Research in Veterinary Science 103 (2015) 170–175

Contents lists available at ScienceDirect

Research in Veterinary Science

journal homepage: www.elsevier.com/locate/yrvsc

Lactobacillus and *Lactobacillus* cell-free culture supernatants modulate chicken macrophage activities

W.M. Quinteiro-Filho, J.T. Brisbin, D.C. Hodgins, S. Sharif *

Department of Pathobiology, Ontario Veterinary College, University of Guelph, Guelph, Ontario N1G 2W1, Canada

ARTICLE INFO

Article history:
Received 8 June 2015
Received in revised form 9 October 2015
Accepted 17 October 2015

Keywords:
Macrophages
Cytokines
Phagocytosis
Nitric oxide
Innate immunity
Lactobacilli

ABSTRACT

Lactobacilli are commensal microbes that reside in the intestines of several species, including chickens. Structural constituents of lactobacilli are able to stimulate the host immune system. Macrophages are crucial players in both innate and adaptive immune systems. Here, we investigated the effects of *Lactobacillus acidophilus*, *Lactobacillus reuteri*, *Lactobacillus salivarius* and their cell-free culture supernatants on the pro-inflammatory gene expression profile, nitric oxide (NO) production and phagocytosis by chicken macrophages. Substantial differences were found among *Lactobacillus* strains in their capacity to induce pro-inflammatory cytokines. *L. acidophilus* only up-regulated interferon (IFN)-γ, while *L. reuteri* and *L. salivarius* up-regulated interleukin (IL)-1β, IL-6, IL-8 and IL-12 expression. Supernatant of *L. salivarius* up-regulated IL-1β, IL-8 and IFN-γ expression, while the other cell-free supernatants did not induce significant changes. Moreover, *L. reuteri* and *L. salivarius* increased macrophage phagocytosis, but all cell-free supernatants increased macrophage NO production and did not change phagocytosis activity.

© 2015 Elsevier Ltd. All rights reserved.

1. Introduction

Members of the intestinal microbiota, including commensal bacteria, play an important role in the development and regulation of the immune system. Commensal microbiota can elicit innate and adaptive immune responses in the host via binding to pattern recognition receptors (PRRs) expressed on different cell types, including cells of the immune system (Wells, 2011). The genus *Lactobacillus* comprises a large heterogeneous group of Gram-positive facultative anaerobic bacteria that are the predominant commensal bacteria in the intestinal tract of broiler chickens (Gong et al., 2002; Amit-Romach et al., 2004; Brisbin et al., 2008a; Brisbin et al., 2010).

Over the past decade, different lactobacilli and other probiotic bacteria have been shown to interact with mucosal immune system cells, modulating the immune system of chickens (Brisbin et al., 2008a; Brisbin et al., 2010; Bai et al., 2014). *Lactobacillus acidophilus*, *Lactobacillus reuteri*, and *Lactobacillus salivarius* are normal inhabitants of the chicken intestine (Gong et al., 2007). Live *L. acidophilus*, *L. reuteri* and *L. salivarius* increase interleukin (IL)-1β expression in spleen cells, and substantial differences have been found among these species in regard to their ability to induce T helper (Th)-1 type cytokine expression (Brisbin et al., 2010). When cultured with cecal tonsil cells, *L. acidophilus*, *L. reuteri* and *L. salivarius* increase expression of IL-12 (Brisbin et al., 2010) and regulate the expression of Toll-like receptor (TLR)21, IL-10, interferon (IFN)-γ and transforming growth factor (TGF)-β (Brisbin et al., 2011).

However, not only live *Lactobacillus* can stimulate the immune system, *L. acidophilus* cellular components, such as DNA and bacterial cell wall components, can activate immune system cells (Brisbin et al., 2008b). Exopolysaccharides (major component of bacterial biofilms) produced by *Lactobacillus* can also effectively stimulate production of inflammatory mediators by macrophages in vitro (Ciszek-Lenda et al., 2011). These findings indicate that live lactobacilli, their bacterial components, and/or their metabolites can have immunomodulatory effects on the immune system.

Macrophages play an important role in innate immunity through mechanisms such as nitric oxide production, phagocytosis, and cytokine production. Upon stimulation, macrophages increase in size and motility, and gain enhanced phagocytic potential as well as bactericidal and tumoricidal activity (Bliss et al., 2005). The commensal microbiota can modulate various signaling pathways in macrophages and have corresponding effects on mucosal immunity. For example, certain *Lactobacillus* strains secrete soluble factors that activate NF-κB and STAT (signal transducer and activation of transcription) signaling in macrophages that consequently induce cytokine production and promote an inflammatory response (Miettinen et al., 2000; Matsuguchi et al., 2003). Moreover, this immunostimulatory signaling may be important in promoting host defense against pathogens (Thomas and Versalovic, 2010).

