

RESEARCH ARTICLE

Zhechko P. Dimitrov
Irina M. Gotova

Authors' address:

LB-Bulgaricum Plc. R&D Center,
12-A Malashevskya Str.,
Sofia 1202, Bulgaria.

Correspondence:

Zhechko Dimitrov
LB-Bulgaricum Plc. R&D Center,
12-A Malashevskya Str.,
Sofia 1202, Bulgaria.
Tel.: +359 896546644
e-mail:
zhechko.dimitrov@lbbulgaricum.bg

Selected lactic acid bacteria strains reduce the expression of interleukin-8 in epithelial cells

ABSTRACT

The proinflammatory chemokine interleukin-8 (IL-8) is produced by the intestinal epithelium especially after stimulation with pro-inflammatory cytokines like TNF- α . The objective of the present work was to evaluate the effect of selected lactic acid bacteria strains on IL-8 secretion in intestinal epithelial cells with and without stimulation by TNF- α . A large number of strains with yoghurt and intestinal origin were evaluated in their ability to reduce the production of IL-8 from human epithelium cell line Caco-2. The strains demonstrating the highest reduction of IL-8 after stimulation of epithelium cells with TNF- α were: *L. gasseri* G7/12, *E. faecalis* E2, *B. longum* Bif6/2, *L. bulgaricus* B67, and *S. thermophilus* T43. The relative reduction of the IL-8 secretion by these strains was between 13.7% and 34.5% after stimulation with TNF- α , and between 2.3% and 12.2% without stimulation with TNF- α . In order to have additional information about anti-inflammatory potential of the strains, the capability in induction of IL-10 by help of a splenocyte model was assessed. It was proven that some LAB strains, even with yoghurt origin, could inhibit the secretion of IL-8 from epithelial cells and could have a beneficial potential against inflammation.

Key words: immunomodulation, probiotics, inflammation

Introduction

Intestinal epithelia constitute mucosal barrier of the bowel, and participate in inflammatory or immune responses in gut (Campbell et al., 1999; Isolauri et al., 2002). In some gastrointestinal infectious and inflammatory conditions, such as inflammatory bowel disease (IBD), inflammatory cells including monocytes, lymphocytes, were activated and accumulated in lamina propria. The cells secrete excessive inflammatory products, such as TH1 type cytokines, chemokines and a lot of active oxides. Overproduction of cytokines could affect the biological action of epithelial cells. For instance, TNF- α could induce epithelial cells to secrete IL-8, and express membrane Toll-like receptor 4 (TLR4) excessively (Hausmann et al. 2002; Wolfs et al., 2002). TLR4 could enable intestinal epithelia hyper reactive in response to lipopolysaccharides (LPS) from the bacteria walls (Aderem & Ulevitch, 2000). When stimulated by cytokines like TNF- α , the intestinal epithelium is capable of releasing some proinflammatory chemokines such as IL-8 (Hausmann et al. 2002). Another cytokine with an importance to the

inflammation is IL-10 which is a multifunctional cytokine secreted by a variety of cells, including T cells, monocytes, macrophages, dendritic cells, and endothelial cells. IL-10 has diverse effects on most hemopoietic cells. It's crucial role is to limit and ultimately terminate immune and inflammatory responses (Moore et al., 2001). Probiotics, including *Bifidobacterium*, *Lactobacillus* play an essential role in the completeness of intestinal mucosa barrier. For instance, some probiotic strains could modulate intestinal mucosal immune response, some could play protective roles by inhibiting the adhesion of pathogenic bacteria to intestinal epithelia (Guandalini, 2002). Some researchers found that manipulating the normal intestinal flora using probiotics had a beneficial effect on health by altering the microbial environment, and some components of the flora could down-regulate inflammation when supplemented to patients with gastrointestinal diseases (Isolauri et al., 2002; Malin et al., 1996). At present, some probiotic compounds have been used in management of some diseases, such as maintenance therapy in IBD (Madsen et al., 1999; Shibolet et al., 2002). The present study was to investigate the effect of selected

RESEARCH ARTICLE

bacterial strains on IL-8 secretion of intestinal epithelium induced by TNF- α . Additionally, selected LAB strains were evaluated in induction of IL-10 using splenocytic cells.

Materials and Methods

Bacteria

Different strains LAB were used from the collection of microorganism of LB Bulgaricum PLC, previously identified at strain level by help of pulsed field gel electrophoresis (Dimitrov, 2012). The strains were grown at 37°C in MRS broth (for lactobacilli), M17 broth (for *S. thermophilus*), and BL broth (for Bifidobacteria), in anaerobic conditions to reach the early stationary phase. Bacteria were harvested by centrifugation at 3000 g for 5 min at 6°C. After two washes with sterile PBS pH 7.2 the bacteria were resuspended in DMEM to reach a final concentration of 10⁸ cfu/ml of medium. Then the bacteria were added to the cell culture wells at appropriate dilution.

