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RAPID COMMUNICATION

Lactobacilli inhibit interleukin-8 production induced by *Helicobacter pylori* lipopolysaccharide-activated Toll-like receptor 4

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Abstract

AIM: To investigate the effect of *Lactobacillus bulgaricus* (LBG) on the Toll-like receptor 4 (TLR4) pathway and interleukin-8 (IL-8) production in SGC-7901 cells treated with *Helicobacter pylori* Sydney strain 1 lipopolysaccharide (*H pylori* SS1-LPS).

METHODS: SGC-7901 cells were treated with *H pylori* SS1-LPS in the presence or absence of pretreatment for 1 h with viable LBG or supernatant recovered from LBG culture MRS broth (LBG-$_S$). Cellular lysates were prepared for Western blot with anti-TLR4, anti-transforming growth factor β-activated kinase 1 (TAK1), anti-phospho-TAK1, anti-nuclear factor κB (NF-κB), anti-p38 mitogen-activated protein kinase (p38MAPK), and anti-phospho-p38MAPK antibodies. The amount of IL-8 in cell culture medium was measured by ELISA.

RESULTS: *H pylori* SS1-LPS up-regulated the expression of TLR4, stimulated the phosphorylation of TAK1, subsequently enhanced the activation of NF-κB and the phosphorylation of p38MAPK in a time-dependent manner, leading to augmentation of IL-8 production in SGC-7901 cells. Viable LBG or LBG-$_S$ pretreatment attenuated the expression of TLR4, inhibited the phosphorylation of TAK1 and p38MAPK, prevented the activation of NF-κB, and consequently blocked IL-8 production.

CONCLUSION: *H pylori* SS1-LPS induces IL-8 production through activating TLR4 signaling in SGC-7901 cells and viable LBG or LBG-$_S$ prevents *H pylori* SS1-LPS-mediated IL-8 production via inhibition of the TLR4 pathway.

Key words: *Lactobacillus*; *Helicobacter pylori*; Lipopolysaccharide; Toll-like receptor 4; Interleukin-8

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INTRODUCTION

Infection with the human gastric pathogen *Helicobacter pylori* (*H pylori*) can develop into chronic gastritis, peptic ulcer and gastric cancer. Some studies[1-5] demonstrated that *H pylori* can stimulate interleukin-8 (IL-8) production in gastric mucosal epithelia, which induces accumulation of neutrophilic granulocytes in mucosa. Chemotactic response initiates inflammatory damage to gastric mucosa, which plays a crucial role in the pathogenesis of *H pylori*. However, signal transduction through which *H pylori* modulates IL-8 production from gastric epithelia is not fully understood.

The product of particular significance for the virulent action of *H pylori* is its cell wall lipopolysaccharide (LPS). The effects of *H pylori* lipopolysaccharide (*H pylori*-LPS) have been manifested by the marked increase of nitric oxide and proinflammatory cytokines including IL-8 in gastric mucosa[6,7], abrogation of proliferation and induction of apoptosis in gastric epithelia[8]. Mammalian Toll-like receptors trigger the signaling pathways involved in innate immune responses to microbial challenge after recognizing pathogen-associated molecular patterns.

H pylori-LPS is the natural ligand for Toll-like receptor 4 (TLR4) in gastric epithelia. It has been proposed that *H pylori*-LPS induces IL-8 production in gastric epithelia through activating the TLR4 signaling pathway[7,9].

Probiotics are living microorganisms with no or low pathogenicity, which exert beneficial effects on the host. *Lactobacillus bulgaricus* (LBG), a bacterium used in the production of yogurt, is one of the best-studied probiotics. There is increasing evidence[10,11] that LBG has therapeutic effects on *H pylori*-related diseases, including enhanced eradication of *H pylori*, amelioration of resistance to antibiotics, down-regulated side effects of antibiotic-based therapy, decreased recurrence of *H pylori* infection, and inhibition of *H pylori*-induced apoptosis. The mechanisms underlying these effects include inhibition of *H pylori* growth and attachment to epithelial cells, inactivation of virulent factors such as urease, and decrease in production of *H pylori*-induced proinflammatory cytokines[12-16]. However, the signaling pathways which are modulated by LBG in gastric epithelia have not been well elucidated.

In this experiment, we demonstrated that viable LBG inhibited the activation of the TLR4 signaling pathway and IL-8 production induced by *H pylori* Sydney strain 1 lipopolysaccharide (*H pylori* SS1-LPS) in SGC-7901 cells. Furthermore, supernatant recovered from the LBG culture MRS broth (LBG-$_S$) also exerted these effects on SGC-7901 cells treated with *H pylori* SS1-LPS. These observations provide the novel insight into the rationale for LBG as a potential treatment for *H pylori*-related diseases.

