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Original Article

Manganese superoxide dismutase induced by lipoteichoic acid isolated from *Staphylococcus aureus* regulates cytokine production in THP-1 cells

Hangeun Kim [a,*,1], Hyeoung-Eun Kim [c,1], Dae Kyun Chung [a,b,c,], Kyoung Ok Jang [c]**

[a] *Research and Development Center, Skin Biotechnology Center Inc., Yongin, 17104, Republic of Korea*
[b] *Skin Biotechnology Center, Kyung Hee University, Suwon, 16229, Republic of Korea*
[c] *Graduate School of Biotechnology, Kyung Hee University, Yongin 17104, Republic of Korea*

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Abstract Lipoteichoic acid isolated from *Staphylococcus aureus* (aLTA) is known to regulate the production of pro-inflammatory cytokines through TLR2-mediated signaling pathways. In our previous study, we found that aLTA significantly increased manganese superoxide dismutase (MnSOD) in the THP-1 human monocyte-like cell line, but the role of MnSOD in the regulation of cytokine production was not elucidated. In the current study, we found that MnSOD was involved in aLTA-mediated cytokine production. The signaling pathways associated with aLTA-mediated MnSOD induction in THP-1 cells included TLR2-MyD88-IRAK2, JNK (c-Jun N-terminal kinases)1/2 and nuclear factor- κB (NF-κB). We also found MnSOD was involved in the regulation of IL-1β and TNF-α, which were induced by early signaling pathways, including JNK1/2, p38, and NF-κB p65. In addition, MnSOD was also involved in the production of IL-6 and CCL2 in aLTA-stimulated THP-1 cells through activation of late signaling pathways such as JAK2-STAT3. Taken together, our data suggest that aLTA-mediated MnSOD production involved in the regulation of cytokine production and it may be the cause of one of the excessive inflammatory reactions caused by *S. aureus*.

* Corresponding author. Research and Development Center, Skin Biotechnology Center Inc., Yongin, 17104, Republic of Korea. Fax: +82 31 888 6177.
** Corresponding author. Graduate School of Biotechnology, Kyung Hee University, Yongin 17104, Republic of Korea. Fax: +82 31 202 3461.
E-mail addresses: hkim93@khu.ac.kr (H. Kim), dkchung@khu.ac.kr (D.K. Chung).
[1] These authors contributed equally in this study.

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Introduction

Lipoteichoic acid (LTA) purified from *Staphylococcus aureus* cell wall (designated as aLTA) has been reported to be a major immunostimulant. In previous studies, aLTA strongly induced the expression of cytokines such as TNF-a and IL-6 in Kupffer cells.[1] Unlike aLTA, pLTA, the LTA isolated from *Lactobacillus plantarum*, only moderately induces inflammatory cytokines, but has strong anti-inflammatory effects in lipopolysaccharide (LPS)-induced septic shock.[2] Generally, LTA-mediated mitogen-activated protein kinase (MAPK) activation is regulated by the toll-like receptor (TLR) 2 receptor, and the interaction between TLR2 and myeloid differentiation primary response 88 (MYD88), interleukin-1 receptor (IL-1R) associated kinases (IRAKs), and TNF-receptor-associated factor 6 (TRAF6).[3] However, LTA seems to activate different signaling mechanisms depending on the target gene. For example, in LTA-treated cells serum response elements (SREs) are regulated by the ERK pathway, including the phosphorylation of ERK-1, MEK1/2, and c-Raf.[4] With regard to the induction of Matrix metalloproteinase (MMP)-9, LTA activates the PKC (alpha)-dependent pathway, which is associated with the activation of ATF2/AP-1 in RBA-1 cells.[5] In addition, LTA isolated from different lactobacilli species have differing immunostimulatory effects, which are mediated by the ability of LTA to activate the MAPK signaling pathway.[6] In addition to TLR2, induction of the LTA-mediated inflammatory response requires lipopolysaccharide-binding protein (LBP), CD14, and nuclear protein high-mobility group box 1 (HMGB1).[7]

Manganese superoxide dismutase (MnSOD) is an essential mitochondrial antioxidant enzyme that detoxifies the superoxide free radical, the major by-product of mitochondrial respiration.[8,9] By catalyzing the dismutation of superoxide to molecular oxygen and H_2O_2, MnSOD controls the generation of H_2O_2, the accumulation of which may lead to abnormal cell proliferation through activation of NF-κB.[10] A role for MnSOD in the production of cytokines has also been postulated. Researchers have shown that an Ala16Val-SOD2 gene polymorphism induced production of pro-inflammatory cytokines, such as IL-1, IL-6, IL-10, TNF-α, and IFN-γ, in human peripheral blood mononuclear cells (PBMCs).[11] In addition, MnSOD negatively regulates the expression of inflammatory cytokines such as TNF-α and IL-1β through the elimination of reactive oxygen species (ROS).[12]

Cytokines, such as IL-1β, TNF-α, and IFN-γ, induce MnSOD messenger RNA (mRNA) expression and its activity.[13,14] However, there have been few studies addressing the relationship between LTA and MnSOD. In this study, we found that aLTA stimulation of THP-1 cells increased MnSOD production, which influenced the secretion of cytokines.

