (10–272)	126 (80–134)	NS
Interleukin-10	113 (108–210)	178 (22–300)*	198 (66–315)	292 (238–422)*	0.001*

* p-values between different indices.

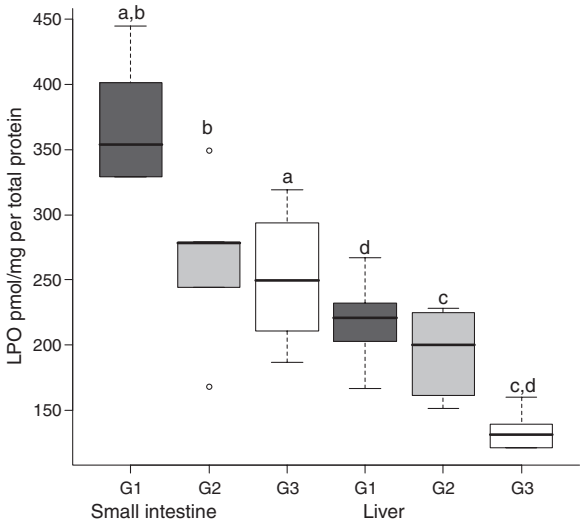

Fig. 2. The values (mean ± standard deviations) of lipid peroxides in the small intestine and liver of *Salmonella* Typhimurium-challenged mice groups (Gr1–Gr3). a: $p = 0.002$; b: $p = 0.007$; c: $p = 0.004$; d: $p < 0.001$. G1, *S*. Typhimurium-challenged mice; G2, *S*. Typhimurium + ofloxacin; G3, *S*. Typhimurium + ofloxacin + *Lactobacillus fermentum* ME-3.

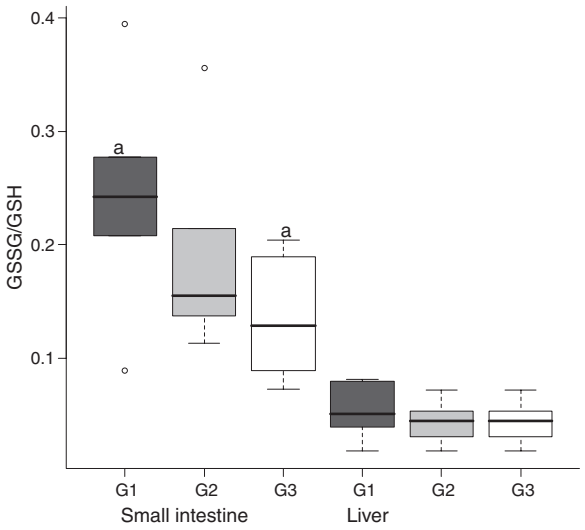

Fig. 3. The values (mean ± standard deviations) of redox ratio GSSG/GSH in the small intestine and liver of *Salmonella* Typhimurium-challenged groups. a: $p = 0.046$. G1, *S*. Typhimurium-challenged mice. G2, *S*. Typhimurium + ofloxacin; G3, *S*. Typhimurium + ofloxacin + *Lactobacillus fermentum* ME-3.

OFX treatment increased the eradication of viable *S*. Typhimurium and reduced the number of mice with typhoid nodules in the liver, important for avoiding persistent infection. The mechanisms of action involve the antagonistic activity of *L. fermentum* ME-3 against *S*. Typhimurium in the gut accompanied by the antioxidative and immuno-modulatory effects in the small intestine and liver.

It is widely accepted that the intestinal microbiota are important in maintaining the health of the host and possess immune-modulating capacities. The activated gut immune system in response to food allergens, pathogens, and normal microbiota leads to physiological inflammation (23). The immune-modulating capacities of probiotics are considered strain-specific (24). The probiotic *L. fermentum* ME-3 demonstrated the ability to induce the production of IL-10 and to reduce TNF-α in the small intestine and liver of uninfected mice. Still, the range of values of both pro- and anti-inflammatory cytokines was large, seemingly because of the variety of intestinal microbiota and health status of the particular host.

The mouse model of *S*. Typhimurium has shown good results for studies of human typhoid fever because of a similar pathogenesis of the disease (4). Mice could be inoculated either by intravenous injection or by an oral route with gastric gavage. Usually the generalized infection of *S*. Typhimurium develops by affecting the liver with the formation of typhoid nodules, yet meningitis has been also described (25). We applied the milder infection model with oral sublethal doses of *S*. Typhimurium, aiming to achieve a persistent granulomatous infection and study the particular responses to treatment with OFX and the probiotic *L. fermentum* ME-3.

