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Scavenger receptor for lipoteichoic acid is involved in the potent ability of *Lactobacillus plantarum* strain L-137 to stimulate production of interleukin-12p40

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ABSTRACT

Heat-killed *Lactobacillus plantarum* strain L-137 (HK L-137) is a more potent inducer of interleukin (IL)-12 than other heat-killed *Lactobacillus* strains. To elucidate the mechanism involved in this IL-12p40 induction, we compared HK L-137 with heat-killed *L. plantarum* strain JCM1149 (HK JCM1149) by nuclear magnetic resonance and mass spectrometry. Results showed that HK L-137 contained lipoteichoic acid (LTA) with a chemical structure similar to that of JCM1149, except for a lower degree of glucosyl substitution in the poly(glycerol phosphate) backbone. Lysozyme sensitivity and electrophoretic moiety analysis revealed that HK L-137 exposed more LTA on its cell surface than HK JCM1149. Phagocytosis of HK L-137 by splenic adherent cells was significantly greater than that of HK JCM1149. Anti-LTA antibody and anti-scavenger receptor-A (SR-A) antibody selectively inhibited phagocytosis of HK L-137, as well as IL-12p40 production, by splenic adherent cells. Thus, a higher efficiency of phagocytosis of HK L-137 *via* SR-A for LTA is responsible for the potent IL-12p40 induction.

1. Introduction

Many lactic acid bacteria are used as probiotics because of their health-promoting effects, including enhancement of the immune system. *Lactobacillus plantarum* L-137, a strain isolated from a fermented fish and rice dish from the Philippines called "burong isda" [1] is a potent inducer of interleukin (IL)-12 *in vitro* as well as in mice *in vivo* [2]. Administration of heat-killed (HK) L-137 suppressed not only IgE production against a natural antigen in a mouse model of food allergy, but also inhibited tumor growth in mice transplanted with syngeneic tumor cells [2,3]. Furthermore, it was demonstrated that, in healthy subjects, daily intake of HK L-137 enhanced acquired immunity, particularly Th1-related immune functions [4].

Lipoteichoic acid (LTA) of lactobacilli [5] is potent inducers of proinflammatory cytokines such as tumor necrosis factor (TNF)-α and IL-6. *Lactobacillus acidophilus* deficient in LTA [6] exhibited an impaired ability to induce production of proinflammatory cytokines such as TNF-α and IL-12 by human peripheral blood mononuclear cells and murine bone marrow-derived dendritic cells. The amount of fatty acid substitution and the content of unsaturated fatty acid in the glycolipid anchor moiety of LTA reportedly influence the cytokine-inducing capacity of LTA [7,8]. The level of D-Ala substitution in the poly(glycerol phosphate) (GroP) backbone was also reported to affect cytokine induction by LTA [8]. The ability of *L. plantarum*, which contains much less D-Ala in its LTA than other lactobacilli [9], to induce proinflammatory cytokines is also impaired. Thus, structural differences in LTA in the cell walls of lactobacilli may affect their immunomodulatory properties.

Several receptors related to pattern recognition in innate immunity have been identified as receptors for LTA. Toll-like receptor (TLR)-2 recognizes various fungal and bacterial cell wall components, including the LTA of Gram-positive bacteria, and activates a myeloid differentiation factor 88 (MyD88) nuclear factor (NF)-κB pathway, leading to cytokine induction [10–12]. CD36 (aka scavenger receptor B3) are co-receptors of TLR2 for LTA in proinflammatory cytokine responses [13]. CD14 is primarily known as a co-receptor of TLR4, and is part of the lipopolysaccharide (LPS) receptor complex [14]. CD14 also assists in LTA signaling, acting as a coreceptor for TLR2 [13]. CD36 acts as a sensor for LTA and diacylated lipopeptides [15], and also acts as a co-receptor for TLR2 in response to microbial diacylglycerides. CD36 also mediates the phagocytosis of various Gram-positive and Gram-negative bacteria, including *Escherichia coli*, *Klebsiella pneumoniae*, *Salmonella typhimurium*, *Staphylococcus aureus*, and *Enterococcus faecalis* [16]. Scavenger receptor A (SR-A aka CD204) binds to a broad range of polyanionic ligands, including modified lipoproteins, the LPS of Gram-negative bacteria, the LTA of Gram-positive bacteria, and bacterial CpG DNA and double-stranded RNA [17,18]. SR-A can also recognize bacterial surface LTA and lipoproteins, and mediate non-opsonic phagocytosis of Gram-positive bacteria such as *Streptococcus pyogenes* and *S. aureus* [17,19,20].

We previously reported that HK L-137 exhibited a stronger ability to produce IL-12 than HK *L. plantarum* JCM1149^T (HK JCM1149) [21]. The

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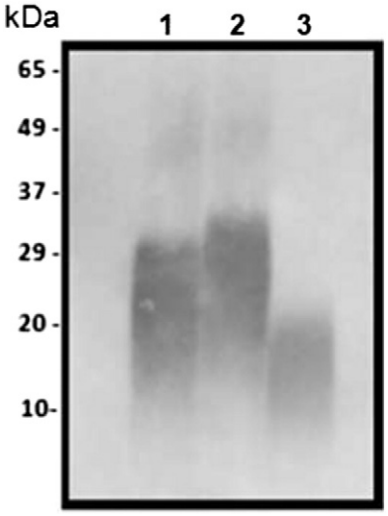

Fig. 1. Western blot analysis of LTA. LTA from *L. plantarum* strains was analyzed by immunoblotting with a mouse anti-LTA antibody (clone 55). Lane 1, LTA obtained from L-137; lane 2, LTA obtained from JCM1149; lane 3, *S. aureus* LTA positive control.

objective of this study was to investigate the mechanisms by which HK L-137 induces potent IL-12p40 production, assessed by comparison of the chemical structure of LTA and recognition receptors between HK L-137 and HK JCM1149.