Methods

Cell culture and treatment

The THP-1 human monocyte-like cell line was maintained in RPMI-1640 medium supplemented with 10% fetal bovine serum (FBS), 2 mM L-glutamine, 100 U/ml penicillin, and 100 μg/ml streptomycin in a humidified 37 °C incubator with 5% CO_2. The THP-1 cells (1×10^4—1×10^6 cells/ml) were stimulated with pLTA or aLTA for the indicated times prior to RNA and protein analyses using several methods, including reverse transcriptase-polymerase chain reaction (RT-PCR), quantitative PCR, enzyme-linked immunosorbent assay (ELISA), and western blotting.

For the RT-PCR and quantitative PCR assays, cells (1×10^5 cells/ml) were seeded into 6- well plates and incubated with the LTAs for the indicated times. For siRNA transfection, cells (1×10^5 cells/ml) were seeded into 12-well plates and transiently transfected with siRNAs for LR2, MyD88, IRAK2, MnSOD, and the negative control (QIAEN, MD, USA) after combination with Lipofectamine® RNAiMAX Transfection Reagent (Thermofisher Scientific, MA, USA). After 36—48 h transfection, transfected cells were incubated with 100 μg/ml of the LTAs for the indicated times to examine the effects on TLR2, MyD88, IRAK2, and MnSOD, as well as cytokines, such as IL-1β, TNF-α, IL-6 and CCL2.

To block LTA-mediated signaling pathways, cells (1×10^4 cells/well) were pre-incubated with inhibitors (10 μM), such as Akt, ERK, JNK, NF-κB, pI3K, and p38 (Sigma—Aldrich, MO, USA), for 30 min, and then incubated with 100 μg/ml of the LTAs for 24 h. In additional studies, cells were pre-incubated with neutralization antibodies for TLR2, TLR4, CD14 (Invivogen, CA, USA) for 30 min prior to LTA stimulation.

LTA preparation

The LTAs were isolated from *L. plantarum* K8 (KCTC 10887BP; pLTA) and *S. aureus* (ATCC 25923; aLTA) as previously described.[2] Protein and endotoxin contamination were assessed using silver staining and an endotoxin assay kit (GenScript, NJ, USA), respectively, and the results confirmed that the purified LTAs were not contaminated.

RT-PCR and quantitative PCR

Total RNA was extracted from THP-1 cells after stimulating with pLTA and aLTA for the indicated times and cDNA synthesis was performed with the cDNA Synthesis Kit (Bio-Rad, CA, USA) according to the manufacturer's instructions. The expression of messenger RNA (mRNA) was examined by RT-PCR and real-time PCR. For RT-PCR, the following sequences for the forward and reverse primer pairs were used: 5′-ACTTTGCTGGCTACTGTGCT-3′ and 5′-GTGCTCTGGGTGCTTCTCAA-3′ for IRAK-1; 5′-CAGCAACTTGTGGACCTCCT-3′ and 5′-CATTGGGGTGGCAGCATCTA-3′ for IRAK-2; 5′-TTGGTCCTGGGCACAGAAAA-3′ and 5′-TCGAATGTGCCAAGGGAGTG-3′ for IRAK-M; 5′-GCTTCCTAGTTCGGCTGGTT-3′ and 5′-GACTTGAGGAGTCAGGTGGC-3′ for IRAK-4; 5′-CCATGGGGAAGGTGAAGGTC-3′ and 5′-AGTGATGGCATGGACTGTGG-3′ for glyceraldehyde-3-phosphate dehydrogenase (GAPDH).

For real-time PCR, the CFX Connect™ Real-Time PCR Detection System (Bio-Rad) was used in the analyses of mRNA variation. The following sequences for the forward and

reverse primer pairs were used: 5′-GGCCTACGTGAACAACCTGA-3′ and 5′-CACGTTTGATGGCTTCCAGC-3′ for MnSOD; 5′-TCCTGCTAAGAGACTCCTCTGT-3′ and 5′-TGGGGAGTGCCCCAAATACT-3′ for TLR2; 5′-ATGAAGTGCTCCTTCCAGGA-3′ and 5′-GCAGGGAACCAGCATCTT-3′ for IL-1β; 5′-CAAGGACAGCAGAGGACCAG-3′ and 5′-TGGCGTCTGGAGGGTTGTTTT-3′ for TNF-α; 5′-GACCCAACCACAAATGCCAG-3′ and 5′-GTGCCCATGCTACATTTGCC-3′ for IL-6; 5′-TCTGTGCCTGCTGCTCATAG-3′ and 5′-GGGCATTGATTGCATCTGGC-3′ for CCL2; 5′-AAGGTCGGAGTCAACGGATT-3′ and 5′-GCAGTGAGGGTCTCTCTCCT-3′ for GAPDH. Expression of mRNA was normalized to GAPDH expression.

