이학석사 학위논문

Phenotypes and functions of human dendritic cells stimulated with teichoic acid-deficient *Staphylococcus aureus*

테이코익산이 결여된 황색포도상구균에 의해 자극된 인간 수지상세포의 표현형과 기능

2014년 8월

서울대학교 대학원

치의과학과 면역 및 분자미생물치의학 전공

홍 성 준

Phenotypes and functions of human dendritic cells stimulated with teichoic acid-deficient *Staphylococcus aureus*

지도교수 : 한 승 현

이 논문을 이학석사 학위논문으로 제출함

2014년 5월

서울대학교 대학원

치의과학과 면역 및 분자미생물치의학 전공

홍 성 준

홍성준의 석사학위논문을 인준함

2014년 6월

위 원 장 석 영 재 (인)

부 위 원 장 윤 철 희 (인)

위 원 한 승 현 (인)

Abstract

Phenotypes and functions of human dendritic cells stimulated with teichoic acid-deficient *Staphylococcus aureus*

Sung Jun Hong

Department of Dental Science, Immunology and Molecular Microbiology in Dentistry, School of Dentistry, Seoul National University

(Directed by Professor **Seung Hyun Han**, Ph.D.)

Dendritic cells (DCs) are professional antigen-presenting cells linking innate and adaptive immunity. The surface molecule expression, production of cytokine, and lymphocyte-stimulating capacity of DCs are dependent on DC maturation triggered by bacterial cell wall components including wall teichoic acid (WTA), lipoteichoic acid (LTA), and peptidoglycan (PGN). Among those cell wall components, role of WTA in the DC maturation has not been fully

understood. In the present study, ethanol-killed *S. aureus* wild-type remarkably induced the expression of co-stimulatory molecules such as CD83, CD86, major histocompatibility complex (MHC) class I, MHC class II, PD-L1 and PD-L2 in human monocyte-derived DCs. In addition, Wild-type *S. aureus* significantly induced the production of pro-inflammatory cytokines such as tumor necrosis factor-α (TNF-α), interleukin (IL)-12, and IL-1β. In contrast, WTA-deficient *S. aureus* (Δ*tagO*) failed to induce the expression of such molecules and cytokines under the same condition. Wild-type *S. aureus*-stimulated DCs significantly increased the expression of T cell activation markers, CD25 and MHC class II, and T cell proliferation when DCs were co-cultured with PBMC. However, Δ*tagO*-stimulated DCs weakly induced the T cell activation and proliferation under the same condition. Wild-type *S. aureus*-stimulated DCs also increased interferon-γ and IL-10 expression in PBMC but Δ*tagO*-stimulated DCs could not. Expression of T cell activation markers, T cell proliferation and cytokine-producing ability of PBMC were restored by DCs stimulated with p*StagO*. Collectively, WTA might be an important cell wall component of *S. aureus* in the differentiation and activation of DCs.

Keyword : Human monocyte-dendritic cells, *Staphylococcus aureus*, Wall teichoic acid, T cell activation

Student Number : 2012-23693

CONTENTS

INTRODUCTION

Staphylococcus aureus is a Gram-positive bacterium which colonizes nasal passage and axillae. It induces inflammation and forms abscesses in the skin and soft tissues at infection (1-4). *S. aureus* is common cause of several infectious diseases such as pneumonia, sepsis, and toxic shock syndrome (5, 6). *S. aureus* has various microbes-associated molecular patterns (MAMP) including lipoprotein, peptidoglycan (PGN), lipoteichoic acid (LTA), and wall teichoic acid (WTA) (7, 8). These virulence factors play important roles in *S. aureus*-induced systemic inflammation. Among those cell wall components, role of WTA in the host immune activation has not been fully understood in spite of its abundant expression on the bacterial cell wall (9).

WTA is covalently attached to C6 hydroxyl group of *N*-acetylmuramic acid residue in PGN layer and has more than 40 ribitol-phosphate-repeating units with D-alanine and *N*-acetyl glucosamine (GlcNAc) (10, 11). WTA biosynthesis is regulated by various genes such as *tarABD* (clustered genes) and *tagO* (separately encoded gene). Among those genes, *tagO* is responsible for the initial biosynthesis pathway of WTA. The *tagO* gene produce UDP-GlcNAc which transfer of GlcNAc-phosphate to bactoprenol. Indeed, *tagO*-deficient *S. aureus* mutant lacks WTA regardless its viability (12). WTA plays important roles in cell division and autolysis of bacteria contributing to induction of resistance to

high temperature (13, 14). In addition, D-alanine-deficient WTAs increase susceptibility to antibiotics, such as vancomycin or teicoplanin, and cationic antimicrobial peptides (15, 16). It contributes to biofilm formation and increases the bacterial adhesion to host cells, which are important in the initiation of severe infectious diseases (13, 17). WTA is recognized by serum immunoglobulin G (IgG) or mannose binding lectin (MBL) complex, and activates classical- or lectin- pathway of complement system, which results in the enhancement of opsonophagocytosis of polymorphonuclear leukocytes (PMN) (18).

Dendritic cells (DCs) are professional antigen-presenting cells linking innate and adaptive immunity (20). Human monocyte-derived DCs express CD1a, CD11c, CD80, CD86, and major histocompatibility complex (MHC) molecules. DCs are widely distributed within almost all tissues (19). Immature DCs efficiently take up invading microbes and malignant cells whereas mature DCs reduce the take up of antigen and increase antigen presentation capacity. The DCs recognize microbial antigens through pattern recognition receptor (PRR) such as Toll-like receptors, NOD-like receptors, or C-type lectin receptors (20-22). Upon recognizing microbial antigens, the DCs up-regulate the expression of co-stimulators (CD80, CD86) and MHC-antigen complex on the cell surface. Also, the activated DCs produce cytokines such as interleukin (IL)-1β, IL-12, tumor necrosis factor-α (TNF-α), IL-6, and IL-10 (23-28). To activate T cells, mature DCs migrate to lymph nodes and present antigen to naive T cells. Therefore maturation of DCs is essential process for antigen-specific T cell

activation and differentiation into effector cells (29).

Although WTA is abundantly expressed in the cell wall and easily exposed to host cells, the roles of WTA in host immune responses has not been elucidated. In the present study, roles of WTA in *S. aureus*-induced DC maturation and DC-mediated T cell activation were investigated using WTA-deficient *S. aureus* strain.