MATERIALS AND METHODS

H pylori SS1 culture and *H pylori* SS1-LPS preparation

H pylori SS1 was kindly offered by Professor Qian Yu (School of Public Health, Sichuan University). *H pylori* SS1 was incubated in Brucella broth (bioMérieux Corporate, La Balme-Les Grottes, France) supplemented with 10% fetal calf serum (FCS; Invitrogen GIBCO, Carlsbad, California, USA), 10 mg/L vancomycin, 10 mg/L amphotericin and 2500 U/L polymycin B in a shaking incubator (100 r/min) at 37℃ in an atmosphere containing 50 mL/L O_2, 100 mL/L CO_2 and 850 mL/L N_2 for 48 h. *H pylori* SS1 was precipitated from Brucella broth by centrifuging at 10000 r/min for 10 min at 4℃ and washed twice with PBS. Then the concentration of *H pylori* SS1 in PBS was adjusted to 10^8 colony forming units (CFU)/mL with optical density determined as 1 at A_{660}. The *H pylori* SS1-containing PBS was used to prepare *H pylori* SS1-LPS with the LPS extraction kit (bioMérieux) following guidelines from its manufacturer. *H pylori* SS1-LPS concentrations were determined with the kinetic Limulus amebocyte lysate assay kit (Cambrex, Walkersville, Maryland, USA) according to the manufacturer's instructions.

LBG culture and LBG-$_S$ preparation

LBG, kindly offered by Professor Qian Yu (School of Public Health, Sichuan University), was incubated in MRS broth (bioMérieux) in a candle jar at 37℃ for 24-48 h, precipitated from MRS broth by centrifuging at 5000 r/min for 10 min and washed twice with PBS. Then the concentration of LBG in PBS was adjusted to 10^7 CFU/mL with optical density determined as 0.5 at A_{600}. LBG was precipitated from PBS by centrifuging at 5000 r/min for 10 min and resuspended with an equivalent volume of RPMI 1640 medium (Invitrogen GIBCO) for pretreatment of SGC-7901 cells.

LBG-$_S$ was generated by centrifuging at 1000 × *g* for 15 min and filtering (0.2 μm) LBG culture MRS broth, then the filtrate was concentrated using Centricon Plus-20 (5-100 kDa; Millipore, Bedford, Massachusetts, USA) by centrifugation at 4000 × *g* for 1 h following guidelines from the manufacturer. Protein concentrations were determined with the Pierce protein assay kit (Pierce, Rockford, Illinois, USA) using MRS broth as the control.

Cell culture

SGC-7901 cell line was established from human gastric adenocarcinoma cells. Though the characteristics of cell apoptosis and proliferation are different from the cell line derived from normal gastric epithelia, SGC-7901 cells have been widely used as models for investigations on *H pylori*-induced gastric epithelial inflammatory responses because their inflammatory responsibility is similar to normal gastric epithelia. Therefore, SGC-7901 cells were used in our experiment. They were grown in RPMI 1640 medium supplemented with 10% FCS, 100 U/mL penicillin and 100 μg/mL streptomycin at 37℃ in an atmosphere containing 50 mL/L CO_2. After 3-4 times of passage, SGC-7901 cells were seeded to generate 1 × 10^6 cells per 6 cm culture dish and incubated in RPMI 1640 medium containing 10% FCS, 100 U/mL penicillin and 100 μg/mL streptomycin at 37℃ in an atmosphere containing 50 mL/L CO_2 for 24 h. Then all cells were serum-starved (0.5% FCS) for 24 h before experimentation.

Treatment of cell line

SGC-7901 cells were treated with 25 endotoxin units (EU)/mL *H pylori* SS1-LPS for 0, 30, 60 min or 120 min in the absence or presence of pretreatment for 1 h with 10^7 CFU/mL viable LBG or 10^{-2} mg/mL LBG-$_S$. At the end of each time point, cells were collected for Western blot, and RPMI 1640 medium was collected for ELISA. Each experiment was in triplicate.

Preparation of cellular lysates and Western blot analysis

Nuclear and cytoplasmic extraction from SGC-7901 cells was performed using the nuclear-cytosol extraction kit (Cell Signaling Technology, Danvers, Massachusetts, USA) following guidelines from the manufacturer. Protein concentrations of all extracts were determined with the Pierce protein assay kit. Each extract was mixed with an equal amount of 2 × loading buffer and heated at 100℃ for 5 min. Thirty micrograms of protein was loaded in each lane, resolved in sodium dodecyl

