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Article

Differential Gene Expression Profiles in Human THP-1 Monocytes Treated with *Lactobacillus plantarum* or *Staphylococcus aureus* Lipoteichoic Acid

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The lipoteichoic acid (LTA) of *Staphylococcus aureus* (aLTA) and *Lactobacillus plantarum* (pLTA) engage the same toll-like receptor 2 (TLR2) signaling pathway but exert different effects on innate immunity and inflammation. The mechanisms underlying these differential effects are not yet clear. Human oligonucleotide microarrays were used to investigate the transcriptome of human THP-1 monocytes upon exposure to aLTA or pLTA, and differential gene expression profiles were observed between the aLTA- and pLTA-treated cells. The expression level of 1,302 genes in aLTA-treated cells increased more than 2-fold; some of which have been implicated in immune or inflammatory responses, cell adhesion, cell signal transduction, transcription factors, anion transport, proteolysis, and oxidative processes. Particularly, a variety of genes that encode cytokines and chemokines, and TLR signaling-related molecules belonging to the tumor necrosis factor receptor-associated factor (TRAF), nuclear factor-kappa B, and signal transducer and activator of transcription families were remarkably up-regulated by aLTA stimulation. In contrast, pLTA treatment altered the expression of only 90 genes by more than 1.5-fold, and these genes were not correlated with innate immunity, inflammation or other related processes. The different effects mediated by aLTA and pLTA were further verified and compared by analysis of the expression of a selected group of genes, including TRAFs and some cytokines and chemokines, using real time-polymerase chain reaction and ELISA. These data suggest that aLTA and pLTA have different immunomodulatory potentials. Compared with pLTA, aLTA is a stronger stimulator and impacts the expression of many innate immunity- and/or inflammation-related genes.

Key words: Human acute monocytic leukemia cell line (THP-1), Gene expression profile, *Lactoabcillus plantarum*, Lipoteichoic acid, Oligonucleotide microarray, *Staphylococcus aureus*

Introduction

Although both S*taphylococcus aureus* and *Lactobacillus plantarum* are Gram-positive bacteria, they have different physiological effects on human innate immunity and inflammation. *S. aureus* is a common pathogen and is a known etiological agent of Gram-positive bacterial sepsis, whereas *L. plantarum* is nonpathogenic and is regarded as a health-promoting probiotic that inhabits the growth of harmful bacteria in the normal human intestine. Many studies suggest that lactobacilli have anticarcinogenic activities and play important roles not only in regulating the immune system [Grangette *et al.*, 2005; Mohamadzadeh *et al.*, 2005] but also in protecting against intestinal infection and lowering total blood cholesterol level [Bloksma *et al.*, 1979; Ha *et al.*, 2006; Lee and Lee, 2006]).

The immunostimulating component of Gram-positive bacteria is lipoteichoic acid (LTA). LTA is the major

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pathogen-associated molecular pattern (PAMP) of Gram-positive bacteria that mediates innate immunity and inflammatory responses [Seo *et al.*, 2008] in a fashion similar to the recognition of lipopolysaccharide (LPS) in Gram-negative bacterial sepsis [Ginsburg, 2002]. Toll-like receptor (TLR) 2 is a cognate pattern recognition receptor (PRR) for the LTA ligand in inflammatory responses to Gram-positive bacteria [Takeuchi *et al.*, 2000; Schroder *et al.*, 2003]. Like LPS, LTA from pathogenic Gram-positive bacteria such as *S. aureus, Streptococcus pneumoniae*, and *Staphylococcus epidermidis* have been shown to activate monocytes, macrophages, and induce the secretion of proinflammatory cytokines including tumor necrosis factor alpha (TNF-α), interleukin-1 beta (IL-1β), IL-6, and IL-8 [Mattsson *et al.*, 1993; Standiford *et al.*, 1994; Ellingsen *et al.*, 2002]. While strong immune responses are seen after challenge with pathogenic Gram-positive bacteria, the probiotic *L. plantarum* LTA (pLTA) only induces TNF-α production at low levels compared to *S. aureus* LTA (aLTA). Moreover, pLTA was shown to efficiently inhibit aLTA- or LPS-induced TNF-α secretion, resulting in a high survival rate among mice subjected to endotoxic shock [Kim *et al.*, 2008a; 2008b]. These results indicate physiological and functional differences in the LTAs of pathogenic and nonpathogenic Gram-positive bacteria, even though they activate the common TLR2 signaling pathway when interacting with innate immune and inflammatory responses [Han *et al.*, 2003; Kim *et al.*, 2007].

Efforts to utilize probiotics in regulating the immune system and treating gastrointestinal diseases have led to clinical trials using various *L. plantarum* strains [Georgieva *et al.*, 2008]. The beneficial and protective properties of certain probiotic Gram-positive bacteria may be mainly due to the LTA present in their cell walls. The key modulator necessary for monocyte stimulation is the LTA anchor that contains two fatty acids and a glycerophosphate backbone with D-alanine substituents [Deininger *et al.*, 2003]. Structural differences in the LTAs from diverse species, particularly in D-alanine content, have been shown to elicit different immunological responses [Grangette *et al.*, 2005; Velez *et al.*, 2007]. In light of these findings, it was proposed that differences in the chemical structures of aLTA and pLTA contribute to the differential immunostimulatory effects of *S. aureus* and *L. plantarum*.