MATERIALS AND METHODS

Bacterial culture

S. aureus including parent strain RN4220 (wild-type), *tagO*-deficient mutant strain (Δ*tagO*), and complemented mutant (p*StagO*) were kindly provided by Dr. Bok Luel Lee (Pusan National University, Busan, Korea) (18, 30, 31). The Δ*tagO* or p*StagO* was prepared by transformation of RN4220 strain with pMutinT3 plasmid harboring the internal region of *tagO* gene (position 36 to 645) or by introduction of pT0702 plasmid containing *tagO* gene (positions-580 to 1268) into the mutant, respectively. *S. aureus* wild-type was grown in Luria-Bertani (LB, Difco Laboratories, Detroit, MI) media at 37°C, and Δ*tagO* was cultured in LB media containing erythromycin (10 μg/ml) and p*StagO* was cultured in LB media containing erythromycin and chloramphenicol (12.5 μg/ml) at 30℃, respectively. To prepare ethanol-killed *S. aureus*, the bacteria were washed three times with PBS, suspended in 70% ethanol and incubated for 1 h with shaking. To ensure that the bacteria were completely killed, all the ethanol-treated *S. aureus* were plated on the LB agar plates containing the appropriate antibiotics followed by incubation overnight. No colonies were observed.

Reagents and chemicals

Ficoll-paque plus was purchased from GE healthcare (Uppsala, Sweden). Anti-

human CD14 antibody-coated magnetic beads were obtained from BD Biosciences (San Diego, CA, USA). Fetal bovine serum (FBS), penicillin-streptomycin solution, and RPMI 1640 were purchased from HyClone (Logan, UT, USA). Recombinant human granulocyte macrophage-colony stimulating factor (GM-CSF) and IL-4 was purchased from Peprotech (Rocky Hill, NJ, USA) and R&D Systems (Minneapolis, MN, USA), respectively. 5-(and-6)-carboxyfluoresceindiacetate succinimidyl ester (CFDA-SE/CFSE) was purchased from Molecular Probes (Eugene, OR, USA). Monoclonal antibodies used for flow cytometric analysis were as follows; FITC-labeled anti-human HLA-DR, -DP, -DQ antibody for MHC class II was obtained from BD Bioscience. PE-labeled anti-human CD83 antibody, APC-labeled anti-human CD86 antibody, PE Cy-5 labeled anti-human HLA-A, -B, -C antibody for MHC class I, APC-labeled anti-human PD-L1 antibody, PE-labeled anti-human PD-L2 antibody, APC-labeled anti-human CD3 antibody, PE-labeled anti-human CD4 antibody, FITC-labeled anti-human CD25 antibody were purchased from BioLegend (San Diego, CA, USA). Enzyme-linked immunosorbent assay (ELISA) kit for quantification of human IL-1β was purchased from R&D Systems and human IL-12p70, IL-10, IL-6, TNF-α, and interferon (IFN)-γ ELISA kits were purchased from BioLegend.

Generation of human monocyte-derived dendritic cell (MoDC)

All the experiments using human blood were conducted under the approval of

Institutional Review Board of Seoul National University (IRB No. S-D 20120003). Peripheral blood was provided from blood center of the Korean Red Cross. Peripheral blood mononuclear cells (PBMCs) were isolated from human blood by density-gradient centrifugation using ficoll-paque plus. To isolate $CD14^+$ monocytes, the PBMCs were incubated with anti-human CD14 antibody-coated magnetic beads for 30 min, then the cells were positively selected through magnetic separation. The isolated $CD14^+$ monocytes were suspended in RPMI-1640 containing 10% FBS and 1% antibiotics at a density of 2×10^6 cells/ml and the cells were plated in 100 mm cell culture dishes (Corning Glass Works, Corning, NY, USA). To differentiate the monocytes into DC, the cells were treated with human recombinant GM-CSF (50 ng/ml) and IL-4 (17.7 ng/ml) and cultured for 6 days.

Phenotypic analysis

DCs (5×10^5 cells/ml) were stimulated with *S. aureus* wild-type, Δ*tagO*, or p*StagO* (30 μg/ml) in the presence GM-CSF (25 ng/ml) and IL-4 (9 ng/ml) for 24 h. The DCs were stained with fluorochrome-conjugated anti-human antibody specific for HLA-A, B, C, HLA-DR, -DP, -DQ, CD83, CD86, PD-L1, and PD-L2 for 30 min at 4℃. Then, the cells were washed with PBS once and fixed with PBS containing 1% paraformaldehyde. All flow cytometric data were analyzed by using FACSCalibur (BD Bioscience, San Diego, CA, USA) and FlowJo software (Tree Star, San Carlos, CA, USA).

ELISA

DCs (5 x 10^5 cells/ml) were stimulated with *S. aureus* wild-type, Δ*tagO*, or p*StagO* (30 μg/ml) for 24 h. Amount of IL-1β, IL-12p70, IL-6, IL-10, and TNF-α in the culture supernatants was quantified by commercial ELISA kits according to the manufacturer`s instruction. To analyze DC-mediated cytokine production by PBMCs, unstimulated- or *S. aureus*-stimulated DCs (1 x 10^5 cells/ml) were co-cultured with autologous PBMCs (1 x 10^6 cells/ml) for three days. The amount of IFN-γ and IL-10 in the culture supernatants was quantified by commercial ELISA kits according to the manufacturer`s instruction. The optical density was measured by VERSA max microplate reader (Molecular Devices, Crawley, West Sussex, UK).

T cell proliferation and activation by DCs

DCs (1 x 10^5 cells/ml) were stimulated with *S. aureus* wild-type, Δ*tagO*, or p*StagO* (30 μg/ml) for 24 h. To label PBMCs, the cells were suspended in PBS containing 10 μM CFSE and incubated at 37°C for 15 min. Then, the cells were washed with PBS and suspended in RPMI-1640 containing 10% FBS and 1% antibiotics at a density of 1 x 10^6 cells/ml. The CFSE-labeled PBMCs (1 x 10^6 cells/ml) were added to the unstimulated- or *S. aureus*-stimulated DCs (1 x 10^5 cells/ml) and were co-cultured for five days. The culture media were changed after three days and the cells were cultured for additional two days. The PBMCs were stained with anti-human CD25 antibody, anti-human HLA-DR, -DP, -DQ

antibody, and anti-human CD3 antibody for 30 min at 4℃. The cells were washed with PBS once and fixed with PBS containing 1% paraformaldehyde. To analyze T cell proliferation and activation marker expression, $CD3^+$ cells were gated and analyzed by flow cytometry and Flow Jo software.

Statistical analysis

All the data were analyzed by Student`s *t*-test using graph pad prism 5 (GraphPad Software Inc, La Jolla, CA). *P*-value under 0.05 was considered statistically significant.

RESULTS

WTA plays a critical role in *S. aureus*-induced maturation of human MoDC.