sulphate-polyacrylamide gels and electrotransferred to polyvinylidene difluoride membrane (1.2 mA/cm^2, 1 h). After blocked with 5% fat-free dried milk in Tris-buffered saline containing 0.1% Tween-20 (TBST) for 1 h, the membrane was incubated overnight at 4℃ with the following primary antibodies: anti-TLR4, anti-transforming growth factor β-activated kinase 1 (TAK1), anti-phospho-TAK1 (p-TAK1), anti-nuclear factor κB (NF-κB), anti-p38 mitogen-activated protein kinase (p38MAPK), anti-phospho-p38MAPK (p-p38MAPK; all at the dilution of 1:1000, Cell Signaling Technology). Anti-β-actin and anti-lamin B1 (both at the dilution of 1:1000, Santa Cruz Biotechnology, Santa Cruz, California, USA) were used for the control of equal protein loading. After washed three times in TBST, the membrane was incubated with horseradish peroxidase-conjugated IgG (1:5000, Santa Cruz Biotechnology) as the secondary antibody at room temperature for 1 h. The photographic film was exposed to bands visualized with the Supersignal West Pico chemiluminescent substrate kit (Pierce). The integrated optical density (I*A*) of each band was quantified using Quantity One software 4.5.0 (Bio-Rad Laboratories, Hercules, California, USA). Each value for TLR4 band was normalized as the ratio of I*A* of TLR4 band to that of β-actin band. Each value for p-TAK1 band was normalized as the ratio of I*A* of p-TAK1 band to that of TAK1 band. Each value for p-p38MAPK band was normalized as the ratio of I*A* of p-p38MAPK band to that of p38MAPK band. Each value for NF-κB band was normalized as the ratio of I*A* of NF-κB band to that of lamin B1 band. Each Western blot analysis of extract samples was performed in triplicate.

Detection of IL-8 production

The concentration of IL-8 in RPMI 1640 medium was determined with a commercially available ELISA kit (R&D Systems, Minneapolis, Minnesota, USA) following guidelines from the manufacturer. Each sample was detected thrice.

Statistical analysis

The data were expressed as mean ± SD, and analyzed by SPSS13.0 software (SPSS, Chicago, Illinois, USA) for One-Way ANOVA test. $P < 0.05$ was considered statistically significant.

RESULTS

Viable LBG or LBG-$_S$ inhibited *H pylori*SS1-LPS-induced activation of TLR4 signaling pathway

Twenty-five EU/mL *H pylori*SS1-LPS up-regulated the expression of TLR4, enhanced the phosphorylation of TAK1 and p38MAPK, and induced the translocation of NF-κB into nuclei in a time-dependent manner (Figure 1A and B, Table 1). These results demonstrate that *H pylori*SS1-LPS could activate the TLR4 signaling pathway. Pretreatment for 1 h with 10^7 CFU/mL viable LBG (Figure 1A) or 10^{-2} mg/mL LBG-$_S$ (Figure 1B) significantly inhibited these effects of *H pylori*SS1-LPS on the TLR4 pathway in SGC-7901 cells to a great extent (Table 1).

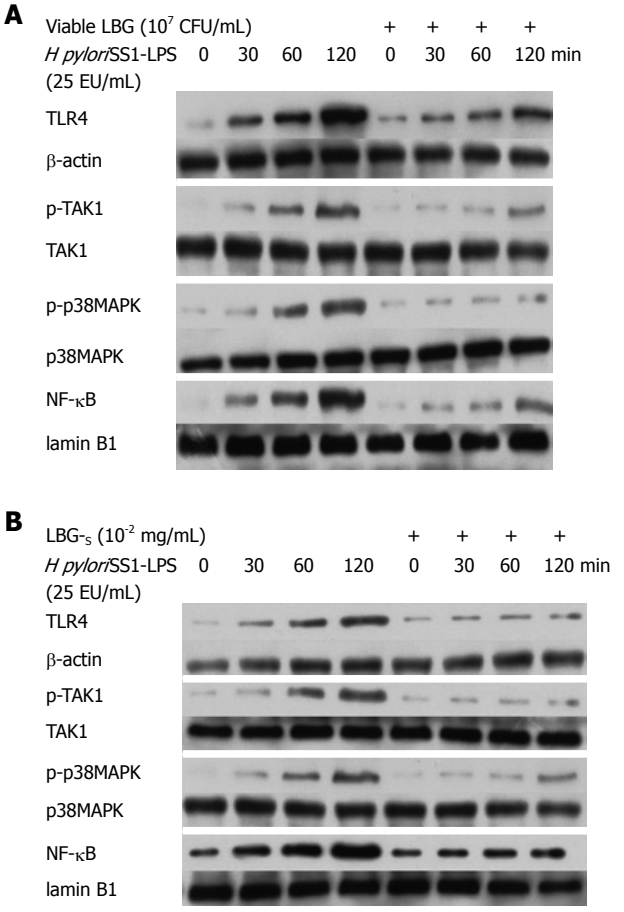

Figure 1 Expression of TLR4 and activation of TAK1, p38MAPK and NF-κB in SGC-7901 cells treated with *H pylori*SS1-LPS in the absence or presence of pretreatment with viable LBG (**A**) and LBG-$_S$ (**B**).