Enzyme-linked immunosorbent assay (ELISA)

Culture supernatants from stimulated cells were used to assess the concentrations of secreted cytokines. Sandwich ELISA was performed with monoclonal anti-human capturing antibodies (Cat # MAB601 for IL-1β, Cat # AF-410-NA for TNF-α, Cat # MAB206 for IL-6, Cat # MAB679 for CCL2) and biotinylated polyclonal antibodies for each cytokine (Cat # BAF201 for IL-1β, Cat # BAF 410 for TNF-α, Cat # BAF206 for IL-6, Cat # BAF279 for CCL2; R and D Systems, MN, USA) according to the manufacturer's instructions.

Western blot analyses

For Western blot analysis, samples were prepared with Laemmli buffer after THP-1 cells were stimulation with aLTA. The proteins were separated by 10%—12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred onto nitrocellulose (NC) membrane. To block NC membrane, it was incubated with TBST buffer (20 mM Tris—HCl, 150 mM NaCl, 0.05% Tween-20, 5% skim milk) for 1 h at room temperature (RT). Membrane was incubated with primary antibodies (1:1000 dilution) for 2 h at RT. After washing three times with TBST buffer, it was incubated with HRP-conjugated secondary antibodies (1:2000) for 1 h at RT. After washing, the membrane was treated with enhanced chemiluminescence (ECL) reagents (GE Healthcare, Buckinghamshire, UK) for 2 min and exposed to x-ray film. Anti-IRAK-2, anti-JAK2, anti-STAT3, anti-phospho-p38, anti-phospho-JNK1/2, anti-phospho-ERK, anti-phospho-IkBα, anti-phospho-Akt anti-phospho-p65, anti-phospho-c-Jun (Cell Signaling Technology, MA, USA) and anti-TLR2, anti-MyD88, anti-c-Jun, anti-p65, and anti-β-actin (Santa Cruz Biotechnology, TX, USA) antibodies were used in this study.

Data analyses

Statistical analyses of the experimental data were performed using the two-tailed Student's *t* test, and significant differences between the group means were assessed using a one-way analysis of variance (ANOVA) or unpaired two-tailed *t* test. The data shown are representative results of the mean ± standard deviation (SD) of triplicate experiments. Differences were considered statistically significant when the p value was <0.05.

Results

LTA induced MnSOD production in THP-1 cells

When THP-1 cells were stimulated with LTAs, MnSOD mRNA expression increased significantly in a concentration-dependent manner. Incubation with 100 μg/ml of aLTA induced a 150-fold increase in the expression of MnSOD mRNA l (Fig. 1A), which peaked after 3 h of stimulation (Fig. 1B). Incubation with 100 μg/ml of pLTA produced only a 9-fold increase in MnSOD mRNA expression (Fig. 1C), which peaked after 3 h of stimulation (Fig. 1D). The production of MnSOD in THP-1 cells was reduced after 6 h stimulation with either LTA. Expression of MnSOD protein was also induced by pLTA and by aLTA, in particular (Fig. 1E and F), in a concentration-dependent manner.

TLR2-mediated pathway was involved in MnSOD production

After incubation with aLTA, TLR2 mRNA expression increased significantly in THP-1 cells, while pLTA did not affect TLR2 mRNA expression (Fig. 2A). To examine the role of TLR2 in aLTA-mediated MnSOD production, THP-1 cells were pre-incubated with neutralizing antibodies, such as anti-TLR2, anti-TLR4, anti-CD14, prior to LTA stimulation. As shown in Fig. 2B, aLTA-mediated MnSOD induction was inhibited in anti-TLR2-antibody-treated cells, indicating that TLR2 was involved in aLTA-mediated MnSOD production in THP-1 cells. Similar results were obtained in the siRNA experiments. Several TLR2-associated genes, including TLR2 and MyD88, were knocked down by the siRNAs and expression of the corresponding proteins was also reduced (Fig. 2C, upper panel). As compared to positive control cells stimulated with aLTA, MnSOD protein was dramatically reduced by siRNAs for TLR2 and MyD88 (Fig. 2C, lower panel). Next, we examined the mRNA expression of the IRAK family including IRAK1, IRAK2, IRAKM, and IRAK4. Among them, only IRAK2 was dramatically increased in aLTA-stimulated cells, suggesting that IRAK2 might play an important role in LTA-mediated MnSOD production in THP-1 cells (Fig. 2D). After IRAK2 knock-down (Fig. 2E, upper panel), cells were stimulated with aLTA. Increased MnSOD in aLTA-stimulated cells was reduced in IRAK2 knock-down cells (Fig. 2E, lower panel). Activation of aLTA-induced signaling by TLR2-MyD88-IRAK2 altered the phosphorylation of JNK1/2 and IκBα (Fig. 2F), and resulted in the activation of AP-1 and NF-κB (Fig. 2G). To confirm the role of JNK and NF-κB in MnSOD production, THP-1 cells were pre-incubated with signaling inhibitors prior to aLTA stimulation. As shown in Fig. 2H, MnSOD production was inhibited in the JNK- and NF-κB-inhibitor-treated cells.