Although pLTA might have the potential to be used as a therapeutic agent for LPS- or aLTA-induced septic shock, further investigation is needed to understand the molecular events triggered by pLTA-TLR2 interaction on host immune cells [Kim *et al.*, 2008a; 2008b]. Whereas LPS-induced cell responses have been characterized [Malcolm *et al.*, 2003], the gene expression profile of human monocytes exposed to aLTA remains incompletely defined. Therefore, in the present study human oligonucleotide DNA microarrays were used to identify genes that are differentially expressed in human acute monocytic leukemia cell line (THP-1) in response to aLTA or pLTA exposure, and the results revealed that aLTA exposure induced a more robust gene expression response than pLTA exposure. These findings provide valuable clues as to the differences in the cellular mechanisms by which aLTA and pLTA execute their functions in innate immunity and inflammation.

Materials and Methods

Cell culture. Human monocytic THP-1 cells were maintained in RPMI 1640 medium supplemented with 5% fetal bovine serum, 2 mM L-glutamine, penicillin (100 U/mL), and streptomycin (100 μg/mL) at 37°C in a humidified 5% (v/v) CO_2 incubator. The cells were passaged every 3 days.

Cell stimulation and RNA isolation. For total RNA isolation, THP-1 cells were seeded at 5×10^5/mL in 6-well tissue culture plates and stimulated for 6 h with highly purified and structurally intact aLTA (25 μg/mL) prepared from *S. aureus* or pLTA (25 μg/mL) from *L. plantarum*, as described previously [Kim *et al.*, 2007; 2008a]. Untreated cells were seeded in three wells and used as the control. In aLTA- or pLTA-treated cells, three replicates were performed for each sample, and each replicate consisted of three wells. After stimulation, total cellular RNA was extracted from THP-1 cells using RNA Isolation Solvent RNA-Bee (TEL-TEST, Friendswood, TX), according to the manufacturer's protocol. Equal amounts of total RNA from each of the three wells of the control or the aLTA- or pLTA-treated samples were pooled. The amount and quality of RNA were determined spectrophotometrically and assessed by agarose gel electrophoresis. Samples with intact 18S and 28S rRNA peaks were used for cDNA microarray and real time-polymerase chain reaction (RT-PCR) analyses.

Preparation of the DNA microarray. A human genome 70-mer oligonucleotide microarray was obtained from CapitalBio Corporation (Beijing, China) [Guo *et al.*, 2005; Patterson *et al.*, 2006]. Briefly, a human genome oligonucleotide set (ver. 2.0) consisting of 70-mer probes and representing 21,522 *Homo sapiens* genes was purchased from Operon (Huntsville, AL). The oligonucleotides were dissolved in EasyArray™ spotting solution (CapitalBio Corp.) and printed on a PolymerSlide (CapitalBio Corp.). The slide consisted of

48 blocks, and each block had 22 columns and 22 rows.

Human oligonucleotide microarray analyses. Pooled total RNA extracted from normal THP-1 cells (control sample) or cells stimulated with aLTA or pLTA were analyzed with the 22K human oligonucleotide DNA microarray. Double-stranded cDNAs (containing the T7 RNA polymerase promoter sequence) were synthesized from 1 μg total RNA using CbcScript reverse transcriptase and the cDNA synthesis system, according to the manufacturer's protocol (CapitalBio Corp.), together with the T7 Oligo (dT). The cDNA labeled with a fluorescent dye (Cy5 and Cy3-dCTP) was prepared by the Eberwine linear RNA amplification method and subsequent enzymatic reaction as described previously [Guo *et al.*, 2005]. The labeled cDNAs were then used for hybridization with the 22 K human oligonucleotide DNA microarray. Two microarray analyses were performed per sample using a dye-swap procedure to help minimize error due to fluor-associated bias. The DNA microarrays were scanned using a confocal LuxScan™ scanner, and the obtained images were analyzed using the LuxScan™ 3.0 software (both obtained from CapitalBio Corp.). Space- and intensity-dependent normalization of the data based on the LOWESS program was performed [Yang *et al.*, 2002].

Real-time PCR. cDNA was generated from THP-1 cell total RNA using the ImProm-IITM reverse transcription system (Promega, Madison, WI) according to the manufacturer's instructions. Relative mRNA levels were determined using the ABI Prism 7000 Sequence Detection System (Applied Biosystems, Carlsbad, CA), and the PCR products were detected with SYBR Green (BioRad, Hercules, CA). Mean relative mRNA expression was calculated from three replicate assays and expressed as the fold-increase relative to mRNA levels of untreated cells. mRNA expression was normalized to that of β-actin.