Maturation of DCs is important process for the induction of antigen-specific adaptive immune responses. Recognizing foreign-antigens, the DCs increase the expression of co-stimulatory molecules, MHC-antigen complexes, and cytokine production, which are important in activation and differentiation of T lymphocytes (19). To examine the role of WTA in the *S. aureus*-induced phenotypic maturation of DCs, immature DCs were stimulated with ethanol-killed *S. aureus* wild-type, Δ*tagO*, or p*StagO* for 24 h, and the expression of maturation markers was analyzed by flow cytometry. *S. aureus* wild-type efficiently up-regulated the expression of CD83 (MFI: from 9 to 171), CD86 (MFI: from 21 to 485), MHC class I (MFI: from 70 to 174), PD-L1 (MFI: from 232 to 1384) and PD-L2 (MFI: from 18 to 52). However, Δ*tagO* poorly induced these proteins on DCs (MFI of CD83: from 9 to 55, MFI of CD86: from 21 to 172, MFI of MHC class I: from 70 to 40, MFI of MHC class II: from 138 to 135, MFI of PD-L1: from 232 to 787, and MFI of PD-L2: from 18 to 33). Restoration in the expression of these molecules was observed when the DCs were stimulated with p*StagO* (Fig. 1). These results suggest that WTA is a critical molecule in the *S. aureus*-induced phenotypic maturation of DCs.

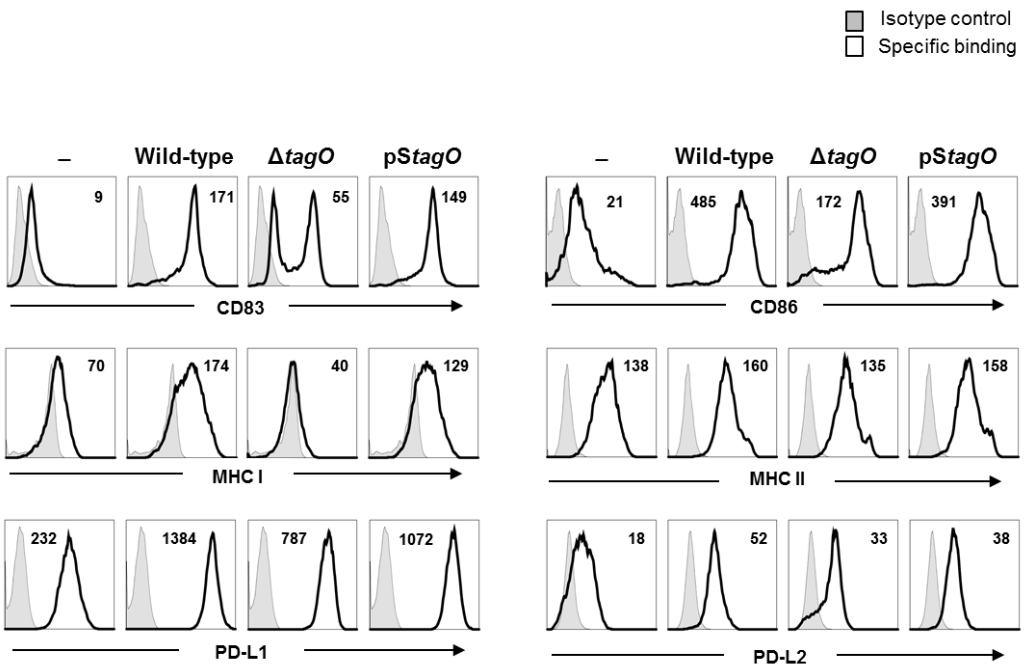

Figure 1. WTA plays a critical role in *S. aureus*-induced maturation of human MoDC. Monocyte-derived dendritic cells (MoDCs) (5 x 10^5 cells/ml) were stimulated with ethanol-killed *S. aureus* wild-type, Δ*tagO*, or p*StagO* for 24 h and then, the expression of co-stimulatory molecules (CD83 and CD86), MHC proteins (MHC class I and MHC class II) and programmed death-ligands (PD-L1 and PD-L2) was analyzed by flow cytometry. Gray-filled area and open area indicate isotype antibody binding and specific target antibody binding, respectively. Numbers in the histograms indicate mean fluorescence intensity (MFI) of the cells. This result is one of eight similar results.

WTA is critical for *S. aureus*-induced IL-1β, IL-12p70 and TNF-α production by human MoDC.

Activated DCs efficiently produce cytokines such as IL-1β, IL-12p70, TNF-α, IL-6 and IL-10, which regulates various immune responses (25). To examine the role of the WTA in cytokine production of DCs, the cells were stimulated with ethanol-killed *S. aureus* wild-type, Δ*tagO*, or p*StagO* and the amount of cytokines in the culture supernatant was analyzed by ELISA. Wild-type *S. aureus*-stimulated DCs efficiently produced IL-1β (352.8 ± 97.3 pg/ml), IL-12p70 (502.1 ± 93.2 pg/ml), TNF-α (3210 ± 848.8 pg/ml), IL-6 (4927 ± 1636 pg/ml), and IL-10 (68.1 ± 14.6 pg/ml) (Fig. 2). However, Δ*tagO* poorly induced these cytokines (IL-1β: 106.3 ± 33.1 pg/ml, IL-12p70: 10.7 ± 7.2 pg/ml and TNF-α: 642.8 ± 155.5 pg/ml) (Fig. 2A, 2B, and 2C). *S. aureus* p*StagO*-stimulated DCs completely restored the expression of IL-1β (432.8 ± 88.4 pg/ml), IL-12p70 (717.8 ± 170.9 pg/ml), and TNF-α (2275 ± 345.4 pg/ml). No significant difference in the expression of IL-6 and IL-10 was observed in DCs stimulated either with *S. aureus* wild-type or with Δ*tagO*. (Fig. 2D, and 2E). These results indicate that WTA is a key molecule in *S. aureus*-induced IL-1β, IL-12p70 and TNF-α production of DCs.