MnSOD was involved in cytokine production in aLTA-treated THP-1 cells

Next, we examined cytokine expression of in aLTA-t stimulated THP-1 cells. As shown in Fig. 3, expression of IL-1β and TNF-α mRNA peaked after 1 h of stimulation and decreased rapidly thereafter (Fig. 3A and B). On the other

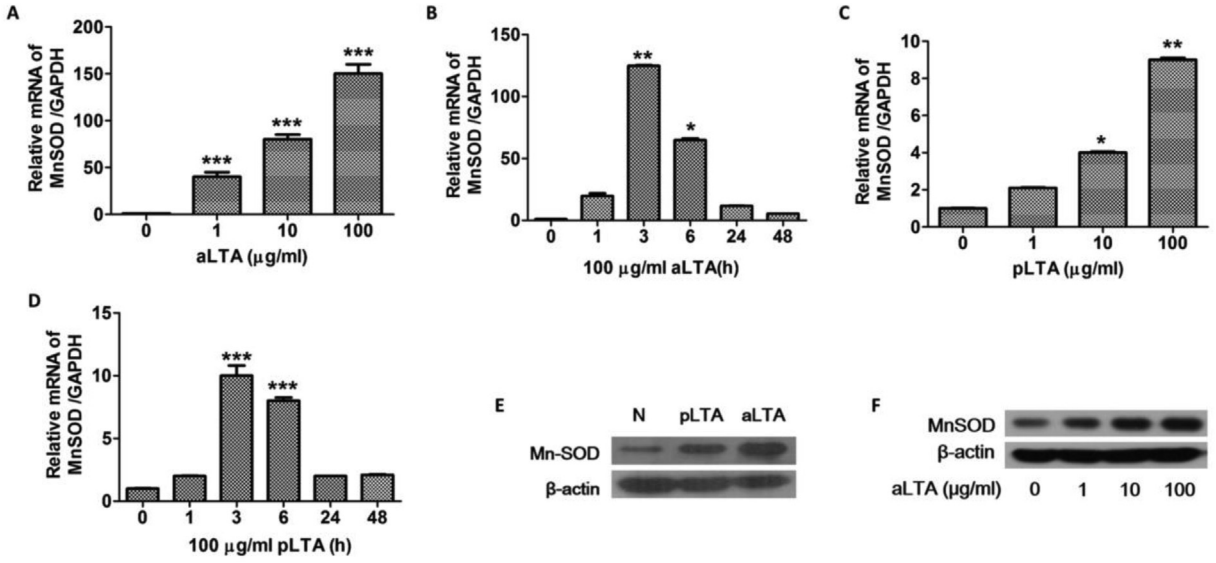

Figure 1. LTA induces MnSOD in THP-1 cells. THP-1 cells were stimulated with the indicated concentration of aLTA and pLTA for the indicated time points. MnSOD mRNA was quantified by real-time PCR and protein was visualized by western blotting. β-actin was used as the internal loading control. Data are displayed as the mean ± SD of three independent experiments. Statistical analyses were conducted using the one-way ANOVA and Tukey's post test. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$ compared to 0.