ELISA assays. THP-1 cells were incubated in the presence or absence of aLTA (25 μg/mL) or pLTA (25 μg/mL) for 16 h. After treatment, the culture supernatants were removed and assayed for cytokine production by standard sandwich ELISA. All antibody pairs used to measure TNF-α, IL-6, IL-8, and CCL20 levels were purchased from R&D Systems (Minneapolis, MN). Cytokine levels were determined according to the manufacturer's instructions. Cytokine concentrations were calculated using standard curves generated from recombinant cytokines, and the results were expressed in ng/mL.

Statistical analysis. All experiments were performed in triplicate and the presented data are representative results of the means ± SD. An unpaired *t*-test was used to determine the significance of the difference between two datasets. A $p \leq 0.05$ was considered statistically significant.

Results

Differential gene expression profiles in aLTA- and pLTA-treated THP-1 cells. In the present study, two highly pure and intact LTAs were used as stimulants; aLTA was extracted from pathogenic *S. aureus* and pLTA prepared from the probiotic *L. plantarum*. THP-1 cells were treated with either aLTA or pLTA, and the gene expression profiles were analyzed using human oligo microarrays. In aLTA-treated cells, the expression of 1302 genes differed from that in unstimulated controls by more than 2-fold. Of these, 569 genes were up-regulated and 733 were down-regulated in response to aLTA. Detailed gene expression profile information for aLTA-treated cells is provided in Table S1 (supplemental data). In contrast, pLTA treatment resulted in a less robust gene expression profile compared to aLTA. Only 90 genes were expressed at greater than a 1.5-fold change over control cells; 75 of these genes were up-regulated, whereas 15 were down-regulated (Table S2). Hierarchical cluster analysis clearly demonstrated the different gene expression profiles of THP-1 cells stimulated with aLTA or pLTA (Fig. 1), illuminating the striking effect of aLTA treatment on gene expression compared to pLTA.

Although most genes were differentially expressed between aLTA- and pLTA-treated cells, 4 genes were up-regulated and 9 genes were down-regulated by both aLTA and pLTA; however, the expression levels of these genes differed between the groups. In addition, 18 genes were inversely regulated by the two LTA treatments. The alpha-2A adrenergic receptor (*ADRA2A*), metalloproteinase inhibitor 3 (*TIMP3*), a disintegrin and metalloproteinase with thrombospondin motifs 1 (*ADAMTS1*), phosphatidylinositol-4-phosphate 5-kinase type-1 beta (*PIP5K1B*), dickkopf-related protein 2 (*DKK-2*), Rho GTPase activating protein 26 (*ARHGAP26*), interleukin-1 receptor accessory protein (*IL1RAP*), carnitine palmitoyltransferase I (*CPT1A*), cyclin-G1 (*CCNG1*), *NP_006451*, T cell receptor gamma variable 9 (*TRGV9*), hypothetical protein *LOC147343* (*homo sapiens*), SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily a, member 2 (*SMARCA2*), and tribbles homolog 3 (*TRIB3*) genes were up-regulated in response to pLTA, but down-regulated by aLTA treatment. Similarly, zinc finger CCHC domain-containing protein 2 (*ZCCHC2*) and inhibitor of DNA binding 2B (*ID2B*) were up-regulated in aLTA-treated cells, but down-regulated in pLTA-stimulated cells (Table S2). The inverse regulation of these genes may impart the different

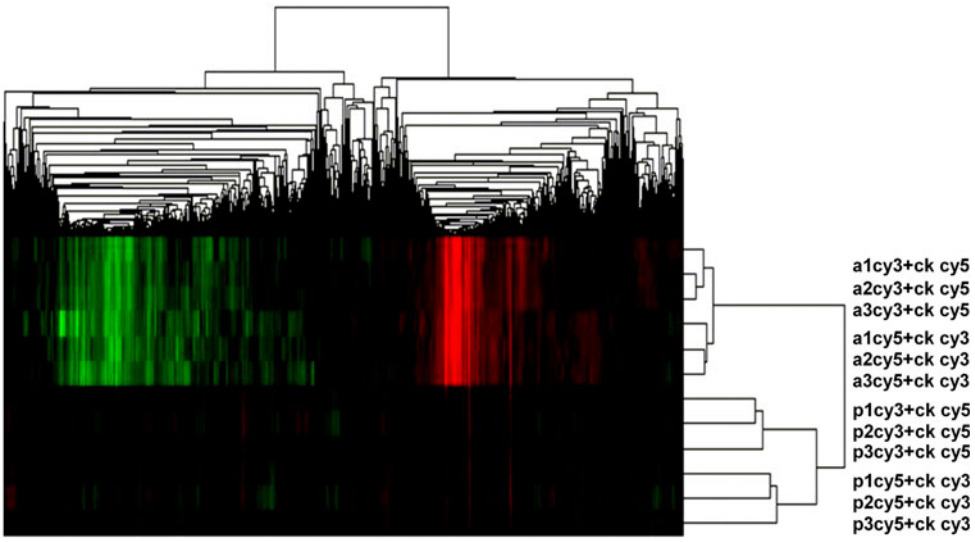

Fig 1. Hierarchical cluster analysis of differentially expressed genes in THP-1 cells stimulated with aLTA (25 μg/mL) or pLTA (25 μg/mL) for 6 h. Genes were categorized into two distinct groups: 1) regulated by aLTA treatment and contained three repeats, i.e., a1, a2, and a3; and 2) regulated by pLTA treatment and contained three repeats, i.e., p1, p2, and p3. Cluster analysis was performed using the publicly available Cluster and TreeView software. Log2-calibrated ratios were clustered with uncentered correlation coefficients and average intercluster linkages. Color in the cluster heat map represents the extent of differential expression: red, induced; green, repressed; and black, unchanged. Branch length in the dendrogram denotes the degree of similarity between clusters.

