



Immunomodulatory effect of *Lactococcus lactis* JCM5805 on human plasmacytoid dendritic cells

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Abstract Plasmacytoid dendritic cells (pDCs) play a crucial role in anti-viral immunity through production of large amounts of interferons (IFNs). A previous study revealed the existence of lactic acid bacteria that directly stimulate pDCs in mice. In this study, we demonstrated that *Lactococcus lactis* JCM5805 activates human pDCs and induces IFN production in vitro. In addition, our randomized, placebo-controlled, double blind test showed that yogurt fermented with *L. lactis* JCM5805 activated pDC activity in vivo. This effect was greater in low pDC subjects, and their ability to produce IFNs was increased from the beginning. Furthermore, the risk of morbidity from the common cold was suppressed in the *L. lactis* JCM5805 group compared with the placebo group. In conclusion, intake of *L. lactis* JCM5805 can directly activate pDCs and increase the ability to produce IFNs in vivo. Therefore, *L. lactis* JCM5805 may be a beneficial tool to enhance anti-viral immunity in humans.

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1. Introduction

Viral infection often impinges on our life and sometimes triggers serious problems. pDCs play a crucial role in antiviral immunity as proficient type I interferon (IFN) producing cells (IPC) [1,2]. pDC derived type I IFNs can induce cellular antiviral responses to restrict viral replication and spread [3].

pDCs and pDC-derived type I IFNs also contribute to other immune mechanisms, such as follows: differentiation and maturation of DCs which induces a T helper 1 (Th1)-mediated immune response and cross-presents antigens to CD8⁺ T cells [4–6] and promotion of CD8⁺ T cell function and polarization of CD4⁺ T cells into Th1 cells [7–9]. Takagi et al. [10] have demonstrated the function of pDCs to suppress the induction of CD4⁺ T cell response and participate in the initiation of CD8⁺ T cell using Siglec-H-deficient mice and pDC-ablated mice. In a non-IPC role, pDCs can produce chemokines and recruit CD4⁺ and CD8⁺ T cells to infection loci [11,12]. Furthermore, type I IFNs stimulate activation of NK cells [13] and differentiation of

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B cells into plasma cells [14,15]. pDCs also play a role in mucosal T cell independent IgA production [16]. Thus, pDCs have many important roles in both innate immunity and adaptive immunity.

pDCs express two distinct types of Toll-like receptors (TLRs), TLR7 and TLR9 [17]. TLR7 plays a role in response to ssRNA viruses, such as influenza virus, by sensing, not only the ssRNA of the virus but also synthetic agents, such as imiquimod and R-848 [18–20]. TLR9 plays a role in response to DNA viruses by sensing ssDNA containing CpG motifs, even in synthetic ODNs [21–23]. After sensing viral nucleic acid, via interaction with MyD88, interferon regulatory factor 7 (IRF7) is activated, phosphorylated, and translocated into the nucleus to initiate the transcription of type I IFNs [24,25]. Since pDCs constitutively express high levels of IRF7 [26], have a unique ability to retain TLR9 ligand DNA for long periods in the early endosome and are able to maintain the activation of IRF7 [27], it can rapidly produce abundant type I IFNs compared with other cells [2].

We previously reported that *Lactococcus lactis* JCM5805 activates pDCs and induces type I and type III IFN production in mice [28]. This effect was invaluable in lactic acid bacteria (LAB), although it is also known to occur in pathogenic bacteria, such as *Staphylococcus aureus* [29]. LAB have traditionally been used in fermented foods, such as yogurt, cheese, and pickles, and in addition, they are taken as a supplement due to their effects on intestinal regulation and immunity. One of the remarkable effects of LAB is improvement of allergic symptoms, and we previously reported LAB which have this effect [30–32]. Recently, many researchers have focused on the effects of LAB on phylaxis using infected mice models [33–35] and human clinical tests [36–39].

L. lactis JCM5805 were discovered to be unique LAB which can directly activate pDCs in mice. In this study, we evaluated the effect of *L. lactis* JCM5805 on human pDCs. In accordance with previous results observed in mice, *L. lactis* JCM5805 could activate human pDCs and induce type I and type III IFNs in vitro. Furthermore, in a group administered *L. lactis* JCM5805, pDC activity was higher compared with a group administered placebo yogurt; the amount of IFN- α mRNA was also higher in the JCM5805 group. Interestingly, the increase in pDC activity was higher in subjects with low pDC activity, i.e. in subjects more susceptible to viral infection. Therefore, oral administration of *L. lactis* JCM5805 might contribute against viral infection.