Viable LBG or LBG-$_S$ inhibited *H pylori*SS1-LPS-induced IL-8 production

The production of IL-8 in SGC-7901 cells treated with 25 EU/mL *H pylori*SS1-LPS for 0, 30 or 60 min in the absence or presence of pretreatment for 1 h with 10^7 CFU/mL viable LBG or 10^{-2} mg/mL LBG-$_S$ was almost undetectable (Table 2). No significant difference in these data was possibly attributed to their extremely small value. Thirty or 60 min of treatment with *H pylori*SS1-LPS might be too short for SGC-7901 cells to produce enough IL-8 for ELISA, because cytokine production usually is posterior to the process of corresponding signal transduction. If we detected IL-8 mRNA in SGC-7901 cells using the retro-transcriptional polymerase chain reaction, results at 30 min or 60 min should have been significantly higher than those at 0 min. Only 120 min of treatment with 25 EU/mL *H pylori*SS1-LPS augmented IL-8 production in SGC-7901 cells (Table 2), which was, however, down-regulated by pretreatment for 1 h with 10^7 CFU/mL viable LBG or 10^{-2} mg/mL LBG-$_S$ (Table 2).

Table 1 Effects of viable LBG, LBG-$_S$ and *H pylori*SS1-LPS on the expression of TLR4 and activation of TAK1, p38MAPK and NF-κB in SGC-7901 cells

	LPS 0 min	LPS 30 min	LPS 60 min	LPS 120 min		LBG + LPS 0 min	LBG + LPS 30 min	LBG + LPS 60 min	LBG + LPS 120 min	LBG-$_S$ + LPS 0 min	LBG-$_S$ + LPS 30 min	LBG-$_S$ + LPS 60 min	LBG-$_S$ + LPS 120 min
TLR4/β-actin	0.014 ± 0.003	0.21 ± 0.03[1]	0.43 ± 0.05[1]	1.21 ± 0.11[1]		0.016 ± 0.003	0.08 ± 0.01[2]	0.15 ± 0.02[2]	0.40 ± 0.05[2]	0.016 ± 0.003	0.088 ± 0.013[2]	0.12 ± 0.02[2]	0.18 ± 0.03[2]
[1]*t*		2.588	3.068	3.502	[2]*t*		2.328	2.425	2.302		2.182	2.733	2.901
[1]*P*		0.012	0.003	< 0.001	[2]*P*		0.031	0.021	0.033		0.041	0.01	0.007
p-TAK1/TAK1	0.008 ± 0.002	0.075 ± 0.009[1]	0.17 ± 0.03[1]	0.49 ± 0.06[1]		0.008 ± 0.001	0.025 ± 0.003[2]	0.051 ± 0.006[2]	0.15 ± 0.02[2]	0.007 ± 0.002	0.031 ± 0.005[2]	0.086 ± 0.011[2]	0.13 ± 0.02[2]
[1]*t*		2.445	2.871	3.421	[2]*t*		2.408	2.502	2.530		2.278	2.108	2.682
[1]*P*		0.018	0.005	< 0.001	[2]*P*		0.022	0.018	0.017		0.035	0.044	0.012
p-p38MAPK/ p38MAPK	0.010 ± 0.002	0.079 ± 0.011[1]	0.18 ± 0.03[1]	0.48 ± 0.08[1]		0.009 ± 0.002	0.031 ± 0.004[2]	0.058 ± 0.008[2]	0.16 ± 0.03[2]	0.013 ± 0.003	0.029 ± 0.004[2]	0.079 ± 0.012[2]	0.12 ± 0.02[2]
[1]*t*		2.401	2.819	3.403	[2]*t*		2.261	2.445	2.506		2.289	2.093	2.704
[1]*P*		0.019	0.006	< 0.001	[2]*P*		0.036	0.02	0.018		0.034	0.046	0.011
NF-κB/lamin B1	0.012 ± 0.002	0.18 ± 0.03[1]	0.32 ± 0.05[1]	1.02 ± 0.13[1]		0.012 ± 0.002	0.042 ± 0.005[2]	0.084 ± 0.009[2]	0.26 ± 0.04[2]	0.014 ± 0.002	0.091 ± 0.012[2]	0.17 ± 0.02[2]	0.23 ± 0.04[2]
[1]*t*		2.523	2.968	3.458	[2]*t*		2.796	2.690	2.711		2.088	2.068	2.787
[1]*P*		0.014	0.004	< 0.001	[2]*P*		0.009	0.012	0.011		0.046	0.048	0.009

[1]*t* and *P* value of each group *vs* corresponding LPS 0 min group; [2]*t* and *P* value of each group *vs* corresponding LPS group at the same time point.