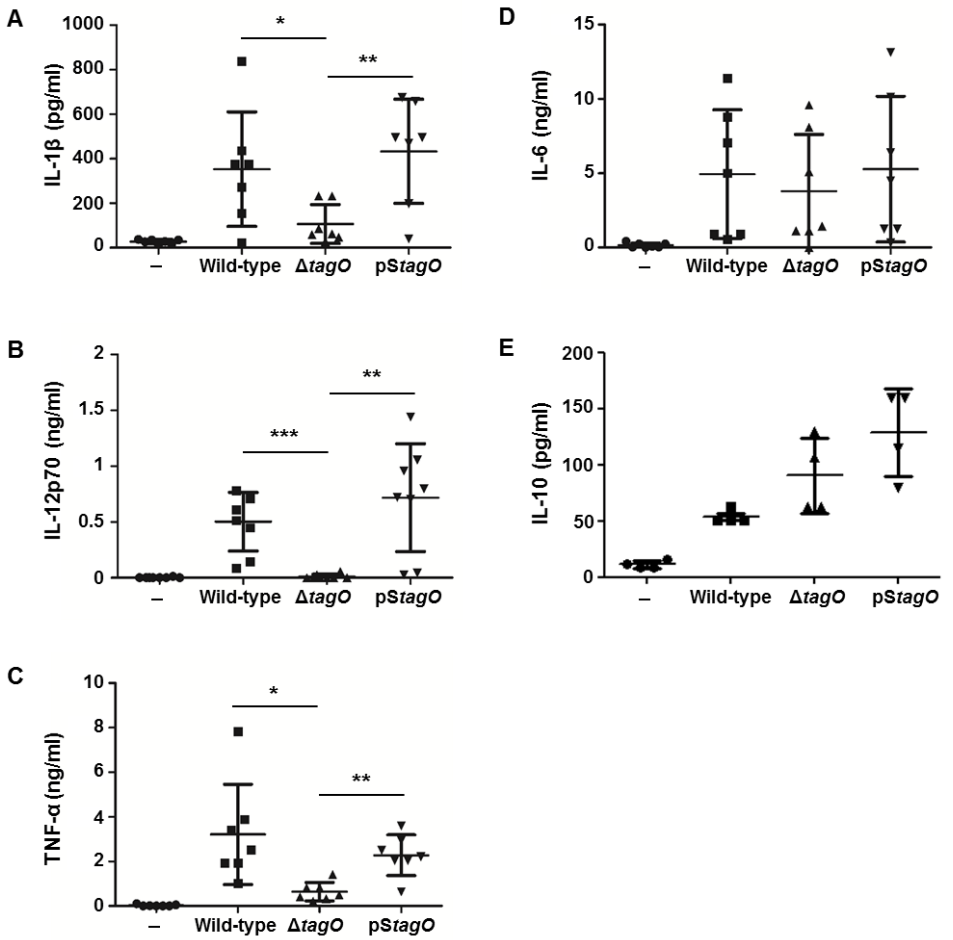

Figure 2. WTA is critical for *S. aureus*-induced IL-1β, IL-12p70 and TNF-α production of human MoDCs. The MoDCs (5 x 10^5 cells/ml) were stimulated with ethanol-killed *S. aureus* wild-type, Δ*tagO* or p*StagO* (30 μg/ml) for 24 h. The amount of IL-1β, IL-12p70, TNF-α, IL-10, and IL-6 in the culture supernatant was quantified by ELISA ($n \geq 5$). Statistical significance between the treatment groups was analyzed by Student`s *t*-test. *, $P < 0.05$, **, $P < 0.01$, and ***, $P < 0.001$.

DC stimulated with *S. aureus* wild-type, but not with WTA-deficient *S. aureus* induced autologous T cell activation.

Next, this study examined lymphocyte-activating ability of the DCs. DCs were stimulated with *S. aureus* wild-type, Δ*tagO*, and p*StagO*. Then, the unstimulated- or *S. aureus*-stimulated DCs were co-cultured with autologous PBMCs at an effector to target ratio 1:10 for five days. Then, the proliferation of T cells and expression of T cell activation markers (CD25 and MHC class II) was analyzed by flow cytometry. Wild-type *S. aureus*-stimulated DCs efficiently increased T cell proliferation. However, Δ*tagO*-stimulated DCs scarcely induced T cell proliferation (Fig. 3A). Wild-type *S. aureus*-stimulated DCs augmented CD25 (MFI: from 22 to 48) and MHC class II (MFI: from 50 to 254) on the T cells, whereas Δ*tagO*-stimulated DCs poorly induced those molecules (MFI of CD25: from 22 to 26 and MFI of MHC class II: from 50 to 82). Proliferation and activation markers on the T cells were partially restored by DCs stimulated with p*StagO* (MFI of CD25: from 22 to 44 and MFI of MHC class II: from 50 to 245) (Fig. 3B). Therefore, WTA is an important molecule of *S. aureus* to stimulate DCs leading to the activation of autologous T cells.

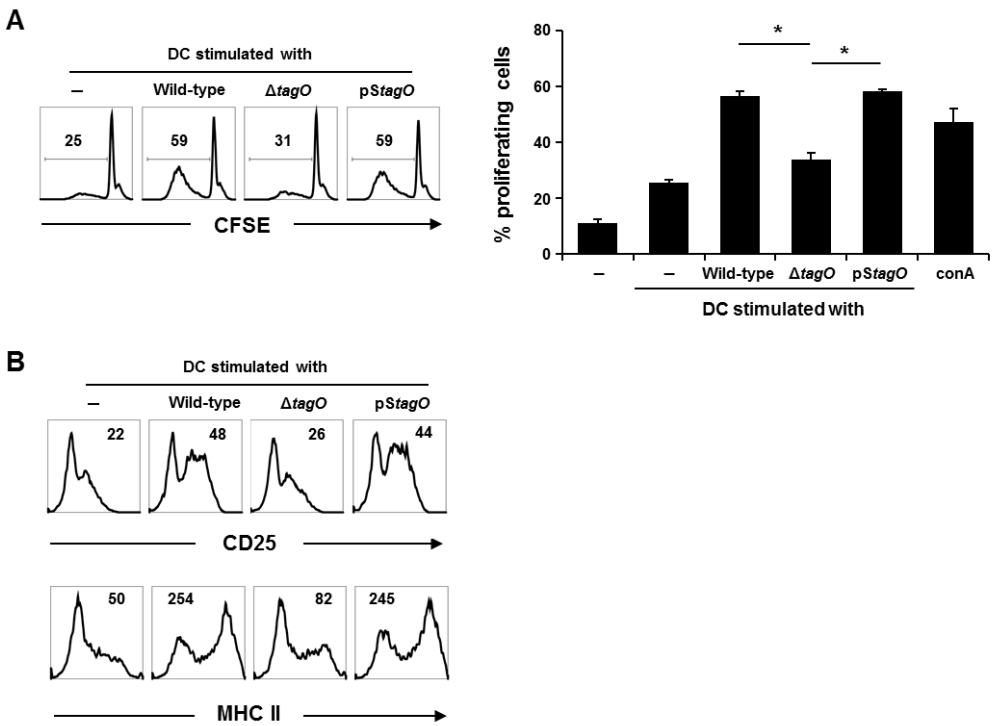

Figure 3. DC stimulated with *S. aureus* wild-type, but not with WTA-deficient *S. aureus,* induced the activation of autologous T cells. The MoDC (1×10^5 cells/ml) were stimulated with ethanol-killed *S. aureus* wild-type, Δ*tagO,* or p*StagO* (30 μg/ml) for 24 h. (A) The unstimulated- or stimulated DCs were co-cultured with CFSE-labeled autologous PBMC (1×10^6 cells/ml) for five days then, T cell proliferation was analyzed by flow cytometry. The number on each histogram indicates percentage of proliferated T cells. (B) The expression of T cell activation markers (CD25 and MHC class II) was analyzed by flow cytometry. The number on each histogram indicates MFI of the cells. These data are representative from six similar experiments. Statistical significance between the treatment groups was analyzed by Student`s *t*-test. *, $P < 0.05$.

