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Short communication: Probiotic induction of interleukin-10 and interleukin-12 production by macrophages is modulated by co-stimulation with microbial components

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ABSTRACT

Probiotic lactobacilli stimulate macrophages and dendritic cells to secrete cytokines and thereby regulate the immune responses of the host. The balance of the IL-10 and IL-12 production induced by a probiotic is crucial for determining the direction of the immune response. In the present study, we examined the ability of microbial components to modify IL-10 and IL-12 production induced by a popular probiotic strain, *Lactobacillus casei* strain Shirota (LcS), which itself predominantly induces IL-12 production. Microbial ligands for toll-like receptor (TLR)3 and TLR5 further enhanced the IL-12 induction by LcS, whereas ligands for TLR2, TLR4, TLR7, and TLR9 converted the cytokine production pattern from IL-12 predominant to IL-10 predominant. These results indicate that the probiotic induction of IL-10 and IL-12 production can be flexibly modified by co-stimulation with microbial components. This could explain the variety of immunomodulatory functions (immunoactivation or anti-inflammation) exerted by this probiotic strain.
Key words: probiotic, interleukin-10, interleukin-12, toll-like receptor, macrophage

Short Communication

Probiotics are live microorganisms that exert health benefits in the host, with currently popular probiotics including some strains of lactobacilli (WHO/FAO, 2006). One of the most attractive health benefits of probiotics is immune modulation. Thus, several strains of probiotics have been used to make functional dairy foods that aim to maintain the immunological homeostasis of the host (van Baarlen et al., 2013; Linares et al., 2017).

Ingested probiotics must encounter macrophages and dendritic cells to induce the production of a range of cytokines, which themselves trigger various immune responses. In particular, the cytokines IL-12 and IL-10 have received special attention because IL-12 is key to augmenting the immune defense against infections and cancers, whereas IL-10 is critical for regulating excessive immune responses to avoid inflammatory diseases (Trinchieri, 2003; Ouyang et al., 2011). The production of the 2 cytokines is reciprocally regulated (Ma et al., 2015), and the IL-10 and IL-12 response varies with each strain of probiotics, which may be responsible for their specific effects on the host immune system (Kaji et al., 2010; Rask et al., 2013).

We previously elucidated that *Lactobacillus casei* strain Shirota (**LcS**), one of the most popular probiotic strains with immunomodulatory activities, can induce a large amount of IL-12 and little IL-10 and that stimulation of macrophages via the 3-dimensional structure of the cell wall is critical for their cytokine-inducing activity (Shida et al., 2006). In addition, the cell wall teichoic acid derived from *Lactobacillus plantarum* can convert the predominant IL-12 response induced by LcS into a predominant IL-10 response via toll-like receptor (**TLR**)2-dependent extracellular signal-regulated kinase (**ERK**) activation in macrophages (Kaji et al., 2010). Modification of the IL-10 and IL-12 response by TLR-mediated stimuli has also been reported in dendritic cells and monocytes (Barkman et al., 2008; Baba et al., 2009). Thus, we hypothesized that some microbial components other than TLR2 ligands might alter the original properties of the IL-12–inducing immunostimulatory LcS. This would help to explain the variety of its clinical efficacies mediated through immune modulation (Shida et al., 2011). In the present study, we examined the modifying effect of various TLR ligands on IL-10 and IL-12 production by using macrophages stimulated with LcS.

Highly purified or chemically synthesized microbial components/mimics and killed lactobacillus cells were used in this study to understand the basic mechanism

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of the flexible cytokine production induced by probiotics. The following TLR ligands were purchased from InvivoGen (San Diego, CA): lipoteichoic acid (**LTA**; TLR2 ligand), poly (I:C) (TLR3 ligand), LPS (TLR4 ligand), flagellin (TLR5 ligand), gardiquimod (TLR7 ligand), and CpG-DNA (TLR9 ligand). Heat-killed LcS (YIT 9029) and *Lactobacillus johnsonii* YIT 0219^{T} were prepared as described previously (Shida et al., 2006). Briefly, LcS and *L. johnsonii* were obtained from the culture collection of the Yakult Central Institute (Tokyo, Japan), cultured for 20 h at 37°C in Lactobacilli-de Man, Rogosa, and Sharpe broth (Difco, Detroit, MI), collected by centrifugation, heated at 100°C for 30 min, and then lyophilized. Peritoneal macrophages were prepared from BALB/c mice (Japan Clea Co., Tokyo, Japan) 4 d after intraperitoneal injection of 4% thioglycollate broth (Difco), as described previously (Shida et al., 2006). Mice were used at 8 to 12 wk of age in accordance with the guidelines for the care and use of laboratory animals established by the Yakult Central Institute.

