International Immunopharmacology 56 (2018) 205–211

Contents lists available at ScienceDirect

International Immunopharmacology

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

Administration of *Lactococcus lactis* strain Plasma induces maturation of plasmacytoid dendritic cells and protection from rotavirus infection in suckling mice

Kenta Jounai[a,b,*], Tetsu Sugimura[b], Yuji Morita[b], Konomi Ohshio[b], Daisuke Fujiwara[b]

[a] *Technical Development Center, Koiwai Dairy Products Co Ltd. Sayama, Saitama 350-1321, Japan*
[b] *Central Laboratories for Key Technologies, Kirin Co. Ltd., Yokohama, Kanagawa 236-0004, Japan*

ARTICLE INFO

Keywords:
Lactococcus lactis subsp. *lactis* JCM 5805
Plasmacytoid dendritic cells
Rotavirus

ABSTRACT

Lactococcus lactis subsp. *lactis* JCM 5805 (LC-Plasma) is a strain of lactic acid bacteria (LAB) that activates murine and human plasmacytoid dendritic cells (pDCs) to express interferons (IFNs). Oral administration of LC-Plasma drastically decreased fatality levels caused by parainfluenza virus infection in a murine model. In this study, we investigated the anti-viral effects of oral administration of LC-Plasma using a suckling mouse model of rhesus rotavirus (RV) infection. LC-Plasma-fed mice showed improvement in retardation of body weight gain, fecal scores, and a reduction in RV titer in the feces when compared to control mice. The mechanism of anti-viral effects elicited by LC-Plasma administration was investigated using naive mice: in the LC-Plasma -fed mice, lamina propria (LP) pDCs resident in the small intestine were significantly matured and the proportion of pDCs was increased. The expression levels of anti-viral factors induced by IFNs, such as *Isg15*, *Mx1*, *Oasl2* and *Viperin*, and an anti-bacterial factor *Reg3*γ, were up-regulated in the small intestinal epithelial cells (IECs) of LC-Plasma-fed mice. The specific LAB strain may affect the anti-viral immunological profile of IECs *via* maturation of LP pDCs, leading to protection from RV virus infection *in vivo*.

1. Introduction

Rotavirus (RV), a double-stranded (ds) RNA icosahedral virus in the *Reoviridae* family, is the leading cause of severe dehydrating diarrhea in young children worldwide, with 500,000 to 600,000 annual deaths attributed to RV infections [1–3]. RV is well adapted to its host; homologous animal models of RV infection have shown that < 10 infectious units of virus replicate vigorously and initiate diarrheal disease, including viral shedding and efficient spread to uninfected mice [4]. Two vaccines (Rotarix and RotaTeq) are available to prevent RV infection but their use may be limited by financial constraints, especially in low-income countries. They are licensed for use only within a very narrow age window (> 6 weeks and ≤26 weeks of age) to avoid the risk of intussusception [5].

Plasmacytoid dendritic cells (pDCs) act in the innate immune system as the first line of defense against viral infection and, triggered by viral nucleic acids, secrete a large amount of interferon-α (IFN-α) [6,7]. pDCs secretion of type I IFNs is most evident at early time-points in systemic infections with viruses, such as murine cytomegalovirus (MCMV), vesicular stomatitis virus (VSV), lymphocytic choriomeningitis virus (LCMV) and herpes simplex virus 1 (HCV1). IFN secretion mediates an immediate containment of viral replication [8–10]. Viral clearance and protection against re-infection with RV, an intestinal pathogen, is highly dependent on B cells. Type I IFN production by human and murine pDCs exposed to RV is necessary for B cell activation, viral antigen-specific antibody secretion and viral clearance during infection [11,12]. The mucosal IFN system has an important role in curtailing early RV replication in its homologous host to minimize tissue damage [13–15]. A consensus is emerging showing that human and murine pDCs produce IFNs [16–18]. Therefore, stimulation of host pDCs is considered to have a protective effect against RV infection through production of IFNs.