2. Materials and methods

2.1. In vitro analysis

2.1.1. Preparation of LAB

L. lactis JCM5805 was purchased from the microorganism collections held at the Japan Collection of Microorganisms (JCM). *L. lactis* JCM5805 used for the in vitro experiment was cultured at 30 °C for 48 h in M17 broth (OXOID) according to their instructions, washed twice with sterile distilled water, heat killed at 100 °C, lyophilized and suspended in PBS.

2.1.2. Human pDC culture

Human peripheral blood mononuclear cells (PBMCs) from healthy, HIV, HBV, HCV and HTLV donors were purchased

from Lonza. pDCs were purified from PBMCs as follows: 1) monocytes, macrophages and mDCs (myeloid dendritic cells) were removed using a CD14 MicroBead kit (Miltenyi Biotec). 2) pDC fractions were enriched using a CD304 (BDCA4/Neuropilin-1) MicroBead Kit (Miltenyi Biotec). 3) Finally, pDCs were purified using FACS Aria (BD Biosciences). The purity of the final pDC fraction was more than 95% (data not shown). mDCs were purified using CD1c (BDCA-1)⁺ Dendritic Cell Isolation Kit (Miltenyi Biotec).

Purified pDCs were cultured with 10 μ g/ml of *L. lactis* JCM5805, *Lactobacillus rhamnosus* ATCC53103 or 1 μ M CpG2216 (Invitrogen), which was used as a positive control. Experiments were performed in triplicate, at a density of 1×10^5 cells/ml in RPMI medium with 10 ng/ml IL-3 (R&D Systems) in a 96 well plate, for 24 h at 37 °C. In the experiment shown in Fig. 2, pDCs and mDCs were mixed at the same rate, 1×10^5 cells/ml, each.

2.1.3. FACS analysis

Cells were stained with a fluorescent dye conjugated to Abs: CD123-FITC (AC145), BDCA4-APC (AD-17F6) (Miltenyi Biotec.), CD86-PE (IT2.2) (eBioscience), and HLA-DR-PerCP (L243) (BD Pharmingen). After staining, cells were washed twice with FACS buffer (0.5% BSA in PBS buffer) and suspended in 4% paraformaldehyde for FACS analysis. Data were collected by FACS Cant II (BD Biosciences) and analyzed by FCS Express software (De Novo Software). pDCs were defined as CD123⁺ BDCA4⁺ cells, and the expression levels of cell surface markers on pDCs were measured.

2.1.4. ELISA

The ELISA kit for human IFN- α (pan specific) was purchased from MABTECH.

2.1.5. 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 manufacturer's protocol. qRT-PCR was performed using SYBR Premix Ex Taq (TaKaRa) using LightCycler 480 (Roche). Methods and primers for IFN- α 1, α 2, β , λ 1, λ 2/3, and TLR9 have been previously described [40,41] and employed with slight modification. Primers for IRF3, 5, 7, and 8 were originally designed, as follows:

IRF3 F (GTGCTGAGCTGCCTGGGTGG), IRF3 R (CCATGCCC GCTGTTGGGGAG)
 IRF7 F (GTGGAATTCCGGGCACGGCA), IRF7 R (CGGCACAG CCAGGGTTCCAG)
 IRF5 F (CTGAAGCCCTGGCTGGTGGC), IRF5 R (CTCCGTCC TGGCTGGGACCA)
 IRF8 F (CCGGAGGCCTGTCTGGAGTCA), IRF8 R (TGCTGAATG GTGCGCGTCGT).

2.1.6. Microscopic observation of phagocytosis

To investigate phagocytosis of LAB by pDCs, fluorescent-labeled LAB were prepared by suspending LAB at 1 mg/ml in a FITC solution (FITC isomer 1 (Sigma) dissolved in 0.1 M NaHCO₃ pH9.0 buffer at 0.1 mg/ml), and incubated for 60 min at 25 °C. LAB were washed three times with PBS and yielded FITC-labeled LAB. pDCs were purified from PBMCs using FACS Aria as mentioned above, and placed onto glass

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