In the initial stage of inflammation, because of the lipopolysaccharide of the cell wall of a Gram-negative pathogen, the pro-inflammatory pathways and cytokines (IFN-γ, IL-1, IL-6, IL-12, and IL-18) are activated (26). When *Salmonella* invades the host, IFN-γ secreted from NK cells activates macrophages. The persistence of the infection due to *S. enterica* serovar Typhimurium is granted with the presence of both IFN-γ and TNF-α, shown in BALB/c mice (27). Similarly, our data depicted the increased values of the pro-inflammatory cytokine IFN-γ in the small intestine and in the liver during the first days of infection and persistence until day 10. Moreover, in the liver, the high values of

IFN-γ correlated with the presence of typhoid nodules of *S.* Typhimurium-infected mice.

In the present study, the values of TNF-α in the gut increased after infection, seemingly reflecting the inflammatory damage of intestinal mucosa in the small intestine enabling the invasion of *S.* Typhimurium into the organs. The same was demonstrated by the high values of lipid peroxides and increased glutathione ratio in the small intestine. To date, the release of reactive oxygen species and disruption of the total antioxidant response pathways have been closely associated with the expression of pro-inflammatory cytokines (28). Hence, our morphological results showed the tight correlation between the degree of necrosis of liver typhoid nodules and high values of TNF-α in the small intestine.

Our data concerning IL-10 are conflicting with the results of Sashinami (27), who suggested that the increased amounts of IL-10 were involved in the maintenance of pathogen growth by controlling IFN-γ and TNF-α production during infection. The administration of the probiotic strain *L. fermentum* ME-3 as an adjunct to OFX treatment improved the eradication of viable *S.* Typhimurium and decreased the number of mice with liver typhoid nodules. When we divided the results of IL-10 according to the presence of typhoid nodules in the liver, the increased values of IL-10 were detected in mice without typhoid nodules. This was accompanied by the lower values of IFN-γ and TNF-α in the liver at the initial stage of infection when compared with the group of *S.* Typhimurium-infected and OFX-treated mice.

The possible mechanisms of the effect of probiotic *L. fermentum* ME-3 against *S.* Typhimurium infection include antagonistic and antioxidative activities and modulation of the immune response. First, the antagonistic activity of the probiotic *L. fermentum* ME-3 of human origin against *S.* Typhimurium has been proved previously *in vitro* and in the experimental model (18, 19, 29, 30). In this study, the probiotic–antibiotic combined treatment eradicated *S.* Typhimurium from the gut, except in a single sample.

Second, the increased antioxidative response shown by the reduction of LPO and glutathione ratio values strengthened the intestinal barrier of gut mucosa and seemingly prevented the invasion of surviving *S.* Typhimurium into the organs. Apparently, this is because of the complete glutathione system: synthesis, uptake, and redox turnover ability of *L. fermentum* ME-3 and expressed manganese superoxide dismutase. The latter enables the reduction of inflammation by inhibiting the neutrophil recruitment (20).

Third, in the infection process, the main immunological response of *L. fermentum* ME-3 was the induction of IL-10 and suppression of the pro-inflammatory cytokines. It is well known that in infection caused by intracellular pathogens such as *Mycobacterium tuberculosis*, the maintenance of the granulomatous lesions, e.g. tubercules, has been accompanied with increased values of TNF-α. (31). We suppose that the regulatory function of IL-10 was involved in the avoidance of overreaction by pro-inflammatory cytokines included into the formation of granulomatous lesions such as typhoid nodules.

The limitation of our study is that we did not assess the strain-specific counts of the probiotic *L. fermentum* ME-3 in the gut. By applying the logistic regression model, we have confirmed that the addition of *L. fermentum* ME-3 to OFX treatment could predict the absence or low number of typhoid nodules in *S.* Typhimurium-infected mice, and the relationship stayed significant even after adjusting for OFX administration. Moreover, the total number of lactobacilli in the small intestine of *L. fermentum* ME-3-administered groups was positively correlated with the values of IL-10 and negatively correlated with those of IFN-γ. The cytokine IL-10 is generally considered an anti-inflammatory regulatory cytokine that acts mostly on antigen-presenting cells by inhibiting antigen presentation and production of inflammatory cytokines.

Dogi et al. (32) have shown that a large range of Gram-positive bacteria – both probiotic and non-probiotic strains – increased the number of Toll-like receptors (TLR2) carrying cells that produced the regulatory cytokine IL-10 in the gut. The induction of IL-10 by *L. fermentum* ME-3 could be associated with specific sugars (D-Gal, DGalNAc) on the glycocalyx recognized by Bandeirarea simplifolica I lectin (33). Smits et al. (14) have shown that some selected probiotic bacteria are able to activate IL-10 by binding to the C-type lectin pattern recognition receptor on dendritic cells.

Thus, the probiotic *L. fermentum* ME-3 combined with OFX enhances the eradication of the experimental infection because of the antagonistic and antioxidative activities of the probiotic. The immunological response includes the reduction in pro-inflammatory cytokines IFN-γ and TNF-α and the increase in anti-inflammatory cytokine IL-10 in the liver of mice without typhoid nodules.

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