To test the response of IL-10 and IL-12 production, macrophages (1×10^5 cells) were cultured with each TLR ligand (1 μg/mL) in the presence or absence of heat-killed lactobacilli (10 μg/mL) in 0.2 mL Roswell Park Memorial Institute 1640 medium containing 10% fetal calf serum in a 96-well culture plate for 24 h. The supernatants were collected, and IL-10 and IL-12p70 levels were analyzed by ELISA. Rat anti-mouse IL-12p35 (clone 9A5) and biotinylated rat anti-mouse IL-12p40 (clone C17.8) monoclonal antibodies were used as capture and detection antibodies, respectively. The antibodies and recombinant mouse IL-12p70 were purchased from BD Pharmingen (San Diego, CA). The mouse IL-10 OptEIA set (BD Bioscience, San Diego, CA) was used to determine IL-10 concentrations.

As shown in Figure 1, TLR ligands alone did not induce substantial levels of IL-12 production, in accordance with previous reports that soluble bacterial cellular components can barely induce IL-12 production in macrophages and monocytes (Shida et al., 2006; Barkman et al., 2008). The LTA, LPS, gardiquimod, and CpG-DNA alone induced substantial levels of IL-10. The LcS alone strongly induced IL-12 production but only weakly induced IL-10 production, indicating that this strain is a potent IL-12 inducer. In combination with LcS, the ligands for TLR3 [poly (I:C)] and TLR5 (flagellin) further enhanced IL-12 production induced by LcS. In contrast, the ligands for TLR2 (LTA), TLR4 (LPS), TLR7 (gardiquimod), and TLR9 (CpG-DNA) synergistically enhanced IL-10 production with LcS but inhibited IL-12 production induced by LcS alone. We previously showed that predominant IL-12 production induced by LcS can be converted to predominant IL-10 production by co-stimulation with a TLR2 ligand, cell wall teichoic acid (Kaji et al., 2010). The present data indicate that some TLR ligands other than TLR2 ligands can also alter the balance of IL-10 and IL-12 production induced by LcS from IL-12 predominance to IL-10 predominance.

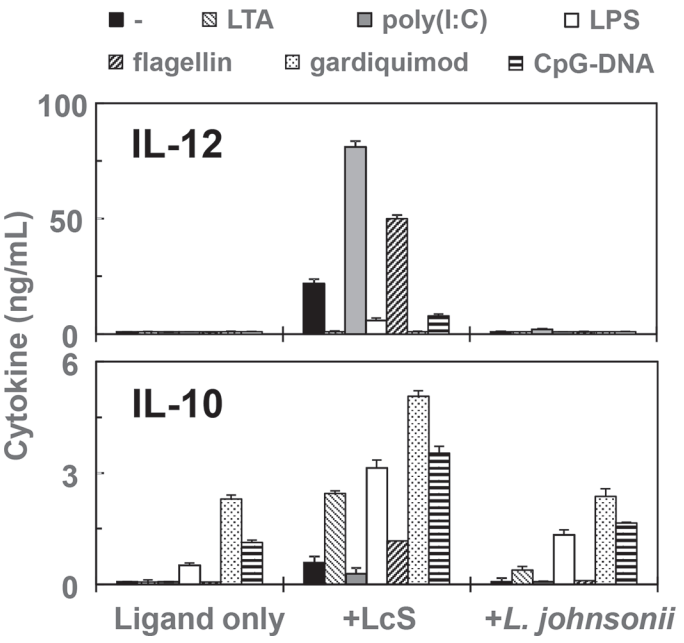

Figure 1. Modification by toll-like receptor (TLR) ligands of IL-10 and IL-12 production induced by *Lactobacillus casei* strain Shirota (LcS). Peritoneal macrophages were cultured with TLR ligands (1 μg/mL) in the presence or absence of lactobacilli (LcS, *Lactobacillus johnsonii*; 10 μg/mL) for 24 h, and the levels of IL-10 and IL-12 in supernatants were determined by ELISA. Data are the mean ± SD of triplicate cultures. Experiments were repeated twice with similar results. − = medium only; LTA = lipoteichoic acid; poly(I:C) = polyinosinic: polycytidylic acid; CpG-DNA = DNA containing unmethylated cytosine-phosphate-guanine motifs.

The modulatory effects of TLR ligands on cytokine production were not observed in the case of co-stimulation with *L. johnsonii*, which originally induced very low levels of IL-10 and IL-12 (Figure 1). We previously showed that *L. johnsonii*, unlike LcS, is digested rapidly in macrophages after phagocytosis and cannot stimulate them via the 3-dimensional structure of the cell wall (Shida et al., 2006). It may be possible that TLR-derived signals modulate only the signals derived from the cell wall structure to lead to flexible IL-10 and IL-12 production.

We previously determined that activation of the ERK pathway via TLR2 is crucial for the conversion of the IL-10 and IL-12 balance from predominantly IL-12 to predominantly IL-10 in LcS-stimulated macrophages (Kaji et al., 2010). Thus, we next examined ERK activation induced by TLR ligands in macrophages. Macrophages (1×10^6 cells) were cultured with each TLR