DCs stimulated with *S. aureus* wild-type, but not with WTA-deficient *S. aureus* induced cytokine production in PBMCs

To examine the role of the WTA in DC-mediated cytokine production by PBMCs, this study analyzed expression of IFN-γ and IL-10 in PBMCs co-cultured with DCs. DCs were stimulated with *S. aureus* wild-type, Δ*tagO*, and p*StagO* then, the cells were co-cultured with autologous PBMCs for three days. Wild-type *S. aureus*-stimulated DCs increased the expression both IFN-γ (3235 ± 135.8 pg/ml) and IL-10 (803.6 ± 40.4 pg/ml), but Δ*tagO*-stimulated DCs induced these proteins to expression lower (IFN-γ: 2538 ± 219.6 pg/ml and IL-10: 446.2 ± 7.1 pg/ml). Cytokine producing ability of PBMCs was restored by DCs stimulated with p*StagO* (IFN-γ: 3785 ± 111.3 pg/ml and IL-10: 1106 ± 66.6 pg/ml) in PBMC (Fig. 4A and 4B). These results suggest that WTA is an important component of *S. aureus* to stimulate DCs for the induction of cytokine production in the co-cultured autologous PBMCs.

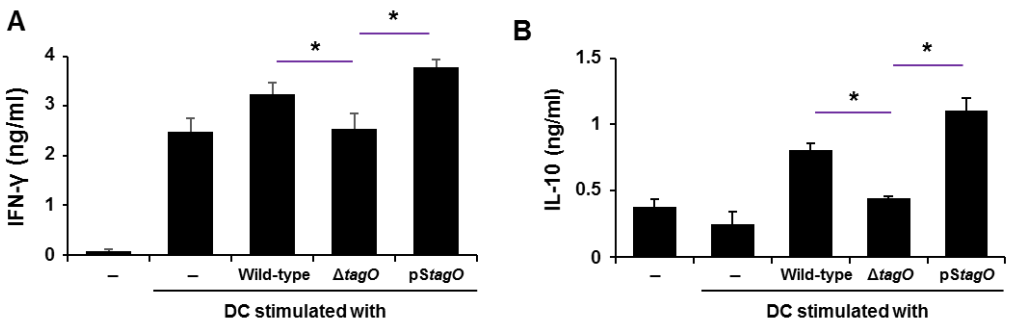

Figure 4. DCs stimulated with *S. aureus* wild-type, but not with WTA-deficient *S. aureus*, induced cytokine production in the co-cultured PBMCs. The MoDC (1 x 10^5 cells/ml) were stimulated with ethanol-killed *S. aureus*, Δ*tagO*, or p*StagO* (30 μg/ml) for 24 h. The DCs were co-cultured with autologous PBMC (1 x 10^6 cells/ml) for three days, then the amount of (A) IFN-γ and (B) IL-10 was analyzed by ELISA. These data are representative of three similar experiments. Statistical significance between the treatment groups was analyzed by Student`s *t*-test. *, $P < 0.05$.

DISCUSSION

WTA is a glycopolymer found in Gram-positive bacterial cell wall (11). It has been reported that WTA contributes to protection of the bacterial cells from environmental stress, antimicrobial peptide, antibiotics and lysozyme (13, 15, 16, 32). However, its roles in the host immune activation have been poorly investigated. Here, this study demonstrated the effect of WTA of *S. aureus* on the human DC maturation and the activation. *S. aureus* WTA played a critical role in the induction of MHC class Ⅰ, MHC class Ⅱ, CD83, CD86, PD-L1, and PD-L2 on DCs. It was important for the production of IL-1β, IL-12 and TNF-α by *S. aureus*-stimulated DCs. In addition, WTA-deficient *S. aureus* was less potent than *S. aureus* wild-type in the activation of DCs for the autologous T cell proliferation and activation. Therefore, these data indicate that WTA is an important molecule of *S. aureus* in the maturation of DCs and T cell activation.

S. aureus has two-types of polyanionic polymers, WTA and LTA in the cell wall (11). These two types of molecules are representative cell wall components of *S. aureus* which have both similarities and differences in terms of the host immune induction. WTA has ribitol-phosphate repeating units covalently attached to PGN layer whereas LTA has glycerol-phosphate repeating units which are non-covalently attached to the cell membrane (12). LTA exhibited immuno-stimulating capacity in human DC activation and these responses were

synergistically potentiated when the cells were co-treated with muramyl dipeptide (MDP) which is a moiety of PGN (33). In DC-mediated T cell activation, Son *et al.* have demonstrated that LTA stimulated DCs induced foxp3 and TGF-β expression in $CD4^+$ T cells (34). Unlike LTA, purified WTA has little immuno-stimulatory effect on THP-1 and RAW 264.7 cell line (35). On the other hand, the present results showed that WTA played an important role in maturation and cytokine production by DCs. Moreover, this study showed that WTA is critical for activation of T cells by DCs. Weidenmaier *et al*. have shown that WTA-deficient *S. aureus* hardly induced $CD4^+$ T cell activation and promoted *S. aureus* induced skin abscesses (35). Since these experiments were conducted by using WTA-deficient *S. aureus*, it is difficult to directly compare the immuno-stimulatory potencies of the WTA and LTA in parallel. Thus, further studies remains to be done, using purified WTA and LTA.

WTA, which is abundantly distributed in the outer layer of Gram-positive bacteria, seems to efficiently evoke host immune responses. High level of WTA-specific antibody is detected in human serum (18). Jung *et al*. have shown that the anti-WTA IgG specifically binds β-*N*-acetylglucosamin residue of ribitol-phosphate repeats of *S. aureus* WTA (36). In addition, the WTA initiates complement activation by binding to the serum antibody or MBL (36, 37). These responses are important to induce complement-mediated opsonophagocytosis of PMNs (38). DCs are highly phagocytic cells which express various kinds of phagocytosis-related receptors such as mannose-binding lectin (CD206) (39, 40).