2. Materials and methods

2.1. Preparation of heat-killed L. plantarum cells

HK L-137 and HK JCM1149 were prepared according to previously described methods [22]. Briefly, *L. plantarum* L-137 and JCM1149 were

Table 1
^{1}H and ^{13}C chemical shift data of LTAs from L-137 and JCM1149.

Strain	Residue	Chemical shifts of proton and carbon in D_2O (δ ppm)					
		1	2	3	4	5	6
L-137							
	GroP-H						
	^{1}H	3.87/3.92	4.02	3.87/3.92	–	–	–
	^{13}C	66.3	69.4	66.3	–	–	–
	GroP-*d*-Ala						
	^{1}H	4.08	5.36	4.08	–	–	–
	^{13}C	63.7	74.1	63.7	–	–	–
	GroP-Glc						
	^{1}H	3.87/3.92	4.09	3.87/3.92	–	–	–
	^{13}C	66.3	75.3	66.3	–	–	–
	Glc						
	^{1}H	5.15	3.52	3.74	3.40	3.89	3.75/3.86
	^{13}C	97.4	71.5	72.9	69.7	71.9	60.5
	d-Ala						
	^{1}H	–	4.26	1.61	–	–	–
	^{13}C	170.0	48.9	15.4	–	–	–
JCM1149							
	GroP-H						
	^{1}H	3.87/3.93	4.02	3.87/3.93	–	–	–
	^{13}C	66.2	69.5	66.2	–	–	–
	GroP-*d*-Ala						
	^{1}H	4.08	5.37	4.08	–	–	–
	^{13}C	63.7	74.2	63.7	–	–	–
	GroP-Glc						
	^{1}H	3.87/3.93	4.09	3.87/3.93	–	–	–
	^{13}C	66.2	75.2	66.2	–	–	–
	Glc						
	^{1}H	5.15	3.51	3.74	3.39	3.91	3.75/3.86
	^{13}C	97.5	71.5	73.0	69.6	71.9	60.5
	d-Ala						
	^{1}H	—	4.27	1.61	—	—	—
	^{13}C	170.0	48.9	15.4	—	—	—

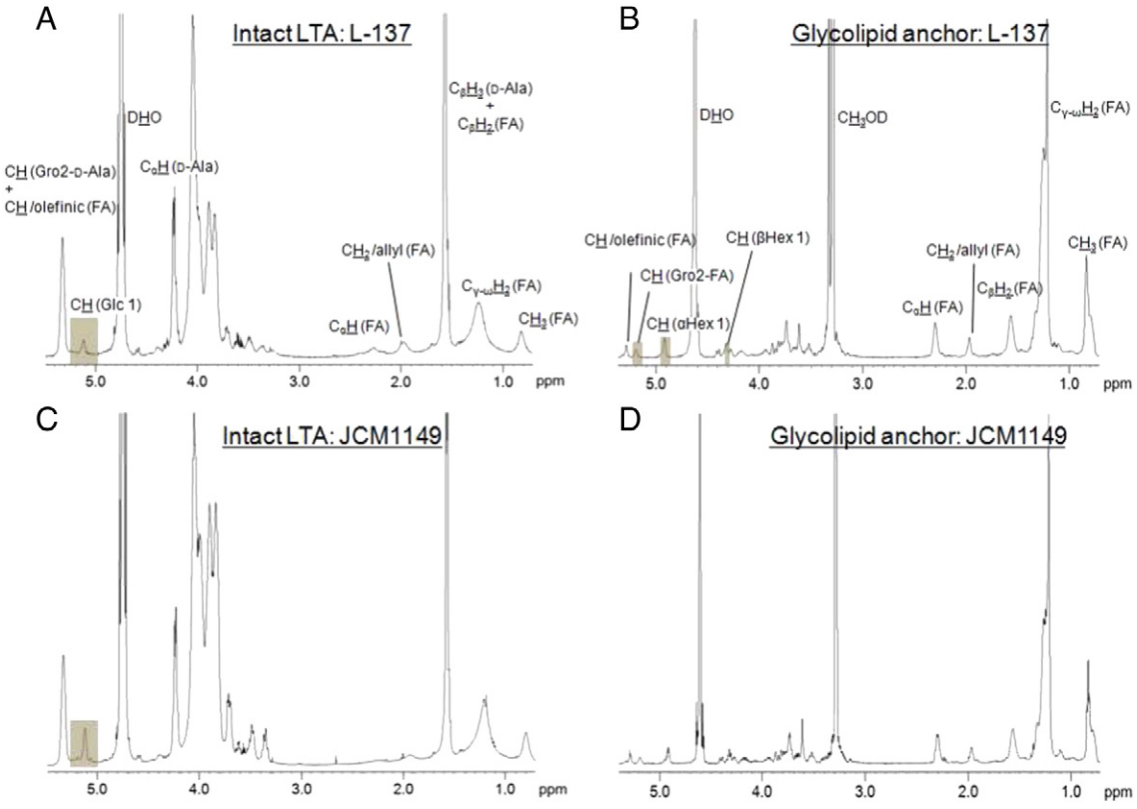

Fig. 2. NMR spectra and specific features of LTA and the glycolipid anchors obtained following HF hydrolysis. ^{1}H NMR spectra (500 MHz, 297 K) of LTA purified from *L. plantarum* L-137 (A) and JCM1149 (C) suspended in D_2O, and those of glycolipid anchors from L-137 (B) and JCM1149 (D) suspended in $CD_3OD/CDCl_3$ (1:1 v/v).