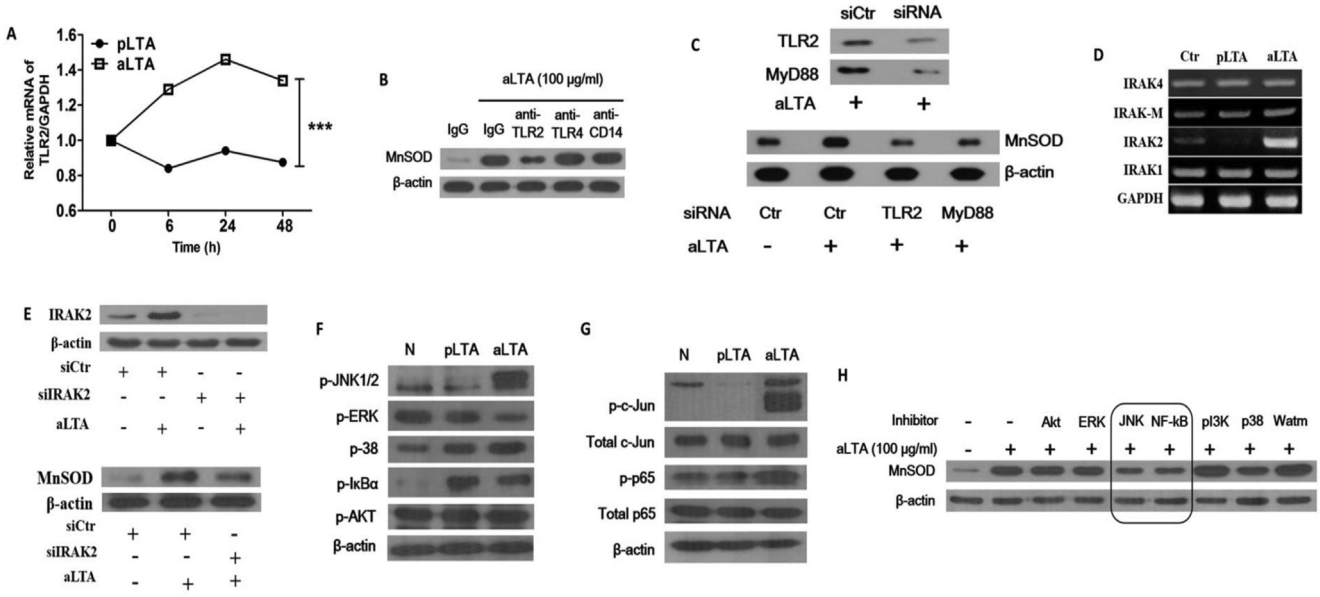

Figure 2. The TLR 2 mediated pathway induces MnSOD. (A) TLR2 mRNA expressionTHP-1 cells was quantified by real-time PCR after incubation with LTA. (B) MnSOD protein expression was visualized by western blotting following pre-incubation with neutralizing antibodies for 30 min and subsequent incubation with aLTA. (C) THP-1 cells were transiently transfected with siRNAs and incubated with 100 μg/ml aLTA for 24 h. MnSOD was detected by western blotting. (D) Gene expression of IRAK family was detected by reverse transcriptase-polymerase chain reaction (RT-PCR) from THP-1 cells incubated with 100 μg/ml pLTA or aLTA for 6 h. (E) Western blots of IRAK2 and MnSOD proteins in THP-1 cells transiently transfected with siIRAK2 RNA followed by incubation with 100 μg/ml aLTA for 24 h. (F) Western blots of signaling molecules in THP-1 cells incubated with 100 μg/ml pLTA or aLTA for 1 h. (G) Western blots of transcription factors in THP-1 cells incubated with 100 μg/ml pLTA or aLTA for 1 h. (H) THP-1 cells were pre-incubated with inhibitors (10 μM) for 30 min, and then stimulated with 100 μg/ml aLTA for 24 h. MnSOD was detected by western blotting. β-actin and GAPDH were used as internal controls. Statistical analyses were conducted using an unpaired two-tailed *t* test. ***$p < 0.001$ compared to 0 h.