Cells and bacteria coculture

Caco-2 cells were grown in DMEM containing 10% fetal calf serum. When grown to confluence in single layer, cells were washed three times with PBS pH 7.2 to remove culture medium and nonadherent cells. The bacteria in culture medium were transferred into individual wells respectively. TNF- α (10 ng/ml) was added into these wells where the effect of stimulation with TNF- α to the IL-8 production would be assessed. The supernatants were collected and centrifuged for measurement of IL-8 after 18 hours.

Splenocytes preparation

Spleens from five eight weeks old balb/c mice were isolated after sacrifice of the mice by CO₂ inhalation. The spleens were washed by RPMI 1640 media containing 10% fetal bovine serum, 4.5 g/l glucose, 10 mM HEPES, 1 mM sodium pyruvate, 2 mM L-glutamine, 1.5 g/l sodium bicarbonate, 100 U/ml penicillin, 100 μ g/ml streptomycin. The spleens were passed through sterile 100 μ m sieve, washed with RPMI 1640, and centrifuged at 135 g for 10 min. The pellets were resuspended in 5 ml 0.87% ammonium chloride for 2 min for removal of erythrocytes. Then the splenocytes were washed three times with RPMI 1640 at 4°C. The concentration of splenocytes was determined by dying with 0.4% trypan-blue and counting using a cytometer. The concentration of splenocytes was adjusted to 1.5 x 10⁶ cells in 1 ml media. One hundred microlitres of suspension of

splenocytes were transferred into the every well of 96-well microplate and 100 μ l of suspension from the evaluated bacterial strain was added. The plates were incubated in CO₂-incubator for 18 hours at 37°C and 5% CO₂. The supernatants were collected and centrifuged for measurement of IL-10.

IL-8 enzyme-linked immunosorbent assays

IL-8 enzyme-linked immunosorbent assays (ELISA) were performed according to the manufacturer's instructions (Diacclone, USA). In short, polyclonal goat anti-human IL-8 antibodies were used as capturing antibodies, biotinylated polyclonal rabbit anti-human IL-8 antibodies as detecting antibodies. Streptavidin-HRP and TMBS were added as color indicator. Plates were read at 450 nm of wavelength right after color reaction was stopped with acid. All procedures were performed at room temperature. Every assay was performed three times.

IL-10 enzyme-linked immunosorbent assays

IL-10 enzyme-linked immunosorbent assays (ELISA) were performed according to the manufacturer's instructions (Diacclone, USA).

Results and Discussion

IL-8 assay

Natural interleukin-8 expression was often found in human epithelium as well in Caco-2 cells. When stimulated by TNF- α (10 ng/ml), Caco-2 cells secreted a high quantity of IL-8 and its concentration was significantly increased than that in control ($P < 0.001$). In Table 1 the concentration of IL-8 in supernatants of each group is shown – with and without stimulation with TNF- α . The concentration of IL-8 in non stimulated by TNF- α Caco-2 cells was only 172 \pm 18.7 ng/l. After stimulation by TNF- α , Caco-2 cells secreted IL-8 and the concentration of IL-8 was elevated to 592 \pm 52.7 ng/l. The incubation with different LAB strains could modulate strain-specifically the secretion of IL-8 in both directions up or down, comparing with the control. After a preliminary assay of modulation properties towards IL-8 we selected those strains which were able to decrease the concentration of IL-8 in the presence of TNF- α . The highest reduction of IL-8 in both groups (with and without TNF- α) was obtained by the strain *Bif. longum* 6/2 – 34.5% and 12.2%, respectively. The relative decrease of IL-8 by each of the selected strains was higher in the case of use of TNF- α co-stimulation. Among many assessed strains with yoghurt origin only a few strains

RESEARCH ARTICLE

demonstrated an ability to decrease the concentration of IL-8. The strains *L.bulgaricus* 67 and *S. thermophilus* 43 were the most effective in their capability to down-regulate the expression of IL-8. The both strains could be included in preparation of starter for yoghurt with a perspective to develop an appropriate product for clinical tests among patients with inflammatory disease.

Table 1. Concentrations of IL-8 with and without stimulation with TNF- α , (mean \pm SD).

Bacterial strain	IL-8, w/o TNF- α , ng/l	IL-8, with TNF- α , ng/l
control, Caco-2 cells	172 \pm 18.7	592 \pm 51.7
<i>Bif. longum</i> 6/2	151 \pm 15.3	388 \pm 36.7
<i>L. gasseri</i> 7/12	159 \pm 15.7	398 \pm 39.8
<i>Ent. faecium</i> 2	162 \pm 16.0	412 \pm 42.1
<i>L.bulgaricus</i> 67	168 \pm 16.8	492 \pm 45.0
<i>S. thermophilus</i> 43	161 \pm 16.6	511 \pm 45.9

In order to imitate the inflammatory condition of gut in vitro, we used TNF- α to stimulate human colonic adenocarcinoma Caco-2 cells, which has the same biological properties as normal colonic epithelia. The selected five strains *B. longum* Bif6/2, *L. gasseri* G7/12, *E. faecalis* E2, *L. bulgaricus* B67, and *S. thermophilus* T43, inhibited the secretion of IL-8 in Caco-2 cells when stimulated with TNF- α between one and two hours after the co-culture with the bacterial strains. It indicated that the strains could trigger anti-inflammatory pathways within the gut epithelium. The epithelia in the presence of the selected bacterial strains showed immune hypo reaction to TNF- α , and produced less IL-8. An additional beneficial property of some of the selected strains is the adhesion to human intestinal cell surface, which was proven for *B. longum* Bif6/2, *L. gasseri* G7/12, and *E. faecalis* E2 (Dimitrov et al., 2014).