immunological effects of aLTA and pLTA on the innate immune and inflammatory responses.

aLTA is a major agonist of the innate immune response and inflammation. Analysis of the oligonucleotide microarrays clarified that aLTA is the key immune-stimulating component of pathogenic *S. aureus*. The genes most highly up-regulated in aLTA-treated cells were those associated with cytokines and chemokines and other innate immune response-related molecules, including IL1, nuclear factor-kappa B1 (NF-κB1), and signal transducer and activator of transcription 4 (STAT4) (complete list in Table 1). Compared with aLTA, pLTA stimulation had little to no effect on the expression of genes responsible for innate immunity and inflammation (Table S2). These data suggest that *L. plantarum,* which is known as a beneficial microbe for human health, could not induce the expression of genes involved in innate immunity or inflammatory responses.

Confirmation of microarray data by RT-PCR and ELISA assays. To verify results of the microarray analysis, RT-PCR was performed to confirm the differential expression of TRAFs. Of the 6 TRAF family members, TRAF1, 2, and 3 were up-regulated by aLTA, but not by pLTA. Moreover, TRAF1 was the most highly induced gene in aLTA-treated cells (Fig. 2). These RT-PCR analysis results were consistent with those obtained from the microarray expression studies.

ELISA assays were used to validate the DNA chip assay results, in which the gene expression of several

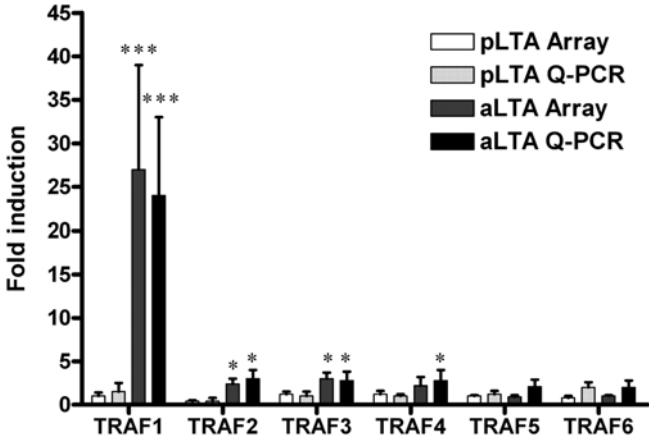

Fig 2. Gene expression changes observed on microarray analysis (Array) and those determined by RT-PCR. THP-1 cells were stimulated with pLTA (25 μg/mL) or aLTA (25 μg/mL) for 6 h prior to cell lysis. Total RNA was extracted and reverse transcribed into cDNA. mRNA levels of the TRAF family genes were quantitatively measured by microarray analysis and RT-PCR, and results are expressed as the fold induction relative to the control. The data shown for RT-PCR represent the mean ± SD of triplicate experiments and were normalized to β-actin. $*p<0.05$, $***p<0.001$ compared to pLTA-treated THP-1 cells.

cytokines and chemokines, including IL-1α, IL-1β, IL-23α, IL-6, IL-8, TNF-α, CCL2, and CCL20, were markedly induced by aLTA, but not by pLTA treatment (Table 1, S2). Among these cytokines and chemokines, TNF-α, IL-6, IL-8, and CCL20 were selected in order to