Table 2 Inhibitory effects of viable LBG or LBG-$_S$ on *H pylori*SS1-LPS-induced IL-8 production (pg/mL)

	*H pylori*SS1-LPS		Viable LBG + LPS	LBG-$_S$ + LPS
0 min	2.62 ± 0.43		2.56 ± 0.46	2.52 ± 0.51
30 min	2.78 ± 0.38		2.67 ± 0.42	2.61 ± 0.47
60 min	3.07 ± 0.35		2.93 ± 0.51	2.89 ± 0.48
120 min	40.39 ± 3.0[1]		24.12 ± 3.05[1,2]	18.41 ± 1.83[1,2]
[1]*t*	2.832		2.601	2.506
[1]*P*	0.006		0.011	0.015
		[2]*t*	2.121	2.175
		[2]*P*	0.047	0.044

[1]*t* and *P* value of each group *vs H pylori*SS1-LPS group at 0 min; [2]*t* and *P* value of each group *vs H pylori*SS1-LPS group at 120 min.

DISCUSSION

H pylori have recently been considered an indigenous biota of human stomach and a dominant niche in gastric microecology including *Lactobacilli* and Sacharomyces with the capability of cross-species communication[17-19]. There is evidence that Helicobacter species are ancient inhabitants of human stomachs for at least 60 000 years which have co-evolved with the host and developed their excellent adaption to humans[20,21]. Though the vast majority of people in developing countries carry *H pylori*, most of them have no clinical manifestations at all[22]. More and more observations are consistent with the hypothesis that *H pylori* have both pathogenetic and symbiotic features, thus relatively balance their cost and benefit[23,24]. Changes in life style and sanitation conditions cause a probable disturbance of gastric microecology, which plays a more important role in the pathogenetic mechanism of *H pylori* in the modern era[25,26]. Therefore, eradiation of *H pylori* does not seem justified for all individuals, especially children. These findings lead to the speculation that we are supposed to domesticate *H pylori* through restoring the homeostasis of the gastric microecosystem. It was reported that administration of exogenous *Lactobacilli* has therapeutic effects on *H pylori*-associated diseases by interfering with the pathogenetic progress of *H pylori*[27-30]. Inhibition of *H pylori*-induced proinflammatory factor production by *Lactobacilli* is a very important aspect. It has been demonstrated that *Lactobacilli* abrogate *H pylori*-mediated IL-8 release *in vitro* and *in vivo*[31,32]. A large body of evidence has shown that *H pylori*-LPS-induced inflammation in gastric mucosa has nearly the same pathological characteristics as the mucosal inflammation initiated by *H pylori* infection[6,7]. Bhattacharyya *et al*[33] reported that pretreatment with LPS inhibitor greatly attenuated *H pylori* extract-mediated gastric mucosal inflammation, suggesting that *H pylori*-LPS may be a major virulent factor for *H pylori*-associated mucosal inflammation, which urged us to research the effect of *Lactobacilli* on *H pylori*-LPS-induced IL-8 production. It has been documented that *H pylori*-LPS induces mucosal inflammation including IL-8 production via TLR4 signaling. In brief, *H pylori*-LPS is recognized by TLR4 of the gastric epithelium, and then activates interleukin-1 receptor- associated kinase, tumor necrosis factor receptor-associated factor-6, TAK1 and TAK1-binding protein 1/2, p38MAPK and NF-κB at last in a cascade mechanism[7,9]. However, whether *Lactobacilli* have the capability of inhibiting *H pylori*-LPS-activated TLR4 pathway through interacting with gastric epithelia directly has not been extensively researched. Our findings demonstrate that viable LBG and LBG-$_S$ can prevent TLR4 signaling activation and IL-8 production mediated by *H pylori*SS1-LPS in SGC-7901 cells, which strongly supports the hypothesis that some soluble proteins secreted by LBG and (or) somatic constituents of LBG exert inhibitory effects on the TLR4 pathways in SGC-7901 cells treated with *H pylori*SS1-LPS. Yan *et al*[34] reported that Lactobacillus rhamnosus GG (LGG) or supernatant recovered from LGG culture MRS

broth (LGG-$_S$) ameliorated apoptosis of young adult mouse colon cells treated with tumor necrosis factor α (TNF-α), interferon-γ or interleukin-1α through blocking p38MAPK and stress-activated protein kinase/c-Jun amino-terminal kinase pathway. In addition, they have identified two proteins in LGG-$_S$ with molecular sizes of 80 and 42 kDa, which may be possible substantial effectors in LGG-$_S$[34]. In a recent study, they purified the two proteins from LGG-$_S$ again, ultimately determined their molecular weight as 75 and 40 kDa, and named them p75 and p40 respectively[35]. Their results demonstrate that both p75 and p40 can inhibit TNF-α-induced apoptosis of intestinal epithelia and promote cell growth through activating Akt[35]. LBG-$_S$ in our experiment probably contained the similar or even same proteins, which could intervene in *H pylori*SS1-LPS-activated TLR4 signaling through modulating other pathways in SGC-7901 cells. Further study is needed to evaluate the hypothesis. In the purification and characterization of the aforementioned potential soluble proteins secreted by LBG, we also detected the effect of heat-killed LBG (hk-LBG) on *H pylori*SS1-LPS-activated TLR4 signaling for evaluating the presumption that some somatic constituents of LBG may inhibit the effect of *H pylori*SS1-LPS. The incomplete data indicate that hk-LBG could also disrupt the *H pylori*SS1-LPS-activated TLR4 pathway. Nevertheless, the effect of hk-LBG was obviously smaller than that of viable LBG at the same concentration.