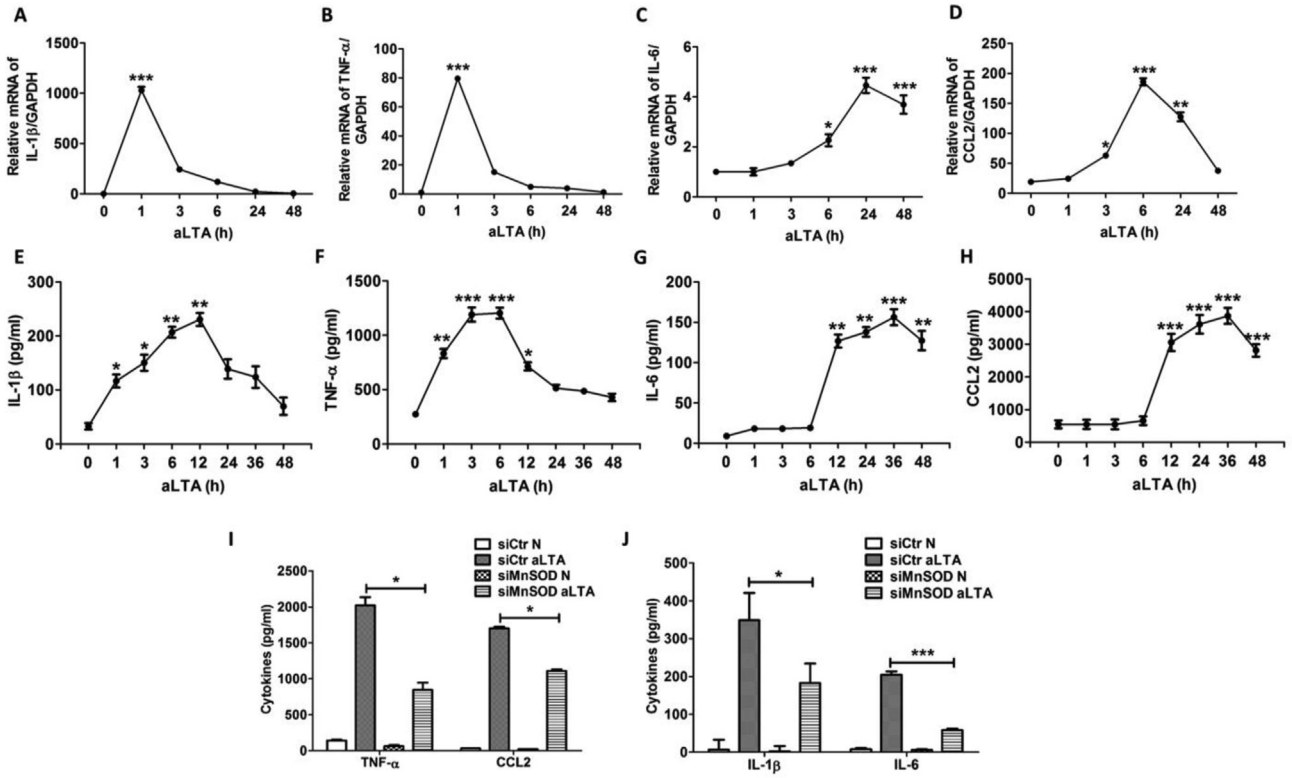

Figure 3. MnSOD was involved in cytokine production in THP-1 cells stimulated with aLTA. (A to H) THP-1 cells were incubated with 100 μg/ml aLTA for the indicated times. Real-time PCR was performed to detect mRNA for IL-1β (A), TNF-α (B), IL-6 (C), and CCL2 (D). Alternatively, ELISA was performed to detect IL-1β (E), TNF-α (F), IL-6 (G), or CCL2 (H) protein. (I to J) THP-1 cells were transiently transfected with siMnSOD RNA and incubated with 100 μg/ml aLTA for 6 h (IL-1β and TNF-α) or 24 h (IL-6 and CCL2). Cytokines in the culture supernatants were detected using an ELISA. Data are displayed as the mean ± SD of three independent experiments. Statistical analyses were conducted using a one-way ANOVA with Tukey's post-hoc test. $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$ compared to 0 h or control.

hand, the production of IL-6 mRNA increased significantly at 6 h and peaked at 24 h. Maximal expression of IL-6 mRNA was maintained for up to 48 h (Fig. 3C). Unlike IL-6, CCL2 mRNA increased significantly after 3 h of stimulation, peaked after 6 h and then decreased slowly for up to 48 h (Fig. 3D). The protein expression pattern of these genes was divided into two. The expression of the corresponding IL-1β and TNF-α proteins increased significantly after 1 h and peaked after 12 h (IL-1β, Fig. 2E) or at 6 h (TNF-α, Fig. 3F) and then decreased rapidly. In contrast, IL-6 and CCL2 protein increased significantly at 12 h and elevated expression was maintained for up to 48 h (Fig. 3G and H).

To examine the role of MnSOD in cytokine expression, THP-1 cells were transiently transfected with siRNA for MnSOD, and then stimulated with aLTA. The induction of these proteins by aLTA was significantly restricted in the transfectants (Fig. 3I and J), with the expression of IL-6 protein being reduced to a greater degree in MnSOD-siRNA-transfected cells.

MnSOD affected signaling pathways involved in cytokine expression in THP-1 cells

When THP-1 cells were stimulated with aLTA, some signaling pathways, including JNK, p38, and NF-κB, were activated in the early phase (Fig. 4A). These pathways were involved in MnSOD production (Fig. 2H). On the other hand, JAK pathways were activated in the late phase (Fig. 4A). Unlike aLTA-stimulation of THP-1 cells, pLTA did not induce dramatic alterations of the signaling pathways (Fig. 4B), which may explain why pLTA is a weaker inducer of MnSOD expression than aLTA. When MnSOD was knocked down with siRNA, aLTA did not activate JNK and NF-κB (Fig. 4C). Similarly, JAK2 and STAT-3 were not activated by aLTA in THP-1 cells transiently transfected with siMnSOD. These results suggest that MnSOD influences both early and late signaling pathways. Given that MnSOD regulates signaling pathways, aLTA-induced MnSOD production was inhibited in siMnSOD-transfected cells (Fig. 4E), suggesting that MnSOD-mediated JNK and NF-κB activation influences MnSOD production in THP-1 cells. Next, we confirmed that the JAK-STAT pathway was involved in the late signaling phase (Fig. 4F). When THP-1 cells were pre-incubated with JAK-STAT inhibitors, IL-6 production in aLTA-stimulated cells was inhibited at 24 and 48 h.