We previously reported that lactic acid bacteria (LAB) strain *Lactococcus lactis* subsp. *lactis* JCM 5805 stimulated murine pDCs to produce IFNs in association with myeloid dendritic cells [19]. *Lactococcus lactis* strain Plasma (LC-Plasma) is a synonym of *Lactococcus lactis* subsp. *lactis* JCM 5805. Additionally, oral administration of LC-Plasma drastically decreased fatality levels in a murine parainfluenza virus-infection model [20]. Recently, Suzuki et al. suggested that LC-Plasma also activated NK activity in association with DCs, including pDCs in mice [21]. LC-Plasma was also shown to activate human pDCs isolated from peripheral blood mononuclear cells (PBMCs) *in vitro* and

* Corresponding author at: Koiwai Dairy Products, Limited, Technical Development Center, 1254, Kamihirose, Sayama-shi, Saitama 350-1321, Japan.
E-mail address: k_jounai@koiwai-dairy.co.jp (K. Jounai).

https://doi.org/10.1016/j.intimp.2018.01.034
Received 16 October 2017; Received in revised form 17 January 2018; Accepted 22 January 2018
1567-5769/ © 2018 Published by Elsevier B.V.

administration of LC-Plasma significantly affected pDCs activity in humans [22]. Anti-viral gene expression in PBMCs elicited by H1N1 (A/PR/8/34) showed that the expression levels of *IFNα* and *ISG15* were higher in the LC-Plasma group compared with the placebo group after the intake period [23].

LAB have been reported to produce potent and diverse immunomodulatory effects and the protective effect of LAB against viral infection is of particular interest: *Lactobacillus pentosus* S-PT84, *L. plantarum* 06CC2, *L. acidophilus* L-92, *Enterococcus faecalis* FK-23 and L. *casei* shirota have all been reported to protect mice from influenza virus infection [24–29]. Oral administration of *Bifidobacterium breve* YIT4064, *Bifidobacterium bifidum, Lactobacillus rhamnosus* GG and VSL#3 has been shown to be effective against RV induced diarrhea [30–35]. Although some LAB has been shown to protect mice from RV infection, little is known about the effect of LAB strain stimulated pDCs on RV infection.

In the present study, we investigated the preventive effects of oral administration of LC-Plasma using a RV infected murine model. We also investigated the mechanism of activation of anti-viral immunity in the intestine induced by oral administration of LC-Plasma. We demonstrated that oral administration of LC-Plasma protected mice against RV infection by activating an immune response in IECs by stimulating pDCs localized in the small intestinal lamina propria (LP).

2. Materials and methods

2.1. Mice

To study the anti-viral effects of LC-Plasma administration in a rhesus RV-infection model, wild-type BALB/c suckling mice (5 days old) with adult female mice (16 weeks old) for childcare were purchased from SLC Japan. BALB/c mice are widely used in RV infection experiments because of their susceptibility to RV. Mice were divided into two groups with an equal average weight. The saline group ($n = 8$) received oral administration of saline (Otsuka Pharmaceutical) and the LC-Plasma group (n = 8) received oral administration of saline containing of 1 mg of heat-killed LC-Plasma/day/mouse. The mice were housed, three per cage, in specific pathogen-free conditions under a 12 h light/dark cycle. The temperature in the room was kept at 21–27 °C and 40–80% humidity. Saline treatment began 2 days before RV infection and continued for 8 days after infection. RV was prepared by the Nihon Bioresearch Inc. Suckling mice (7 days old) were infected by oral application of 50 μl of diluted virus stock at 3.3×10^7 plaque-forming units (PFU)/ml (Fig. 1).

Animal procedures and experiments were approved by the Laboratory Animal Care Committee of the Nihon Bioresearch Inc. The approval ID of these experiments was 081233. Animals were monitored for their conditions: body weight and fecal scores were recorded every day after RV infection. Animals surviving the infection were sacrificed anaesthetically at day 8 using isoflurane.

To study the effect of LC-Plasma administration on immunity in naive mice, wild-type BALB/c suckling mice (5 days old) with adult female mice (16 weeks old) for childcare were purchased from Charles River Japan. Mice were divided into two groups with an equal average weight. The saline group of suckling mice were orally administered saline (Otsuka Pharmaceutical) and the LC-Plasma group of suckling mice were orally administered saline containing of 1 mg of heat-killed LC-Plasma/day/mouse. The mice were housed, one per cage, in specific pathogen-free conditions under a 12 h light/dark cycle. The temperature in the room was kept at 25 ± 1 °C and 60 ± 15% humidity. Animal procedures and experiments were approved by the Laboratory Animal Care Committee of Central Laboratories for Key Technologies, Kirin Co., Ltd. The approval ID of these experiments was YO14-00174. Adequate measures were taken to minimize pain and discomfort, taking into account human endpoints for animal suffering and distress.