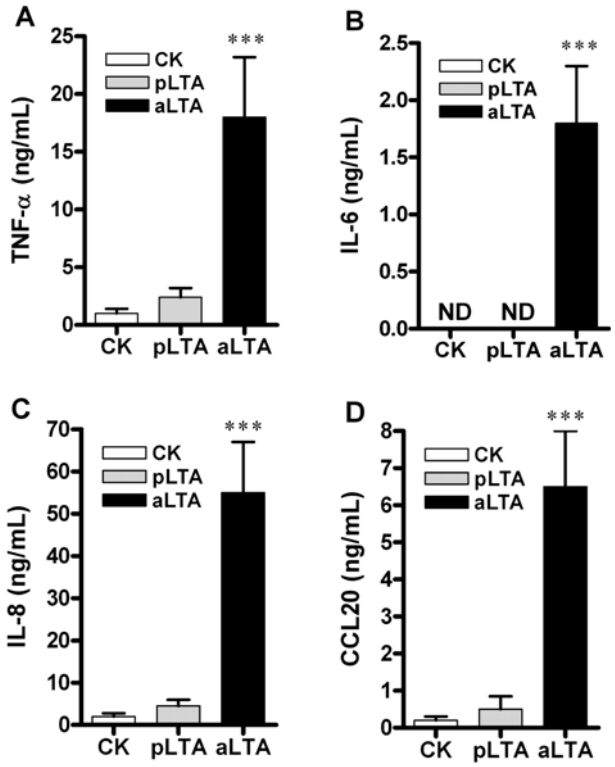

Fig 3. Differential effects of pLTA and aLTA treatment on the production of TNF-α, IL-6, IL-8, and CCL-20 in THP-1 cells. THP-1 cells were stimulated with pLTA (25 μg/mL) or aLTA (25 μg/mL) for 18 h. Untreated cells were used as the control (CK). ELISA experiments were used to measure TNF-α, IL-6, IL-8, and CCL-20 levels in the culture supernatants. Data represent the mean ± SD of triplicate experiments. ND, not detectable. ***p <0.001 compared to CK- or pLTA-treated cells.

investigate the effects of aLTA and pLTA treatment on their secretion in THP-1 cells via ELISA. The results showed that aLTA treatment significantly induced the secretion of TNF-α, IL-6, IL-8, and CCL20, whereas pLTA treatment induced low levels of TNF-α, IL-8, and CCL20 and barely affected IL-6 secretion (Fig. 3). The ELISA data further confirmed that of the DNA chip analysis, in which the mRNA levels of TNF-α, IL-6, IL-8, and CCL20 were significantly elevated in the aLTA- but not in pLTA-treated cells.

Discussion

LTA is a structural component in the cell wall of both pathogenic and nonpathogenic Gram-positive bacteria; however, LTAs from different Gram-positive species are found to differ in the chemical composition of repeating units in their polymeric backbone [Fischer *et al.*, 1990; Behr *et al.*, 1992; Greengerg *et al.*, 1996]. Recent reports on discrepancies in the immunological behavior of LTAs from different pathogenic Gram-positive species might be explained on the basis of these differences in LTA chemical structure [Bhakdi *et al.*, 1991; Han *et al.*, 2003; Schroder *et al.*, 2003]. Our results indicate that two different LTAs purified from the pathogenic *S. aureus* (aLTA) and the probiotic *L. plantarum* (pLTA) have very different effects on cellular responses. Compared to pLTA, aLTA alters gene expression to a greater extent and is a stronger inducer of proinflammatory cytokines. The differentially expressed genes in aLTA-treated cells were shown to be involved in the immune and inflammatory responses, cytoskeleton, cell adhesion, cell cycle, cell signal transduction, transcription factors, anion transport, proteolysis, and oxidative processes. These results shed light on further research opportunities to investigate genes involved in the pathogenesis of *S. aureus*.

Although studies indicate that LTAs from different Gram-positive bacteria exhibit distinct immunostimulatory effects [Han *et al.*, 2003; Schroder *et al.*, 2003; Kim *et al.*, 2008a; 2008b], little is known about their underlying mechanism(s). The differential gene expression profiles observed in aLTA- and pLTA-treated cells illustrate the diversity of the immunological properties of different LTAs. As an analog of LPS, aLTA is a much more potent agonist than pLTA and induces a more robust and varied gene expression. In aLTA-treated cells, genes that were significantly up-regulated encode cytokines and chemokines and other immune-related molecules, such as IL-1, IL-8, TNF-α, CCL20, NF-κB1, NF-κB2, STAT4, STAT5A, TRAF1 and interleukin-1 receptor-associated kinase-like (IRAK) 2. These results further confirm the observation that aLTA is the main factor responsible for Gram-positive bacteria-induced sepsis, similar to LPS from Gram-negative bacteria [Ginsburg, 2002]. In comparison with aLTA treatment, pLTA had a marginal effect on the gene expression profile and had little effect on gene expression of immune or inflammatory response-related molecules in THP-1 cells. These studies support the suggestion that LTA from the probiotic *L. plantarum* could be used as a safe and promising therapeutic agent for curing septic shock [Kim *et al.*, 2008a].

Like LPS, aLTA stimulated the secretion of cytokines and chemokines through the regulation of many immune-response molecules, such as NF-κB, activator protein 1 (AP-1),STAT, TRAF, and IRAK that are indispensable and play important roles in the LTA-triggered TLR2 signaling pathway [Beutler, 2004; Fujioka *et al.*, 2004; Miggin and O'Neill, 2006]. The NF-κB family includes members of inducible proteins that regulate gene expression events involved in immune responses, cell survival, differentiation, and proliferation. This family consists of five members-V-rel reticuloendotheliosis viral oncogene

Table 1. Some up-regulated genes mainly related with innate immune/inflammatory responses in the aLTA-treated THP-1 cells*