Given the importance of macrophages in the immune system and the involvement of lactobacilli in the modulation of the immune system, the objective of the current study was to examine the ability of three commensal bacteria, *L. acidophilus*, *L. reuteri*, *L. salivarius* and their

* Corresponding author at: Department of Pathobiology, Ontario Veterinary College, University of Guelph, 419 Gordon Street, Building 89, Guelph, Ontario N1G 2W1, Canada.
E-mail address: shayan@uoguelph.ca (S. Sharif).

http://dx.doi.org/10.1016/j.rvsc.2015.10.005
0034-5288/© 2015 Elsevier Ltd. All rights reserved.

respective cell-free culture supernatants to alter NO production, phagocytosis and cytokine expression in chicken macrophages.

2. Materials and methods

2.1. MQ-NCSU cells

MQ-NCSU cells, a chicken macrophage cell line (Qureshi et al., 1990), were provided by Dr. Juan Carlos Rodriguez-Lecompte, University of PEI, Canada. Cells were cultured as described previously by Kramer et al. (2003). Briefly, cells were grown in LM-HAHN medium containing Lebovitz with glutamine (L-15 medium; Sigma Ca # L11518), McCoy's 5A modified medium (Invitrogen; 16600-082), 1% tryptose phosphate broth (Sigma-Aldrich, St. Louis, MO), 1% sodium pyruvate (Sigma; S8636), 8% fetal bovine serum (Invitrogen; 12483020), 10% heat inactivated chicken serum (Invitrogen; 16110-082), containing 200 U/ml penicillin, 80 μg/ml streptomycin. The cells were maintained at 41 °C in a 5% humidified CO_2 incubator. During experiments, cells were cultured in RPMI medium containing 10% fetal bovine serum in addition to penicillin and streptomycin.

2.2. Lactobacillus strains, media, growth conditions and cell-free supernatant harvest

In this study, we used strains from three different *Lactobacillus* species: *L. acidophilus* JTB05, *L. reuteri* JTB07, *L. salivarius* JTB07 (herein referred to as *L. acidophilus*, *L. reuteri* and *L. salivarius*, respectively), and PT (phage type) 193 nalidixic acid-resistant *Salmonella enterica* serovar Typhimurium (referred to as *S.* Typhimurium herein). *L. acidophilus* was isolated from a commercial probiotic product (Merck Animal Health, Canada), whereas *L. reuteri* and *L. salivarius* were isolated in our laboratory from intestinal contents of broiler chickens (Brisbin et al., 2010). All strains of lactobacilli were cultured in MRS broth grown at 41 °C without shaking for 6 h, while *S.* Typhimurium was grown in LB broth at 37 °C under aerobic conditions. All bacteria were harvested by centrifugation (3000 ×*g* for 15 min) at the beginning of the stationary growth phase (6 h of growing). Pelleted bacteria were then washed three times in phosphate-buffered saline (PBS) and diluted in RPMI (Invitrogen) containing 10% fetal bovine serum. *S.* Typhimurium was heat killed (HK) prior to use.

The cell-free supernatants of *Lactobacillus* cultures were processed using an Ultracel® YM50 membrane (MILLIPORE®). Briefly, after centrifugation of the *Lactobacillus* strains, the supernatant was collected and stored in fresh tubes. The supernatant was pipetted into an Ultracel® YM50 device reservoir (0.5 ml) and centrifuged at 12,000 *g* for 12 min. The Ultracel® devices containing Ultracel YM-50 membrane are intended for removing particles larger than 50,000 Daltons (Da) of weight. The filtered supernatant aliquot was harvested, transferred to a fresh tube and stored (−20 °C) until use.

2.3. In vitro stimulation of MQ-NCSU cells

One milliliter of the MQ-NCSU cell suspension (5×10^6 cells/ml) was seeded into a 24-well flat-bottom plate for in vitro stimulation with 1×10^6 CFU of heat-killed *S.* Typhimurium, 1×10^8 CFU live *L. acidophilus*, 1×10^8 CFU live *L. reuteri*, 1×10^8 CFU live *L. salivarius*, 5 μg/mL of LPS, or 10 μl of Supernatant of *L. acidophilus*, Supernatant of *L. reuteri* or Supernatant of *L. salivarius*. The cells were incubated at 41 °C in a humidified 5% CO_2 environment and harvested for RNA extraction at 3, 6, 12, and 18 h post-treatment. Supernatants were collected at 24 h post-treatment for nitrite determination. This experiment was repeated twice each time with 4 replicates.