In previous study, *Drosophila* hemocytes and human PMNs less phagocytosed Δ*tagO* compared with *S. aureus* wild-type (36, 41). Concordantly, a decrease in the phagocytosis of DCs against Δ*tagO* mutant was observed in comparison with that of DCs against *S. aureus* wild-type (data not shown). Considering these results, WTAs seem to play an important role in phagocytosis of *S. aureus* by DCs.

S. aureus induces both T helper 1 cells (Th1) and Th17 (42). Major function of Th1 is to remove the invading microbes (43). IFN-γ produced by Th1 activates cytotoxic T lymphocyte and enhances their cytotoxic effects against infected cells. Moreover, Th1-derived IFN-γ potentiates phagocytic ability and cytokine producing capacity of DCs and macrophage, which promote effective clearance of invading microbes (44). Th17 not only related autoimmunity diseases but also bacterial clearance (45). Th17-derived IL-17 enhances the innate immune response via recruit of neutrophil and NK cells. T cell differentiation is mainly regulated by cytokines produced by DCs. IL-12, IL-1β, and TNF-α are key cytokines that induce Th1 differentiation (46). On the other hand, Th17 differentiation needs IL-6 and IL-23 (47). Here, this study has shown that Δ*tagO*-stimulated DCs could not induce IL-1β, IL-12p70, and TNF-α but efficiently produce IL-6. Based on this result, WTA may be involved in the regulation of Th1 rather than Th17. Regulatory mechanism of WTA in DC-mediated Th polarization should be further investigated.

WTA regulates not only innate immunity but also adaptive immunity. Zwitterionic polysaccharide (ZPS) has been characterized by zwitterionic charge motif which is composed of positive- and negative charge groups on each repeat unit, whereas almost bacterial polysaccharides mainly exhibit negative charges. These ZPS molecules are capable of complex formation with MHC class II in APCs and induce the APC-mediated T cell activation (48). *S. aureus* WTA also has a zwitterionic charge in ribitol-phosphate repeat unit and it is able to be associated with MHC class II molecule of DCs (8, 35). Substantially, purified *S. aureus* WTA evoked $CD4^+$ T cell responses. Mice administered with *S. aureus* Δ*tagO* poorly induced skin abscess mediated by $CD4^+$ T cell dependent manner in comparison with *S. aureus* wild-type injected mice (35). Other bacterial ZPS including *Bacteroides fragilis* polysaccharide A (PSA) and *Streptococcus pneumoniae* Sp1 can also induce DC maturation (48, 49). PSA is recognized by TLR2 and induces DC maturation. DCs can process and present the carbohydrate antigen to T cells activation via PSA-MHC class II complex. In addition, PSA-stimulated DCs can up-regulate IL-12 production, which lead to Th1 differentiation (49). Sp1 can be also presented to naïve T cells by DC via MHC class II, Inducing T cell, proliferation and activation (50). These results indicate that *S. aureus* WTA may have important function in DC-mediated immune responses.

The present study demonstrated the functions of *S. aureus* WTA in DC maturation and DC-mediated T cell activation using WTA-mutant- and complemented *S. aureus* strain. This study is the first attempt to elucidate the roles of WTA in human DCs. However, this study has some limitations to understand the exact function of WTA. First, this study could not show DC responses to purified WTA. Second, all the experiments were conducted using killed bacteria. Thus, the present results may not be sufficiently applicable to the immune responses induced at *S. aureus* infection. Third, this study did not have *in vivo* experimental model for confirming the *in vivo* relevance. Despite of these limitations, the current results suggest that WTA is an important component of *S. aureus* for DC maturation and T cell activation.

REFERENCES

1. Dancer S & Noble W (1991), Nasal, axillary, and perineal carriage of *Staphylococcus aureus* among women: identification of strains producing epidermolytic toxin. *Journal of Clinical Pathology* 44(8):681-684.
2. Leach R, Eykyn SJ, Phillips I, Corrin B, & Taylor EA (1979), Anaerobic axillary abscess. *British Medical Journal* 2(6181):5.
3. Noble W (1998), Skin bacteriology and the role of *Staphylococcus aureus* in infection. *British Journal of Dermatology* 139(53):9-12.
4. Weidenmaier C, *et al.* (2004), Role of teichoic acids in *Staphylococcus aureus* nasal colonization, a major risk factor in nosocomial infections. *Nature Medicine* 10(3):243-245.
5. Shands KN, *et al.* (1980), Toxic-shock syndrome in menstruating women: association with tampon use and *Staphylococcus aureus* and clinical features in 52 cases. *New England Journal of Medicine* 303(25):1436-1442.
6. Wardenburg JB, Patel RJ, & Schneewind O (2007), Surface proteins and exotoxins are required for the pathogenesis of *Staphylococcus aureus* pneumonia. *Infection and Immunity* 75(2):1040-1044.
7. Van Amersfoort ES, Van Berkel TJ, & Kuiper J (2003), Receptors, mediators, and mechanisms involved in bacterial sepsis and septic shock.

Clinical Microbiology Reviews 16(3):379-414.

8. Weidenmaier C & Peschel A (2008), Teichoic acids and related cell-wall glycopolymers in Gram-positive physiology and host interactions. *Nature Reviews Microbiology* 6(4):276-287.
9. Song MD, Wachi M, Doi M, Ishino F, & Matsuhashi M (1987), Evolution of an inducible penicillin-target protein in methicillin-resistant *Staphylococcus aureus* by gene fusion. *FEBS letters* 221(1):167-171.
10. Ward J (1981), Teichoic and teichuronic acids: biosynthesis, assembly, and location. *Microbiological Reviews* 45(2):211.
11. Neuhaus FC & Baddiley J (2003), A continuum of anionic charge: structures and functions of D-alanyl-teichoic acids in Gram-positive bacteria. *Microbiology and Molecular Biology Reviews* 67(4):686-723.
12. Xia G, Kohler T, & Peschel A (2010), The wall teichoic acid and lipoteichoic acid polymers of *Staphylococcus aureus*. *International Journal of Medical Microbiology* 300(2):148-154.
13. Vergara-Irigaray M, *et al.* (2008), Wall teichoic acids are dispensable for anchoring the PNAG exopolysaccharide to the *Staphylococcus aureus* cell surface. *Microbiology* 154(3):865-877.
14. Brown S, *et al.* (2012), Methicillin resistance in *Staphylococcus aureus* requires glycosylated wall teichoic acids. *Proceedings of the National Academy of Sciences* 109(46):18909-18914.
15. Collins LV, *et al.* (2002), *Staphylococcus aureus* strains lacking D-alanine modifications of teichoic acids are highly susceptible to human

neutrophil killing and are virulence attenuated in mice. *Journal of Infectious Diseases* 186(2):214-219.