Gene Name	Description	Accession No	Fold Change
Cytokine/chemokine and receptor			
IL1α	Interleukin 1 alpha precursor	NM_000575	17.8
IL1β	Interleukin-1 beta precursor	NM_000576	42.8
IL6	Interleukin 6 precursor	NM_000600	3.1
IL8	Interleukin 8 precursor	M17017	34.1
IL23α	Interleukin 23,alpha subunit p19 precursor	NM_016584	34.7
CCL2	Small inducible cytokine A2 precursor	NM_002982	32.1
CCL20	Small inducible cytokine A20 precursor	NM_004591	19.5
TNF-α	Tumor necrosis factor precursor	NM_000594	9.2
TNFRSF5	TNF receptor superfamily member 5 precursor	NM_001250	11.2
CXCL1	Chemokine (C-X-C motif) ligand 1	NM_001511	5.5
ICAM1	Intercellular adhesion molecule 1 (CD54)	NM_000201	13.7
IL10Rα	Interleukin-10 receptor alpha chain precursor	NM_001558	9.1
IL15Rα	Interleukin 15 receptor alpha isoform 1 precursor	NM_172200	15.7
Signal transduction			
STAT1	Signal transducer and activator of transcription 1	NM_007315	2.1
STAT4	Signal transducer and activator of transcription 4	NM_003151	13.2
STAT5A	Signal transducer and activator of transcription 5A	NM_003152	22.0
DUSP2	Dual specificity protein phosphatase 2	NM_004418	10.7
DUSP4	Dual specificity protein phosphatase 4	NM_001394	9.2
DUSP6	Dual specificity protein phosphatase 6	NM_001946	5.1
IFITM11	Interferon-induced transmembrane protein	NM_003641	8.3
GADD45β	Growth arrest and DNA-damage-inducible 45 beta	NM_015675	4.6
Transcriptional regulation			
NF-κB1	Nuclear factor NF-kappa-B p105 subunit	NM_003998	23.7
NF-κB2	Nuclear factor NF-kappa-B p100 subunit	NM_001077493	20.6
REL	C-Rel proto-oncogene protein	NM_002908	7.2
Rel B	V-rel reticuloendotheliosis viral oncogene homolog B	M83221	4.2
NFKBIA	NF-kappaB inhibitor alpha	NM_020529	6.5
IRF1	Interferon regulatory factor 1	NM_002198	2.3
IRF7	Interferon regulatory factor 7	NM_001572	10.8
Adaptor and other molecules			
SH2B2	SH2 and PH domain-containing adapter protein APS	NM_020979	2.3
TRAF1	SH2 and PH domain-containing adapter protein APS	NM_005658	27.5
TRAF3	TNF receptor associated factor-3	AF110908	2.8
TNFAIP3	TNF alpha-induced protein 3	NM_006290	15.2
MyD88	Myeloid differentiation primary response protein	NM_002468	2.5

*The data of genes changed more than 2.0-fold in the aLTA-treated cells are available in the supplemental material, Table S1.

homolog (Rel) A (p65), NF-κB1 (p50), NF-κB2, C-Rel, and RelB [Hayden and Ghosh, 2008]. In the present study, the gene expression of all NF-κB family members, except for RelA, was significantly up-regulated in aLTA-treated cells in comparison with pLTA-treated cells (Table 1). Similarly, NF-κB activity was also markedly increased by aLTA treatment (data not shown). Higher NF-κB expression level and activity in aLTA-treated cells result in increased cytokine production, because the secretion of many cytokines, such as IL-8 and TNF-α, is mostly NF-κB-dependent [Matsusaka *et al.*, 1993]. Although NF-κB activity is mainly regulated by the interaction of the molecule with a family of NF-κB inhibitors known as inhibitor of kappa B (IκB) [Baeuerle and Baltimore, 1998], transcriptional up-regulation of NF-κB could increase their protein levels and promote cytokine secretion in aLTA-treated cells. Conversely, it is intriguing that aLTA also induces the expression of genes that encode negative

regulators of NF-κB, such as tumor necrosis factor, alpha-induced protein 3 (*TNFAIP3:* A20) and nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, alpha (*NFκBIA:* IκBα), similar to LPS [Jang *et al.*, 2009]. Collectively, these data suggest that aLTA stimulation could simultaneously activate pro- and anti-inflammatory pathways, which may facilitate the maintenance of the innate immune response or inflammation.

TRAF proteins are critical intermediates in almost all NF-κB signaling pathways and are believed to be important regulators of cell death and cellular responses to stress [Bradley and Pober, 2001]. The present study demonstrated that TRAF1 was only up-regulated in aLTA-treated cells. The remarkably increased expression of TRAF1 in aLTA-treated cells suggests that TRAF1 could be an essential mediator in aLTA-elicited TLR2 signaling and could possibly be involved in *S. aureus*-induced septic shock. Since TRAF proteins are expressed in a regulated fashion in normal and diseased tissues, and each family member could play a distinctive role [Chung *et al.*, 2002; Oyoshi *et al.*, 2008], it is important to understand the mechanisms by which TRAF proteins are regulated in physiological and pathological processes and the involvement of these proteins in cytokine and chemokine secretion.

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References

Baeuerle PA and Baltimore D (1998) I kappa B: A specific inhibitor of the NF-kappa B transcription factor. *Science* **242**, 540-546.