2.4. RNA extraction and cDNA synthesis

Total RNA was extracted from MQ-NCSU cells using TRIzol® (Invitrogen, Carlsbad, CA) according to the manufacturer's protocol and treated with DNA Free® (Ambion, Austin, TX) DNAse. Subsequently, 500 ng of purified RNA was reverse transcribed to cDNA using Superscript® II First Strand Synthesis kit (Invitrogen, Carlsbad, CA) and oligo-dT primers, according to the manufacturer's recommended protocol. The resulting cDNA was subsequently diluted 1:10 in DEPC treated water.

2.5. Real-time RT-PCR

Quantitative real-time reverse-transcriptase PCR using SYBR Green was performed on diluted cDNA using the LightCycler® 480 II (Roche Diagnostics GmbH, Mannheim, DEU) as previously described (Villanueva et al., 2011). Briefly, each reaction involved a pre-incubation at 95 °C for 10 min, followed by 45 cycles of 95 °C for 10 min, 55 °C–64 °C (Time of Annealing (T_A) as per primer) for 5 s, and elongation at 72 °C for 10 s. Subsequent melt curve analysis was performed by heating to 95 °C for 10 s, cooling to 65 °C for 1 min, and heating to 97 °C. Primers were synthesized by Sigma-Aldrich-Canada (Oakville, ON), and their specific sequences and accession numbers are outlined in Table 1. Relative expression levels of all genes were calculated relative to the housekeeping gene β-actin using the LightCycler® 480 Software (Roche Diagnostics), based on the formula developed by Pfaffl (2001).

2.6. Nitrite assay

Nitrite in cell culture supernatants was quantified using the Griess reagent (Promega, Madison, WI) according to the manufacturer's instructions. Briefly, 100 μl of Griess reagent (1% sulfanilamide and 0.1% naphthalenediamine in 5% phosphoric acid) was added to 50 μl of culture supernatant or culture medium (control) and absorbance at 570 nm was measured using a microplate reader. Nitrite concentrations were determined from a standard curve of sodium nitrite in culture medium.

2.7. Phagocytosis assay

The phagocytosis assay was carried out using fluorescent latex beads (Cayman Chemical, Michigan, USA) according to the manufacturer's instructions and previously described by Brisbin et al. (2015). Briefly, macrophages were seeded into 96-well black polystyrene plates at 1×10^5 cells/well and allowed to adhere for 2 h. The macrophages were then incubated with LPS, heat-killed *Salmonella*, live lactobacilli and *Lactobacillus* cell-free supernatants as described above, and the fluorescent latex beads for 3 h. Cells were incubated for 3 h at 40 °C in a humidified 5% CO_2 environment, centrifuged (400 ×*g* for 10 min),

Table 1
Primer sequences and accession numbers used for real-time PCR.

Target gene	Primer sequence	GenBank accession number
IL-1β	F: 5′-*GTGAGGCTCAACATTGCGCTGTA*-3′ R:5′-*TGTCCAGGCGGTAGAAGATGAAG*-3′	Y15006
IL-6	F: 5′-*CGTGTGCGAGAACAGCATGGAGA*-3′ R: 5′-*TCAGGCATTTCTCCTCGTCGAAGC*-3′	NM_204628.1
IL-8	F: 5′-*CCAAGCACACCTCTCTTCCA*-3′ R: 5′-*GCAAGGTAGGACGCTGGTAA*-3′	AJ009800
IL-12p40	F: 5′-*CCAAGACCTGGAGCACACCGAAG*-3′ R: 5′-*CGATCCCTGGCCTGCACAGAGA*-3′	AY262752.1
IFN-γ	F: 5′-ACACTGACAAGTCAAAGCCGC-3′ R: 5′-AGTCGTTCATCGGGAGCTTG-3′	FJ891061.1
β-Actin	F: 5′-*CAACACAGTGCTGTCTGGTGGTA*-3′ R: 5′-*ATCGTACTCCTGCTTGCTGATCC*-3′	X00182

Reference: Villanueva et al. (2011) and Brisbin et al. (2010).

Download English Version:

https://daneshyari.com/en/article/2454696

Download Persian Version:

https://daneshyari.com/article/2454696

Daneshyari.com