16. Koprivnjak T, Peschel A, Gelb MH, Liang NS, & Weiss JP (2002), Role of charge properties of bacterial envelope in bactericidal action of human group IIA phospholipase A_2 against *Staphylococcus aureus*. *Journal of Biological Chemistry* 277(49):47636-47644.

17. Weidenmaier C, *et al.* (2005), Lack of wall teichoic acids in *Staphylococcus aureus* leads to reduced interactions with endothelial cells and to attenuated virulence in a rabbit model of endocarditis. *Journal of Infectious Diseases* 191(10):1771-1777.

18. Park K-H, *et al.* (2010), Human serum mannose-binding lectin senses wall teichoic acid glycopolymer of *Staphylococcus aureus*, which is restricted in infancy. *Journal of Biological Chemistry* 285(35):27167-27175.

19. Caroll MC & Prodeus AP (1998), Linkages of innate and adaptive immunity. *Current Opinion in Immunology* 10(1):36-40.

20. Robinson MJ, Sancho D, Slack EC, LeibundGut-Landmann S, & e Sousa CR (2006), Myeloid C-type lectins in innate immunity. *Nature Immunology* 7(12):1258-1265.

21. Hemmi H, *et al.* (2000), A Toll-like receptor recognizes bacterial DNA. *Nature* 408(6813):740-745.

22. Fritz JH, Ferrero RL, Philpott DJ, & Girardin SE (2006), Nod-like proteins in immunity, inflammation and disease. *Nature Immunology*

7(12):1250-1257.

23. Cresswell P (1994), Assembly, transport, and function of MHC class II molecules. *Annual Review of Immunology* 12(1):259-291.

24. Turley SJ, *et al.* (2000), Transport of peptide-MHC class II complexes in developing dendritic cells. *Science* 288(5465):522-527.

25. Trombetta ES, Ebersold M, Garrett W, Pypaert M, & Mellman I (2003), Activation of lysosomal function during dendritic cell maturation. *Science* 299(5611):1400-1403.

26. Greenwald RJ, Freeman GJ, & Sharpe AH (2005), The B7 family revisited. *Annu. Rev. Immunol.* 23:515-548.

27. Fujii S-i, Liu K, Smith C, Bonito AJ, & Steinman RM (2004), The linkage of innate to adaptive immunity via maturing dendritic cells in vivo requires CD40 ligation in addition to antigen presentation and CD80/86 costimulation. *The Journal of Experimental Medicine* 199(12):1607-1618.

28. Jonuleit H, *et al.* (1997), Pro-inflammatory cytokines and prostaglandins induce maturation of potent immunostimulatory dendritic cells under fetal calf serum-free conditions. *European Journal of Immunology* 27(12):3135-3142.

29. Langenkamp A, Messi M, Lanzavecchia A, & Sallusto F (2000), Kinetics of dendritic cell activation: impact on priming of TH1, TH2 and nonpolarized T cells. *Nature Immunology* 1(4):311-316.

30. Novick RP, *et al.* (1993), Synthesis of staphylococcal virulence factors

is controlled by a regulatory RNA molecule. *The EMBO Journal* 12(10):3967.

31. Kaito C & Sekimizu K (2007), Colony spreading in *Staphylococcus aureus*. *Journal of Bacteriology* 189(6):2553-2557.

32. Bera A, *et al.* (2007), Influence of wall teichoic acid on lysozyme resistance in *Staphylococcus aureus*. *Journal of Bacteriology* 189(1):280-283.

33. Kim HJ, *et al.* (2007), Lipoteichoic acid and muramyl dipeptide synergistically induce maturation of human dendritic cells and concurrent expression of proinflammatory cytokines. *Journal of Leukocyte Biology* 81(4):983-989.

34. Son YM, Park SM, & Yun CH (2013), Lipoteichoic Acid Suppresses Effector T Cells Induced by *Staphylococcus aureus*-Pulsed Dendritic Cells. *Journal of Microbiology and Biotechnology* 23(7):1023-1030.

35. Weidenmaier C, McLoughlin RM, & Lee JC (2010), The zwitterionic cell wall teichoic acid of *Staphylococcus aureus* provokes skin abscesses in mice by a novel CD4+ T-cell-dependent mechanism. *PLoS One* 5(10):e13227.

36. Jung D-J, *et al.* (2012), Specific serum Ig recognizing staphylococcal wall teichoic acid induces complement-mediated opsonophagocytosis against *Staphylococcus aureus*. *The Journal of Immunology* 189(10):4951-4959.

37. Takahashi K, *et al.* (2013), Intradermal Immunization with Wall Teichoic

Acid (WTA) Elicits and Augments an Anti-WTA IgG Response that Protects Mice from Methicillin-Resistant *Staphylococcus aureus* Infection Independent of Mannose-Binding Lectin Status. *PLoS One* 8(8):e69739.

38. Kurokawa K, *et al.* (2013), Glycoepitopes of staphylococcal wall teichoic acid govern complement-mediated opsonophagocytosis via human serum antibody and mannose-binding lectin. *Journal of Biological Chemistry* 288(43):30956-30968.

39. Cochand L, Isler P, Songeon F, & Nicod LP (1999), Human lung dendritic cells have an immature phenotype with efficient mannose receptors. *American Journal of Respiratory Cell and Molecular Biology* 21(5):547-554.

40. Mellman I, Turley SJ, & Steinman RM (1998), Antigen processing for amateurs and professionals. *Trends in Cell Biology* 8(6):231-237.

41. Hashimoto Y, *et al.* (2009), Identification of lipoteichoic acid as a ligand for draper in the phagocytosis of *Staphylococcus aureus* by Drosophila hemocytes. *The Journal of Immunology* 183(11):7451-7460.

42. Lin L, *et al.* (2009), Th1-Th17 cells mediate protective adaptive immunity against *Staphylococcus aureus* and *Candida albicans* infection in mice. *PLoS Pathogens* 5(12):e1000703.

43. Mailliard RB, *et al.* (2003), Dendritic cells mediate NK cell help for Th1 and CTL responses: two-signal requirement for the induction of NK cell helper function. *The Journal of Immunology* 171(5):2366-2373.

44. Kidd P (2003), Th1/Th2 balance: the hypothesis, its limitations, and implications for health and disease. *Alternative Medicine Review* 8(3).

45. Khader SA, Gaffen SL, & Kolls JK (2009) Th17 cells at the crossroads of innate and adaptive immunity against infectious diseases at the mucosa. *Mucosal Immunology* 2(5):403-411.

46. Murphy KM & Stockinger B (2010), Effector T cell plasticity: flexibility in the face of changing circumstances. *Nature Immunology* 11(8):674-680.

47. McKenzie BS, Kastelein RA, & Cua DJ (2006), Understanding the IL-23–IL-17 immune pathway. *Trends in Immunology* 27(1):17-23.