2.2. Body weight measurements

Net body weights were measured daily during the course of the experiments and those with severe weight loss, 25% or more, were sacrificed anesthetically.

2.3. Fecal scores after infection with RV

Fecal scores were recorded daily by two independent observers with the same observation criteria to eliminate possible bias associated with individuals. A six-point rating system was used to characterize diarrhea [36]: 1, no stool; 2, normal brown formed stool; 3, soft brown stool; 4, soft-mucous brown-yellow stool; 5, muddy-mucous yellow stool; 6, liquid-mucous yellow stool.

2.4. Virus titer

Fecal virus titer was determined using MA104 cells. Plaque assays were performed using a 1:1 mixture of 3% AVICEL (Thermo Fisher Scientific), 2 ×-concentrated DMEM medium (Invitrogen) and 0.1% BSA. Cells were infected for 1 h at room temperature with virus diluted in 0.1% BSA in PBS.

2.5. Isolation of LP and IECs

LP was isolated as described previously [37]. To prepare neonatal IECs, total small intestinal tissue was cut into small pieces and incubated for 10 min in 30 mM EDTA. After vigorous shaking, epithelial cells were separated from the underlying tissue by filtration through a 100 μm pore size cell strainer (BD Falcon). RNA was isolated from isolated epithelial cell preparations with TRIzol (Invitrogen) according to the manufacturer's instructions.

2.6. Flow cytometry analysis

LP cells were stained with a fluorescent dye conjugated to an antibody as follows: I-A/I-E-FITC (M5/114.15.2) (eBiosciences), CD86-PE (GL1) (eBiosciences), 7-AAD (BD Pharmingen), Siglec-H-APC (551.3D3) (Miltenyi Biotec), and CD11c-PE-Cy7 (N418) (eBiosciences). After staining, the cells were washed twice with FACS buffer (0.5% BSA in PBS buffer) and suspended in FACS buffer for FACS analysis. 7-AAD$^-$ CD11c$^+$ Siglec-H$^+$ cells were defined as pDCs.

To measure intracellular cytokine production, LP cells were treated with a leukocyte activation cocktail using BD GolgiPlug (BD Biosciences) for 4.5 h and with a BD Cytofix/Cytoperm Fixation/Permeabilization kit (BD Biosciences), and then stained with following antibodies: CD4-FITC (RM4-5) (eBiosciences), IL-17A-PE (TC11-18H10) (BD Pharmingen), 7-AAD (BD Pharmingen), IL-22-APC (IL22JOP) (eBiosciences), and CD3ε-APC-Cy7 (145-2C11) (eBiosciences). 7-AAD$^-$ CD3ε$^+$ CD4$^+$ cells were defined as CD4$^+$ T cells.

Data were collected using a FACS Canto II (BD Biosciences) and analyzed by FCS Express software (*De Novo* Software).

2.7. Gene expression analysis

Total RNA was extracted using an RNeasy Kit (Qiagen), and cDNA was prepared using an iScript cDNA synthesis kit (BioRad), according to the manufactures protocols. Quantitative RT-PCR (qRT-PCR) was performed using SYBR Premix Ex Taq (TaKaRa) and a LightCycler 480 (Roche). The methods and primers for qRT-PCR of *Gapdh*, *Isg15*, *Mx1*, *Oasl2* and *Viperin* have been previously described [13,38]. mRNA expression of GAPDH was used as the internal control for normalization of gene expression analysis. The nucleotide sequences of primers were follows: *Gapdh* forward (F) (AACGACCCCTTCATTGAC) and *Gapdh* reverse (R) (TCCACGACATACTCAGCAC), *Isg15* F (GAGCTAGAGCCTGCAGCAAT) and *Isg15* R (TTCTGGGCAATCTGCTTCTT), *Mx1*

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