In conclusion, our evaluation of LBG as a model probiotic organism reveals an important and novel relationship between *H pylori*-LPS-activated TLR4 signaling and selective microflora, and further our understanding of the signal pathways in gastric epithelia involved in inflammatory responses that are regulated by probiotics and pathogenic bacteria composing the gastric microecosystem.

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COMMENTS

Background

Some studies have demonstrated that *Helicobacter pylori* (*H pylori*) can stimulate the release of interleukin-8 (IL-8) from gastric epithelia, which initiates inflammatory damage to gastric mucosa and plays a crucial role in the pathogenesis of *H pylori* infection. *H pylori* lipopolysaccharide (*H pylori*-LPS) is a major virulent factor for *H pylori*-associated mucosal inflammation, which induces IL-8 production via activation of the Toll-like receptor 4 (TLR4) signaling pathway in gastric epithelia. Since *H pylori* is an indigenous biota in gastric microflora including *Lactobacilli* and disturbance of the gastric microecosystem plays a more important role in pathogenetic mechanisms of *H pylori*, eradiation of *H pylori* in all individuals does not seem justifiable. The gastric microecosystem was restored after treatment with exogenous *Lactobacilli*. However, whether *Lactobacilli* inhibit *H pylori*-LPS-induced IL-8 production through blocking *H pylori*-LPS-activated TLR4 pathway needs further study.

Research frontiers

Though there is more and more evidence that *Lactobacilli* have beneficial effects on gastrointestinal diseases, the molecular mechanisms are not well understood, especially in *H pylori*-associated diseases. Whether certain soluble proteins secreted by *Lactobacillus bulgaricus* (LBG) and somatic constituents of LBG directly interact with some signaling pathways in gastric epithelia has not been clearly demonstrated. Our study indicated that Lactobacillus rhamnosus GG secreted p75 and p40, two proteins with a molecular size of 75 and 40 kDa respectively, could ameliorate apoptosis of intestinal epithelia treated with tumor necrosis factor α, interferon-γ or interleukin-1α and promote cell growth through activating Akt, blocking p38 mitogen-activated protein kinase and stress-activated protein kinase/c-Jun amino-terminal kinase signaling. The supernatant recovered from LBG culture MRS broth (LBG-$_S$) in our experiment may contain the similar or even same proteins, which could intervene in *H pylori* Sydney strain 1 lipopolysaccharide (*H pylori*SS1-LPS)-activated TLR4 signaling through modulating other pathways in SGC-7901 cells, suggesting that probiotic bacterial components may be useful in preventing *H pylori*-associated diseases.

Innovations and breakthroughs

Viable LBG or LBG-$_S$ blocked the *H pylori*SS1-LPS-activated TLR4 pathway in SGC-7901 cells, leading to their inhibitory effects on IL-8 production induced by *H pylori*SS1-LPS.

Applications

This study implied that certain soluble proteins secreted by *Lactobacilli* could directly interact with some signaling pathways in gastric epithelia and partly block the noxious effects of *H pylori*. This may benefit the exploration of new drugs for the treatment of *H pylori*-associated diseases.

Peer review

The results of this study indicate that *H pylori*SS1-LPS could increase IL-8 production through TLR4 signaling in gastric epithelia and probiotic bacteria attenuate IL-8 production via inhibition of the TLR4 pathway. The manuscript contains some interesting data, viable probiotics and their extracts against *H pylori*-LPS cytotoxicity.

REFERENCES

1 **Felley CP**, Pignatelli B, Van Melle GD, Crabtree JE, Stolte M, Diezi J, Corthesy-Theulaz I, Michetti P, Bancel B, Patricot LM, Ohshima H, Felley-Bosco E. Oxidative stress in gastric mucosa of asymptomatic humans infected with Helicobacter pylori: effect of bacterial eradication. *Helicobacter* 2002; **7**: 342-348

2 **Yoshimura N**, Suzuki Y, Saito Y. Suppression of Helicobacter pylori-induced interleukin-8 production in gastric cancer cell lines by an anti-ulcer drug, geranylgeranylacetone. *J Gastroenterol Hepatol* 2002; **17**: 1153-1160