48. Cobb BA & Kasper DL (2005), Microreview: Zwitterionic capsular polysaccharides: the new MHCII-dependent antigens. *Cellular Microbiology* 7(10):1398-1403.

49. Wang Q, *et al.* (2006), A bacterial carbohydrate links innate and adaptive responses through Toll-like receptor 2. *The Journal of Experimental Medicine* 203(13):2853-2863.

50. Kalka-Moll WM, *et al.* (2002), Zwitterionic polysaccharides stimulate T cells by MHC class II-dependent interactions. *The Journal of Immunology* 169(11):6149-6153.

국문초록

테이코익산이 결여된 황색포도상구균에 의해 자극된 인간 수지상세포의 표현형과 기능

홍 성 준

서울대학교 대학원

치의과학과 면역 및 분자미생물 치의학 전공

(지도교수 : 한승현)

1. 연구목적

인간 수지상세포는 전문적인 항원제시세포로서 선천성면역계와 적응면역계를 연결한다. 수지상세포의 표면분자발현, 싸이토카인의 분비 및 T 세포 활성화 능력은 박테리아 세포벽에 위치한 펩티도글리칸, 리포테이코익산, 테이코익산을 포함하는 다양한 병독력인자에 의한 수지상세포의 성숙을 통해 조절된다. 그람양성균의 주요 병독력인자중 하나인 테이코익산의 경우, 수지상세포의 성숙 및 활성화에 미치는 영향이 아직 밝혀지지 않았다. 본 연구에서는 테이코익산이 결여된 돌연변이

황색포도상구균을 사용하여 수지상세포의 활성화에서 테이코익산의 역할을 규명하였다.

2. 연구방법

인간 혈액을 ficoll을 이용한 밀도구배원심분리법으로 분리한 혼합백혈구에서 마그네틱 비드를 이용하여 $CD14^+$단핵구만을 분리하였다. 분리한 단핵구를 GM-CSF (50 ng/ml)와 IL-4 (17.7 ng/ml)를 첨가한 RPMI-1640에서 6일간 배양하여 단핵구 유래 수지상세포로 분화시켰다. 수지상세포의 성숙과정에 있어서 황색포도상구균 테이코익산의 역할을 연구하기 위하여 수지상세포(1 x 10^5 cells)에 에탄올로 사멸시킨 황색포도상구균(wild-type)과 테이코익산이 결여된 황색포도상구균 (Δ*tagO*) 그리고 *tagO* 유전자 보완 황색포도상구균 (p*StagO*) 30 μg/ml을 24시간 동안 처리한 후, 수지상세포의 성숙마커인 CD83, CD86, MHC class Ⅰ, MHC class II, PD-L1, PD-L2의 발현을 유세포 분석기로 분석하였다. 또한, 효소결합면역분석법(ELISA)을 이용하여 황색포도상구균 자극에 따른 싸이토카인 IL-1β, IL-12, TNF-α, IL-6, IL-10의 발현을 측정하였다. 수지상세포를 매개한 T 세포 활성화에서 테이코익산의 역할을 알아보기 위하여, wild-type 또는 Δ*tagO*로 24시간 동안 자극한 수지상세포(2 x 10^4 cells)를 자가 유래 혼합백혈구(2 x 10^5 cells)와 5일간 공동 배양한 후, $CD3^+$ T 세포의 활성화 마커인 CD25와 MHC class II의 발현을 유세포 분석기로 분석하였다. 또한 T 세포의 증식양상을 조사하기 위해서 wild-type 또는 Δ*tagO*로 자극한 수지상세포와 CFSE로 염색된 혼합백혈구를 공동 배양한 후, $CD3^+$ T 세포를 유세포 분석기로 분석하였다. 마지막으로 wild-type 또는 Δ*tagO* 로 자극한 수지상세포와 공동 배양한 혼합백혈구의

싸이토카인 IFN-γ 와 IL-10의 발현을 ELISA를 이용하여 측정하였다.

3. 연구결과

Wild-type 황색포도상구균은 인간 단핵구 유래 수지상세포 표면에 동시자극분자 CD83, CD86과 주조직적합복합체 분자 MHC class I, MHC class II 그리고 PD-L1, PD-L2 분자들의 발현을 현저하게 증가시켰다. 하지만 Δ*tagO*로 자극한 수지상세포의 경우, wild-type으로 자극한 수지상세포 대비 50% 수준 이하의 CD83, CD86, MHC class I, PD-L1 발현을 보였다. MHC class II와 PD-L2의 경우, wild-type 대비 유의적 차이가 관찰되지 않았다. 또한, wild-type은 수지상세포의 IL-1β, IL-12, TNF-α, IL-6, IL-10의 생성을 모두 증가시켰지만, Δ*tagO*는 IL-1β, IL-12, TNF-α의 생성을 유도하지 못하였다. 반면, IL-6와 IL-10의 생성은 유의적 차이가 나타나지 않았다. 수지상세포의 표면분자 발현과 싸이토카인의 분비는 p*StagO*로 자극시, wild-type과 유사한 수준으로 회복되는 것을 확인하였다. Wild-type으로 자극된 수지상세포는 T 세포 활성화 마커인 CD25와 MHC class II의 발현 및 T 세포의 증식을 유의적으로 증가시키지만 Δ*tagO*로 자극된 수지상세포는 T 세포 활성화마커 발현과 증식이 wild-type에 비하여 현저히 낮은 것을 관찰할 수 있었다. p*StagO* 자극을 받은 수지상세포에서는 T 세포의 활성 마커 발현 및 증식이 wild-type과 유사한 수준으로 회복되는 것을 확인하였다. 마지막으로 wild-type으로 자극된 수지상세포는 혼합백혈구의 IFN-γ 와 IL-10의 발현을 증가시켰지만, Δ*tagO*로 자극된 수지상세포는 혼합백혈구의 IFN-γ 와 IL-10의 발현을 증가시키지 못하였고 p*StagO*로 자극된 수지상세포는 혼합백혈구의 IFN-γ 와 IL-10의 발현을 wild-type과 유사한 수준으로 증가시키는 것을

확인하였다.

4. 결 론

황색포도상구균의 테이코익산은 인간 수지상세포의 동시자극분자, 주조직적합복합체 분자의 증가 및 염증성 싸이토카인 IL-1β, IL-12, TNF-α의 생성을 유도하는데 필수적이고, 수지상세포를 매개한 T 세포의 활성화 및 증식 조절에서 중요한 역할을 수행한다는 결론을 얻었다.

주요어 : 단핵구 유래 수지상세포, 황색포도상구균, 테이코익산, T세포 활성화

학 번 : 2012-23693