Behr T, Fischer W, Peter-Katalinic J, and Egge H (1992) The structure of *pneumococcal* lipoteichoic acid: Improved preparation, chemical and mass spectrometric studies. *Eur J Biochem* **207**, 1063-1075.

Beutler B (2004) Inferences, questions and possibilities in Toll-like receptor signaling. *Nature* **430**, 257-283.

Bhakdi S, Klonisch T, Nuber P, and Fischer W (1991) Stimulation of monokine production by lipoteichoic acids. *Infect Immunol* **59**, 4614-4620.

Bloksma N, de Heer E, van Dijk H, and Willers JM (1979) Adjuvanticity of lactobacilli. I. Differential effects of viable and killed bacteria. *Clin Exp Immunol* **37**, 367-375.

Bradley JR and Pober JS (2001) Tumor necrosis factor receptor-associated factors (TRAFs). *Oncogene* **20**, 6482-6491.

Chung JY, Park YC, Ye H, and Wu H (2002) All TRAFs are not created equal: Common and distinct molecular mechanisms of TRAF-mediated signal transduction. *J Cell Sci* **115**, 679-688.

Deininger S, Stadelmaier A, von Aulock S, Morath S, Schmidt RR, and Hartung T (2003) Definition of structural prerequisites for lipoteichoic acid-inducible cytokine induction by synthetic derivatives. *J Immunol* **170**, 4134-4138.

Ellingsen E, Morath S, Flo T, Schromm A, Hartung T, Thiemermann C, Espevik T, Golenbock D, Foster D, Solberg R, Aasen A, and Wang J (2002) Induction of cytokine production in human T cells and monocytes by highly purified lipoteichoic acid: Involvement of Toll-like receptors and CD14. *Med Sci Monit* **8**, 149-156.

Fischer W, Mannsfeld T, and Hagen G (1990) On the basic structure of poly (glycerophosphate) lipoteichoic acids. *Biochem Cell Biol* **68**, 33-43.

Fujioka S, Niu JG, Schmidt C, Sclabas GM, Peng BL, Uwagawa T, Li ZK, Evans DB, Abbruzzese JL, and Chiao PJ, (2004) NF-κB and AP-1 connection: Mechanism of NF-κB-dependent regulation of AP-1 activity. *Mol Cell Biol* **24**, 7806-7819.

Georgieva RN, IIiev IN, Chipeva VA, Dimitonova SP, Samelis J, and Danova ST (2008) Identification and in vitro characterization of *Lactobacillus plantarum* strains from artisanal Bulgarian white brined cheeses. *J Basic Microbiol* **48**, 234-244.

Ginsburg I (2002) Role of lipoteichoic acid in infection and inflammation. *Lancet Infect Dis* **2**, 171-179.

Grangette C, Nutten S, Palumbo E, Morath S, Hermann C, Dewulf J, Pot B, Hartung T, Hols P, and Mercenier A (2005) Enhanced antiinflammatory capacity of *Lactobacillus plantarum* mutant synthesizing modified teichoic acids. *Proc Natl Acad Sci USA* **102**, 10321-10326.

Greengerg JW, Fischer W, and Joiner KA (1996) Influence of lipoteichoic acid structure on recognition by the macrophage scavenger receptor. *Infect Immunol* **64**, 3318-3325.

Guo Y, Guo H, Zhang L, Xie H, Zhao X, Wang F, Li Z, Wang Y, Ma S, Tao J, Wang W, Zhou Y, Yang W, and Cheng J (2005) Genomic analysis of anti-hepatitis B virus (HBV) activity by small interfering RNA and lamivudine in stable HBV-producing cells. *J Virol* **79**, 14392-14403.

Ha CG, Cho JK, Lee CH, Chai YG, Ha YA, and Shin SH (2006) Cholesterol lowering effect of *Lactobacillus plantarum* isolated from human feces. *J Microbiol Biotechnol* **16**, 1201-1209,

Han SH, Kim JK, Martin M, Michalek SM, and Nahm MH (2003) *Pneumococcal* lipoteichoic acid (LTA) is not as potent as *Staphylococcal* LTA in stimulating Toll-like receptor 2. *Infect Immunol* **71**, 5541-5548.

Hayden MS and Ghosh S (2008) Shared principles in NF-κB signaling. *Cell* **132**, 344-362.

Jang HS, Chung HS, Ko EJ, Shin JS, Shin MK, Hong MC, Kim YS, Min BI, and Bae HS (2009) Microarray analysis of gene expression profiles in response to treatment with

bee venom in lipopolysaccharide activated RAW 264.7 cells. *J Ethnopharmacol* **121**, 213-220.

Kim HG, Gim MG, Kim JY, Hwang HJ, Ham MS, Lee JM, Hartung T, Park JW, Han SH, and Chung DK (2007) Lipoteichoic acid from *Lactobacillus plantarum* elicits both the production of Interleukin-23p19 and suppression of pathogen-mediated Interleukin-10 in THP-1 cells. *FEMS Immunol Med Microbiol* **49**, 205-214.