3 **Ismail S**, Hampton MB, Keenan JI. Helicobacter pylori outer membrane vesicles modulate proliferation and interleukin-8 production by gastric epithelial cells. *Infect Immun* 2003; **71**: 5670-5675

4 **Nozawa Y**, Nishihara K, Akizawa Y, Orimoto N, Nakano M, Uji T, Ajioka H, Kanda A, Matsuura N, Kiniwa M. Lafutidine inhibits Helicobacter pylori-induced interleukin-8 production in human gastric epithelial cells. *J Gastroenterol Hepatol* 2004; **19**: 506-511

5 **Lopes AI**, Quiding-Jarbrink M, Palha A, Ruivo J, Monteiro L, Oleastro M, Santos A, Fernandes A. Cytokine expression in pediatric Helicobacter pylori infection. *Clin Diagn Lab Immunol* 2005; **12**: 994-1002

6 **Slomiany BL**, Piotrowski J, Slomiany A. Up-regulation of endothelin-converting enzyme-1 in gastric mucosal inflammatory responses to Helicobacter pylori lipopolysaccharide. *Biochem Biophys Res Commun* 2000; **267**: 801-805

7 **Ogawa T**, Asai Y, Sakai Y, Oikawa M, Fukase K, Suda Y, Kusumoto S, Tamura T. Endotoxic and immunobiological activities of a chemically synthesized lipid A of Helicobacter pylori strain 206-1. *FEMS Immunol Med Microbiol* 2003; **36**: 1-7

8 **Kawahara T**, Teshima S, Oka A, Sugiyama T, Kishi K, Rokutan K. Type I Helicobacter pylori lipopolysaccharide stimulates toll-like receptor 4 and activates mitogen oxidase 1 in gastric pit cells. *Infect Immun* 2001; **69**: 4382-4389

9 **Su B**, Ceponis PJ, Lebel S, Huynh H, Sherman PM. Helicobacter pylori activates Toll-like receptor 4 expression in gastrointestinal epithelial cells. *Infect Immun* 2003; **71**: 3496-3502

10 **Johnson-Henry KC**, Mitchell DJ, Avitzur Y, Galindo-Mata E, Jones NL, Sherman PM. Probiotics reduce bacterial colonization and gastric inflammation in H. pylori-infected mice. *Dig Dis Sci* 2004; **49**: 1095-1102

11 **Sheu BS**, Wu JJ, Lo CY, Wu HW, Chen JH, Lin YS, Lin MD. Impact of supplement with Lactobacillus- and Bifidobacterium-containing yogurt on triple therapy for Helicobacter pylori eradication. *Aliment Pharmacol Ther* 2002; **16**: 1669-1675

12 **Mukai T**, Asasaka T, Sato E, Mori K, Matsumoto M, Ohori H. Inhibition of binding of Helicobacter pylori to the glycolipid receptors by probiotic Lactobacillus reuteri. *FEMS Immunol Med Microbiol* 2002; **32**: 105-110

13 **Oh Y**, Osato MS, Han X, Bennett G, Hong WK. Folk yoghurt kills Helicobacter pylori. *J Appl Microbiol* 2002; **93**: 1083-1088

14 **Aiba Y**, Suzuki N, Kabir AM, Takagi A, Koga Y. Lactic acid-mediated suppression of Helicobacter pylori by the oral administration of Lactobacillus salivarius as a probiotic in a gnotobiotic murine model. *Am J Gastroenterol* 1998; **93**: 2097-2101

15 **Michetti P**, Dorta G, Wiesel PH, Brassart D, Verdu E, Herranz M, Felley C, Porta N, Rouvet M, Blum AL, Corthesy-Theulaz I. Effect of whey-based culture supernatant of Lactobacillus acidophilus (johnsonii) La1 on Helicobacter pylori infection in humans. *Digestion* 1999; **60**: 203-209

16 **Linsalata M**, Russo F, Berloco P, Caruso ML, Matteo GD, Cifone MG, Simone CD, Ierardi E, Di Leo A. The influence of Lactobacillus brevis on ornithine decarboxylase activity and polyamine profiles in Helicobacter pylori-infected gastric mucosa. *Helicobacter* 2004; **9**: 165-172

17 **Blaser MJ**, Parsonnet J. Parasitism by the "slow" bacterium Helicobacter pylori leads to altered gastric homeostasis and neoplasia. *J Clin Invest* 1994; **94**: 4-8

18 **Sathar MA**, Gouws E, Simjee AE, Mayat AM. Seroepidemiological study of Helicobacter pylori infection in South African children. *Trans R Soc Trop Med Hyg* 1997; **91**: 393-395

19 **Hadley C**. The infection connection. Helicobacter pylori is more than just the cause of gastric ulcers--it offers an unprecedented opportunity to study changes in human microecology and the nature of chronic disease. *EMBO Rep* 2006; **7**: 470-473

