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ORIGINAL ARTICLE

Immunomodulation and signaling mechanism of *Lactobacillus rhamnosus* GG and its components on porcine intestinal epithelial cells stimulated by lipopolysaccharide

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Abstract *Background/purpose:* This study aimed to evaluate the immunomodulatory effects and signaling mechanisms of *Lactobacillus rhamnosus* GG (LGG) and its components [surface-layer protein (SLP), DNA, exopolysaccharides, and CpG oligodeoxynucleotides] on lipopolysaccharide (LPS)-stimulated porcine intestinal epithelial cell (IEC) IPEC-J2.
Methods: The mRNA expressions of inflammatory cytokines and Toll-like receptors (TLRs) were measured by quantitative real-time polymerase chain reaction. Activation of mitogen-activated protein kinase (MAPK) and nuclear factor kappa B (NF-κB) signaling was detected by western blot and immunofluorescence.
Results: Pretreatment of IPEC-J2 cells with LGG, SLP, or exopolysaccharides significantly alleviated LPS-induced inflammatory cytokines and TLR activation at mRNA level. LGG, SLP, and exopolysaccharides also attenuated LPS-induced MAPK and NF-κB signaling activations. CpG oligodeoxynucleotides significantly increased the interleukin 12, tumor necrosis factor α, and TLR9 mRNA levels and enhanced NF-κB signaling activation in LPS-stimulated cells.
Conclusion: LGG had immunomodulatory effects on LPS-induced porcine IECs by modulating TLR expressions and inhibiting MAPK and NF-κB signaling to decrease inflammatory cytokine

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expressions. Components of LGG exerted immunomodulatory effects on porcine IECs, especially immunostimulatory CpG oligodeoxynucleotides.

Introduction

Intestinal epithelial cells (IECs) play an important role in the innate immune response to pathogens.[1] The porcine small intestinal epithelial cell line (IPEC-J2), which was isolated from the small intestines of neonatal piglets, has features similar to those of porcine primary IECs.[2] IPEC-J2 was used as a model *in vitro* for studying immune responses in pathogen—host interactions.[3]

Lactic acid bacteria (LAB) are one of the commensal bacteria living in the intestinal tracts of piglets.[4] LAB have beneficial effects on its host, but the immunomodulatory effects of LAB on the innate immune system vary between strains.[5–7] *Lactobacillus rhamnosus* GG (LGG) is a well-known probiotic strain isolated from healthy adult feces by Goldin and Gorbach two decades ago.[8] LGG could alleviate inflammation or pathogen-induced barrier dysfunction,[8–10] and could also prevent intestinal injuries induced by rotavirus diarrhea.[11]

Toll-like receptors (TLRs) are a group of pattern recognition receptors and play a critical role in mucosal immune responses.[12] TLRs can recognize microbe-associated molecular patterns; lipopolysaccharides (LPSs) as one of microbe-associated molecular patterns can cause inflammation in IECs through TLR4.[13] Researches have demonstrated that TLR2, TLR4, and TLR9 are involved in the LAB modulation for intestinal inflammation.[2,5,7] Receptors can activate downstream signaling. Nuclear factor kappa B (NF-κB) signaling is known to play an imperative role in immune responses.[14] In response to inflammatory signals, mitogen-activated protein kinase (MAPK) signaling is also activated by phosphorylation of p38MAPK, extracellular signal-regulated kinase (ERK1/2), and Jun N-terminal protein kinase. Previous reports have indicated that LAB modulate the immune responses in IECs through the NF-κB and MAPK signaling.[15–17] Cytokines are produced by the activation of such signaling as part of the IECs' innate immune response to stimuli.[18] Studies have demonstrated that LAB strains could trigger IECs to produce inflammatory cytokines.[2,7,15]

Components of LAB strains exert various effects on IECs, resulting in various effects of LAB strains on intestines. Surface-layer protein (SLP) from LAB had adhesive properties to prevent pathogen invasion.[19] Exopolysaccharides (EPSs) of LGG form a protective shield against inflammatory factors in the intestines.[20] LGG DNA had immunomodulatory effects on TNF-α-induced IECs.[21] The CpG-oligodeoxynucleotides (CpG-ODNs) 5′-ACTTTCGTTTTCTGCGTCAA-3′ from LGG had immunostimulatory effects on immune cells.[22]

The aims of this study are to evaluate the effects of LGG and its components on the expression of cytokines and TLRs, and to elucidate the mechanisms for comprehensive probiotic modulation of IECs by lactobacilli.

Materials and methods

Reagents and antibodies

LPSs were purchased from Sigma-Aldrich (St Louis, MO, USA). TLR4 inhibitor polymyxin B was purchased from InvivoGen (San Diego, CA, USA). NF-κB inhibitor Pyrrolidinedithiocarbamic acid,ammonium salt (PDTC), ERK inhibitor U0126, and p38MAPK kinase inhibitor SB203580 were purchased from Sigma-Aldrich. The anti-phospho-p65, anti-I-κBα, anti-phospho-p38MAPK, anti-p38MAPK, anti-phospho-ERK1/2, anti-ERK1/2, and anti-glyceraldehyde-3-phosphate dehydrogenase (GAPDH) antibodies were purchased from Cell Signalling Technology (Danvers, MA, USA). Horseradish peroxidase- or Fluorescein Isothiocyanate (FITC)-conjugated secondary antibodies were obtained from Jackson ImmunoResearch (West Grove, PA, USA).

Bacterial strain and culture conditions

The LGG was a gift from Professor Jinru Chen at the University of Georgia, Athens, GA, USA. The bacterium was anaerobically grown at 37°C in de Man, Rogosa, and Sharp broth (MRS broth; Hope Bio, Qingdao, Shandong, China) for 18 hours, and then harvested in the logarithmic growth phase and stored at −80°C. Prior to use, the bacteria were thawed and washed with Dulbecco's Modified Eagle Medium/Ham's F-12 (1:1; DMEM/F12; Gibco, Carlsbad, CA, USA). The number of bacterial cells was determined by the plate-counting agar method. The bacterial counts were expressed as colony forming units per milliliter.

Cell culture

The IPEC-J2 cells were a generous gift from Dr Yizhen Wang (Zhejiang University, Hangzhou, P.R. China) and were originally generated in the laboratory of Dr Anthony Blikslager at North Carolina State University (Raleigh, NC, USA).[23] The cells were maintained in an incubator at 37°C in 5% CO_2 in DMEM/F12 supplemented with 10% (v/v) fetal bovine serum (Gibco). Cells (1×10^5 cells/well) were seeded n plastic six-well culture plates (Corning, Tewksbury, MA, USA) and maintained for 14 days (1×10^6 cells/well).

Preparation of components from LGG

The LGG was grown as described above. A bacterial genomic DNA extraction kit (Aidlab, Beijing, China) was used to extract genomic DNA according to previous methods.[24] SLP was obtained from LGG using ultrafiltration with 5M LiCl.[25] EPSs were extracted using trichloroacetic