Kim HG, Kim NR, Gim MG, Lee JM, Lee SY, Ko MY, Kim JY, Han SH, and Chung DK (2008a) Lipoteichoic acid isolated from *Lactobacillus plantarum* inhibits lipopolysaccharide-induced TNF-α production in THP-1 cells and endotoxin shock in mice. *J Immunol* **180**, 2553-2561.

Kim HG, Lee SY, Kim NR, Ko MY, Lee JM, Yi TH, Chung SK, and Chung DK (2008b) Inhibitory effects of *Lactobacillus plantarum* lipoteichoic acid (LTA) on *Staphylococcus aureus* LTA-induced tumor necrosis factor-alpha production. *J Microbiol Biotechnol* **18**, 1191-1196.

Lee HM and Lee YH (2006) Isolation of *Lactobacillus plantarum* from kimchi and its inhibitory activity on the adherence and growth of *Helicobacter pylori*. *J Microbiol Biotechnol* **16**, 1513-1517.

Malcolm KC, Arndt PG, Manos EJ, Jones DA, and Worthen GS (2003) Microarray analysis of lipopolysaccharide-treated human neutrophils. *Am J Physiol Lung Cell Mol Physiol* **284**, 663-670.

Matsusaka T, Fujikawa K, Nishio Y, Mukaida N, Matsushima K, Kishimoto T, and Akira S (1993) Transcription factors NF-IL6 and NF-kappa B synergistically activate transcription of the inflammatory cytokines, interleukin 6 and interleukin 8. *Proc Natl Acad Sci USA* **90**, 10193-10197.

Mattsson E, Verhage L, Rollof J, Fleer A, Verhoef J, and van Dijk H (1993) Peptidoglycan and teichoic acid from *Staphylococcus epidermidis* stimulate human monocytes to release tumour necrosis factor-alpha, interleukin-1β and interleukin-6. *FEMS Immunol Med Microbiol* **7**, 281-287.

Miggin SM and O'Neill LAJ (2006) New insights into the regulation of TLR signaling. *J Leukoc Biol* **80**, 1-7.

Mohamadzadeh M, Olson S, Kalina WV, Ruthel G, Demmin GL, Warfield KL, Bavari S, and Klaenhammer TR (2005) Lactobacilli activate human dendritic cells that skew T cells toward T helper 1 polarization. *Proc Natl Acad Sci USA* **102**, 2880-2885.

Oyoshi MK, Bryce P, Goya S, Pichavant M, Umetsu DT, Oettgen HC, and Tsitsikov EN (2008) TNF receptor-associated factor 1 expressed in resident lung cells is required for the development of allergic lung inflammation. *J Immunol* **180**, 1878-1885.

Patterson TA, Lobenhofer EK, Fulmer-Smentek SB, Collins PJ, Chu TM, Bao W, Fang H, Kawasaki ES, Hager J, Tikhonova IR, Walker SJ, Zhang L, Hurban P, de Longueville F, Fuscoe JC, Tong W, Shi L, and Wolfinger RD (2006) Performance comparison of one-color and two-color platforms within the MicroArray Quality Control (MAQC) project. *Nat Biotechnol* **24**, 1140-1150.

Schroder NWJ, Morath S, Alexander C, Hamann L, Hartung T, Zahringer U, Gobel UB, Weber JR, and Schumann RR (2003) Lipoteichoic acid (LTA) of *Streptococcus pneumoniae* and *Staphylococcus aureus* activates immune cells via Toll-like receptor (TLR)-2, lipopolysaccharide-binding protein (LBP), and CD14, whereas TLR-4 and MD-2 are not involved. *J Biol Chem* **278**, 15587-15594.

Seo HS, Michalek SM, and Nahm MH (2008) Lipoteichoic acid is important in innate immune responses to Gram-positive bacteria. *Infect Immunol* **76**, 206-213.

Standiford TJ, Arenberg DA, Danforth JM, Kunkel SL, Van Otteren GM, and Strieter RM (1994) Lipoteichoic acid induces secretion of interleukin-8 from human blood monocytes: A cellular and molecular analysis. *Infect Immunol* **62**, 119-125.

Takeuchi O, Hoshino K, and Akira S (2000) Cutting edge: TLR2-deficient and MyD88-deficient mice are highly susceptible to *Staphylococcus aureus* infection. *J Immunol* **165**, 5392-5396.

Velez MP, Verhoeven TLA, Draing C, Aulock SV, Pfitzenmaier M, Geyer A, Lambrichts I, Grangette C, Pot B, Vanderleyden J, and De Keersmaecker SCJ (2007) Functional analysis of D-alanylation of lipoteichoic acid in the probiotic strain *Lactobacillus rhamnosus* GG. *Appl Environ Microbiol* **73**, 3595-3604.

Yang YH, Dudoit S, Luu P, Lin DM, Peng V, Ngai J, and Speed TP (2002) Normalization for cDNA microarray data: a robust composite method addressing single and multiple slide systematic variation. *Nucleic Acids Res* **30**, e15.