20 **Falush D**, Wirth T, Linz B, Pritchard JK, Stephens M, Kidd M, Blaser MJ, Graham DY, Vacher S, Perez-Perez GI, Yamaoka Y, Megraud F, Otto K, Reichard U, Katzowitsch E, Wang X, Achtman M, Suerbaum S. Traces of human migrations in Helicobacter pylori populations. *Science* 2003; **299**: 1582-1585

21 **Linz B**, Balloux F, Moodley Y, Manica A, Liu H, Roumagnac P, Falush D, Stamer C, Prugnolle F, van der Merwe SW, Yamaoka Y, Graham DY, Perez-Trallero E, Wadstrom T, Suerbaum S, Achtman M. An African origin for the intimate association between humans and Helicobacter pylori. *Nature* 2007; **445**: 915-918

22 **Go MF**. Review article: natural history and epidemiology of Helicobacter pylori infection. *Aliment Pharmacol Ther* 2002; **16** Suppl 1: 3-15

23 **Rajendra S**, Ackroyd R, Robertson IK, Ho JJ, Karim N, Kutty KM. Helicobacter pylori, ethnicity, and the gastroesophageal reflux disease spectrum: a study from the East. *Helicobacter* 2007; **12**: 177-183

24 **Ackermark P**, Kuipers EJ, Wolf C, Breumelhof R, Seldenrijk CA, Timmer R, Segeren KC, Kusters JG, Smout AJ. Colonization with cagA-positive Helicobacter pylori strains in intestinal metaplasia of the esophagus and the esophagogastric junction. *Am J Gastroenterol* 2003; **98**: 1719-1724

25 **Blaser MJ**. Hypothesis: the changing relationships of Helicobacter pylori and humans: implications for health and disease. *J Infect Dis* 1999; **179**: 1523-1530

26 **Tovey FI**, Hobsley M, Kaushik SP, Pandey R, Kurian G, Singh K, Sood A, Jehangir E. Duodenal gastric metaplasia and Helicobacter pylori infection in high and low duodenal ulcer-prevalent areas in India. *J Gastroenterol Hepatol* 2004; **19**: 497-505

27 **Sakamoto I**, Igarashi M, Kimura K, Takagi A, Miwa T, Koga Y. Suppressive effect of Lactobacillus gasseri OLL 2716 (LG21) on Helicobacter pylori infection in humans. *J Antimicrob Chemother* 2001; **47**: 709-710

28 **Shimizu T**, Haruna H, Hisada K, Yamashiro Y. Effects of Lactobacillus gasseri OLL 2716 (LG21) on Helicobacter pylori infection in children. *J Antimicrob Chemother* 2002; **50**: 617-618

29 **Gotteland M**, Cruchet S. Suppressive effect of frequent ingestion of Lactobacillus johnsonii La1 on Helicobacter pylori colonization in asymptomatic volunteers. *J Antimicrob Chemother* 2003; **51**: 1317-1319

30 **Armuzzi A**, Cremonini F, Bartolozzi F, Canducci F, Candelli M, Ojetti V, Cammarota G, Anti M, De Lorenzo A, Pola P, Gasbarrini G, Gasbarrini A. The effect of oral administration of Lactobacillus GG on antibiotic-associated gastrointestinal side-effects during Helicobacter pylori eradication therapy. *Aliment Pharmacol Ther* 2001; **15**: 163-169

31 **Sgouras DN**, Panayotopoulou EG, Martinez-Gonzalez B, Petraki K, Michopoulos S, Mentis A. Lactobacillus johnsonii La1 attenuates Helicobacter pylori-associated gastritis and reduces levels of proinflammatory chemokines in C57BL/6 mice. *Clin Diagn Lab Immunol* 2005; **12**: 1378-1386

32 **Bergonzelli GE**, Granato D, Pridmore RD, Marvin-Guy LF, Donnicola D, Corthesy-Theulaz IE. GroEL of Lactobacillus johnsonii La1 (NCC 533) is cell surface associated: potential role in interactions with the host and the gastric pathogen Helicobacter pylori. *Infect Immun* 2006; **74**: 425-434

33 **Bhattacharyya A**, Pathak S, Datta S, Chattopadhyay S, Basu J, Kundu M. Mitogen-activated protein kinases and nuclear factor-kappaB regulate Helicobacter pylori-mediated interleukin-8 release from macrophages. *Biochem J* 2002; **368**: 121-129

34 **Yan F**, Polk DB. Probiotic bacterium prevents cytokine-induced apoptosis in intestinal epithelial cells. *J Biol Chem* 2002; **277**: 50959-50965

35 **Yan F**, Cao H, Cover TL, Whitehead R, Washington MK, Polk DB. Soluble proteins produced by probiotic bacteria regulate intestinal epithelial cell survival and growth. *Gastroenterology* 2007; **132**: 562-575

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