Discussion

Early and late signaling pathways activate different cytokines. For example, in the early signaling pathway, JNK and

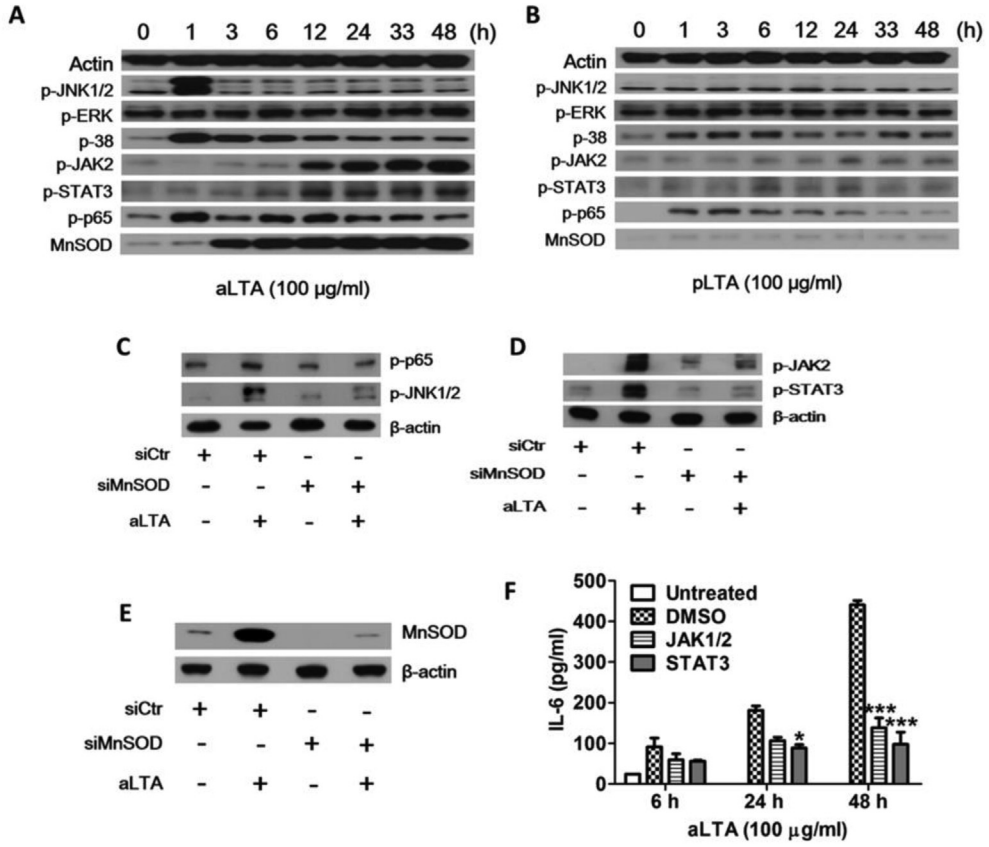

Figure 4. MnSOD influenced signaling pathways involved in cytokine expression in THP-1 cells. (A and B) THP-1 cells were incubated with 100 μg/ml aLTA (A) or pLTA (B) for the indicated times and signaling molecules were detected by western blotting. (C to E) THP-1 cells were transiently transfected with siMnSOD RNA and incubated with 100 μg/ml aLTA for 1 h. Western blotting was performed to detect phospho-p65 and phosphor-JNK (C) and phosphor-JAK2 and phosphor-STAT3 (D). MnSOD was also detected by western blotting (E). β-actin was used as the internal loading control. (F) THP-1 cells were pre-incubated with inhibitors of JAK1/2 and STAT3 for 30 min, and then incubated with 100 μg/ml aLTA for the indicated times. The IL-6 protein concentration in culture supernatants was assessed using an ELISA. Statistical analyses were conducted using one-way ANOVA and Tukey's post-hoc test. *$p < 0.05$; ***$p < 0.001$ compared to DMSO.

NF-κB were activated within one hour of aLTA stimulation, inducing the expression of TNF-α and IL-1β. On the other hand, the late signaling pathway appears to be involved in the expression of IL-6 and CCL2, and induces activation of JAK-STAT pathway. Early activation of NF-κB in response to lipopolysaccharide (LPS) stimulation is dependent upon the MyD88-dependent pathway, while late NF-κB activation is controlled by the TRIF-dependent pathway.[15] The late NF-κB pathway is thought to be activated by a secondary response to newly synthesized TNF-α.[16,17] The results of the current study are consistent with those of previous studies. Phosphorylation of the p65 subunit of NF-κB peaked after 1 h of stimulation and then decreased, but then increased again after 6 and 12 h of stimulation (Fig. 4A). Unlike NF-κB, JNK and p38 were not reactivated. Interestingly, we found that JAK2 was activated after 12 h stimulation and in turn induced STAT3 activation followed by the release of IL-6. The late activation of JAK2 appears to be controlled by cytokines including TNF-α and IL-1β, which are synthesized by early signaling pathways. To confirm that the results were not due to side effects of MnSOD knock down, we performed the cytokine array and examined the alteration of signaling molecules in the cells transfected with siCtr and siMnSOD RNA. Similar to the previous results, most cytokines were increased by aLTA in siCtr RNA-transfected cells, but it was decreased in siMnSOD RNA-transfected cells. However, IL-18 was rather increased in siMnSOD RNA-transfected cells by aLTA treatment. In addition, the phosphorylation of Akt and p38 was not altered by MnSOD knock down. These results suggest that our results were not caused by the side effect of knock down.