IL-10 assay

As the induction of the cytokine IL-10 is recognized to play anti-inflammatory properties, we evaluated the IL-10 induction effect by the five LAB strains, which previously demonstrated the best inhibition of the synthesis of IL-8, on splenocyte model. Concentrations of IL-10 in supernatants after 18 hours co-culture with the each strain are shown in Table 2. When stimulated by the five LAB strains, the splenocytes secreted a different quantity of IL-10. The highest ability to increase the quantity of IL-10 was demonstrated by *Bif. longum* 6/2 and *L. gasseri* 7/12. The

same strains possessed the highest ability to decrease the IL-8, and taking into account their good adhesion properties to epithelium, these strains could be proposed for further clinical trials as probiotics with anti-inflammatory potential.

Table 2. Concentrations of IL-10, (mean \pm SD).

Bacterial strain	IL-10, ng/l
control, splenocytes	20 \pm 1.8
<i>Bif. longum</i> 6/2	350 \pm 21.2
<i>L. gasseri</i> 7/12	274 \pm 12.8
<i>Ent. faecium</i> 2	230 \pm 17.6
<i>L. bulgaricus</i> 67	70 \pm 3.8
<i>S. thermophilus</i> 43	30 \pm 2.1

Conclusions

In the present work the selected five LAB strains successfully inhibited IL-8 secretion in intestinal epithelium model. Three of them proved an ability to increase significantly the IL-10 concentration. These five LAB strains are appropriate to be included in products for further clinical trials of their anti-inflammatory effect.

References

- Aderem A, Ulevitch RJ. 2000. Toll-like receptors in the induction of the innate immune response. *Nature*, 406(6797): 782-787.
- Campbell N, Yio XY, So LP, Li Y, Mayer L. 1999. The intestinal epithelial cell: processing and presentation of antigen to the mucosal immune system. *Immunol. Rev.*, 172: 315-324.
- Dimitrov Zh. 2012. Development of strain-discriminative amplified fragment length polymorphic DNA for bifidobacteria. Design of strain specific markers. *Biotechnol. & Biotechnol. Eq.*, 26(1): 35-38.
- Dimitrov Zh, Gotova I, Chorbadjiyska E. 2014. Characterization of the adhesins of selected probiotics to human epithelium. *Biotechnol. & Biotechnol. Eq.*, *in-press*.
- Guandalini S. 2002. Use of Lactobacillus-GG in paediatric Crohn's disease. *Dig. Liver Dis.*, 34(2): S63-S65.
- Hausmann M, Kiessling S, Mestermann S, Webb G, Spottl T, Andus T, Scholmerich J, Herfarth H, Ray K, Falk W, Rogler G. 2002. Toll-like receptors 2 and 4 are up-regulated during intestinal inflammation. *Gastroenterology*. 122(7): 1987-2000.
- Isolauri E, Kirjavainen PV, Salminen S. 2002. Probiotics: a role in the treatment of intestinal infection and inflammation? *Gut*, 50(3): iii54-iii59.
- Madsen KL, Doyle JS, Jewell LD, Tavernini MM, Fedorak RN. 1999. Lactobacillus species prevents colitis in interleukin 10 gene-deficient mice. *Gastroenterology*. 116(5): 1107-1114.

RESEARCH ARTICLE

- Malin M, Suomalainen H, Saxelin M, Isolauri E. 1996. Promotion of IgA immune response in patients with Crohn's disease by oral bacteriotherapy with *Lactobacillus GG*. *Ann. Nutr. Metab.*, 40(3): 137-145.
- Moore KW, De Waal MR, Coffman RL, O'Garra A. 2001. Interleukin-10 and the interleukin-10 receptor. *Annu. Rev. Immunol.*, 19: 683-765.
- Shibolet O, Karmeli F, Eliakim R, Swennen E, Brigidi P, Gionchetti P, Campieri M, Morgenstern S, Rachmilewitz D. 2002. Variable response to probiotics in two models of experimental colitis in rats. *Inflamm. Bowel Dis.*, 8(6): 399-406.
- Wolfs TG, Buurman WA, van Schadewijk A, de Vries B, Daemen MA, Hiemstra PS, Van't Veer C. 2002. In vivo expression of Toll-like receptor 2 and 4 by renal epithelial cells: IFN- γ and TNF- α mediated up-regulation during inflammation. *J. Immunol.*, 168(3): 1286-1293.