MnSOD, also known as superoxide dismutase 2 (SOD2), is an essential mitochondrial antioxidant enzyme that protects cells from the superoxide radical ($O_2^{\cdot}$), which is a major by-product of mitochondrial respiration. Hydrogen peroxide generated by MnSOD leads to activation of redox-dependent transcription factors such as NF-κB and AP-1, which induce the expression of immediate-early CD95L/IL-2 genes.[18] In our previous study, we found that H_2O_2 concentrations were dramatically increased in aLTA-stimulated THP-1 cells. As compared to unstimulated cells, H_2O_2 increased 28.3-fold after aLTA stimulation, and MnSOD was the most expressed gene among the genes examined in the experiment. On the other hand, pLTA stimulation increased MnSOD 3.3-fold.[19] These results indicate increased MnSOD in aLTA-stimulated THP-1 cells

controls cytokine production: aLTA stimulation activated NF-κB and JNK1/2, which resulted in the induction of MnSOD in THP-1 cells, while MnSOD activated the same signaling pathways, including NF-κB and JNK1/2, and controlled the production of proinflammatory cytokines. These pro-inflammatory cytokines were involved in the activation of the JAK-STAT3 pathway, which was associated with IL-6 production.

It is well known that MnSOD plays a role in apoptosis. Kwak and colleagues have shown that the pro-apoptotic markers Bax and caspase-3 cleavage were reduced and apoptosis was mitigated in MnSOD Tg hearts compared with wild-type hearts.[20] Liu et al. also reported that MnSOD inhibited the release of cytochrome c to cytosol by proline oxidase (POX), which plays a role in p53-induced apoptosis.[21] It is known that reactive oxygen species are involved in TRAIL-induced Smac/DIABLO release and in TRAIL-triggered apoptosis. Overexpression of MnSOD-reduces cytochrome c in cells and inhibits Smac/DIABLO release into the cytosol, because MnSOD catalyze the decomposition and neutralization of reactive oxygen species.[22] Cells with greater MnSOD expression are more resistant to the cytotoxic effects of H_2O_2, O_2^- and UV-B radiation, while MnSOD-deficient cells are more susceptible to apoptotic cell death, which is accompanied by cytochrome C leakage, and caspase 3 activation when cells are challenged with O_2^-.[23] However, there have been few studies addressing the role of MnSOD in the regulation of cytokine production.

Compared to pLTA, aLTA is a stronger inducer of MnSOD expression, likely due to the structural characteristics of aLTA. Generally, LTA consists of a polyglycerolphosphate backbone chain that is linked to the membrane by a glycolipid. The glycolipid of aLTA consists of two fatty acid chains, whereas pLTA contains two or three fatty acid chains.[24] This structural difference in glycolipids leads to a difference in affinity for TLR2. In addition, TLR1, TLR6, CD14 and CD36, all of which interact with TLR2, can also influence the effects of aLTA and pLTA.[25] A previous study found that blocking of CD14 or CD36 inhibited LTA binding for TLR2 and TLR2-mediated TNF release from monocytes.[26] The difference in LTA affinity for TLR2 affects the recruitment of downstream MyD88 and IRAK2.

In conclusion, *S. aureus* secretes a variety of toxins and suppresses the immune system. In this paper, we showed that *S. aureus* LTA can regulate cytokine expression through MnSOD. MnSOD protects cells by converting O_2^- to H_2O_2. We postulate that aLTA acts through MnSOD to induce the expression of cytokines such as TNF-α and IL-1β in the early phase and IL-6 and CCL2 in the late phase, thereby inducing an excessive host inflammatory response. Induction of cytokine expression by LTA can activate the immune system in response to invading pathogens, but it may also cause excessive cytokine storms and cause inflammatory diseases.

Author contributions

H.E.K, K.O.J and H.K performed experiments; H.K and D.K.C wrote the main manuscript text and prepared figures, H.K developed the theory, D.K.C supervised the findings of this work. All authors reviewed the manuscript.

Declaration of competing interest

The authors declare no competing interests.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.jmii.2020.12.011.