Staphylococcus aureus from atopic dermatitis skin alters cytokine production triggered by monocyte-derived Langerhans cell

HIROSHIMA UNIVERSITY

Kazumasa Iwamoto¹, Masaya Moriwaki¹, Yoshie Niitsu^{1,2}, Masachika Saino¹,

Junzo Hisatsune², Motoyuki Sugai², Michihiro Hide¹

1) Department of Dermatology, 2) Department of Bacteriology,

Graduate school of Biomedical and Health Sciences, Hiroshima University, Japan

Background / Question addressed

Patients with atopic dermatitis (AD) have frequent bacterial infections and AD skin harbors an increased level of *Staphylococcus aureus* (*S. aureus*). However, skin immune responses via activated Langerhans cell (LC) by different strains of *S. aureus*, especially **clinically isolated strains from AD skin**, have not been studied.

The aim of this study is to explore whether *S. aureus* isolated from AD skin is able to skew T cell responses via LC as compared to a control strain of *S. aureus* and *S. epidermidis*.

Material & Method - 1

CD14^{pos} monocytes and CD4^{pos} T cells were sorted from peripheral blood mononuclear cells of healthy controls (HC) and patients with AD. Monocyte-derived Langerhans cells (MoLC) were generated by culturing monocytes with GM-CSF, IL-4 and TGF- β for 7 days.

MoLC were cultured with or without *S. aureus* (control and AD strains), *S. epidermidis* and Pam3CSK4 for 24 h, then washed and subsequently sorted by CD1a^{pos} by FACSaria™. Sorted CD1a^{pos} MoLC were co-cultured with autologous CD4^{pos} T cells, which were isolated and kept frozen until Mixed lymphocyte reaction. After co-culture, T cell functions were analyzed. This study was approved by the local ethics committee of Hiroshima University and informed consent was obtained from all participants.

Material & Method - 2

The detailed backgrounds of participants and the sequence types of *S. aureus* strains are listed below.

	HC (n = 24)	AD (n = 13)	<i>S. aureus</i> strain	Sequence type
Male : Female	14 : 10	10 : 3	NCTC8325	8
Age	29 $\pm$ 2	30 $\pm$ 2	TF3378*	59
IgE (IU/ml)	133 $\pm$ 59	13667 $\pm$ 5105	TF2962*	59
TARC (pg/ml)	265 $\pm$ 24	3744 $\pm$ 1240	TF2868*	121
			TF2870*	20

* isolated from AD skin

Statistical analyses; Paired samples were evaluated using the Repeated Measures ANOVA followed by the Newman-Keuls Multiple Comparison Test or the Wilcoxon matched-pairs signed rank test. For independent samples, the Mann-Whitney *U* test was used. Graphs were prepared as scatter plots with mean $\pm$ SEM. Significance was indicated compared to unstimulated control ($\emptyset$) and between groups by **p* < 0.05, ***p* < 0.01 and ****p* < 0.001.

Result 1 : *S. aureus* induces maturation of MoLC.

(a) The surface expression of TLR2 on MoLC from HC (n = 12) and AD (n = 8) was analyzed by flow cytometry. (b) MoLC was cultured $\pm$ *S. aureus* for 24 h. Cell surface expression on MoLC from HC ($\circ$; n = 12) and AD ($\bullet$; n = 10) was analyzed by flow cytometry.

Result 2 : *S. aureus* from AD skin enhance T cell proliferation.

MoLC was cultured $\pm$ *S. aureus* for 24 h. After *S. aureus* stimulation, CD1a-sorted MoLC were co-cultured with autologous CD4^{pos} T cells for 7 days. After co-culture, accurate numbers of proliferated alive CD4^{pos} T cells (7-AAD^{neg}) were measured by FACSaria™ II. (a) MoLC was stimulated by a standard strain of *S. aureus* NCTC8325 or *S. aureus* strains (TF3378, TF2962, TF2868 and TF2870) from AD skin and the number of proliferated T cells was counted, n = 6 (HC). (b) T cell proliferation by *S. aureus*-stimulated MoLC derived from HC and AD were compared, n = 13 ($\circ$; HC) and n = 10 ($\bullet$; AD). (c) The time course of divided T cells was assessed by CFSE, n = 9–13 (HC) and n = 6–10 (AD). (d) A representative dot plot is shown. The percent of divided T cells is indicated.

Result 3 : *S. aureus* TF3378 from AD skin stimulated-MoLC trigger altered cytokine production from T cells.

Cytokine production in the supernatant from co-culture was quantified by a BD™ Cytometric Bead Array kit, n = 13 ($\circ$; HC) and n = 9–10 ($\bullet$; AD). The Y-axis represents cytokine production per 50,000 live CD4pos T cells.

Result 4 : Quantitative real-time PCR analysis of cytokine mRNAs.

After co-culture, alive CD4^{pos} T cells (7-AAD^{neg}) were collected by FACSaria™ II and mRNA was extracted. (a) The expression of *TBX21* and *GATA3* was analyzed by quantitative real-time PCR, n = 10 (HC) and n = 8 (AD). The expression levels of each gene were normalized using *ACTB* and untreated cells were used as control. (b) The ratio of *TBX21* divided by *GATA3*, indicating Th1/Th2 balance, was shown.

Result 5 : *S. epidermidis* induce T cell proliferation via MoLC comparable to a control strain of *S. aureus*

MoLC was cultured $\pm$ *S. aureus* (NCTC8325 or TF3378), *S. epidermidis*, or TLR1/2 ligand Pam3CSK4 for 24 h. (a) Stimulated MoLC was sorted by CD1a and then co-cultured with autologous CD4^{pos} T cells for 7 days. After co-culture, the number of proliferated alive CD4^{pos} T cells (7-AAD^{neg}) were counted by FACSaria™ II, n = 7 ($\circ$; HC) and n = 6 ($\bullet$; AD). (b) Cytokine production in the supernatant from co-culture was quantified by a BD™ Cytometric Bead Array kit, n = 7 ($\circ$; HC) and n = 6 ($\bullet$; AD). The Y-axis represents cytokine production per 50,000 live CD4pos T cells.

Result 6 : Proteinase K treatment of *S. aureus* TF3378 diminish the capacity for activating MoLC.

MoLC was stimulated by *S. aureus* NCTC8325 or TF3378 with or without proteinase K treatment for 24 h. (a) A representative histogram from is shown. After co-culture of *S. aureus* stimulated MoLC and autologous CD4^{pos} T cells, (b) Accurate numbers of live CD4^{pos} T cells were measured. (c) IL-2 production in the supernatant from co-culture. (d) Silver staining of cell wall proteins extracted from *S. aureus* is shown.

Conclusion

TLR2 stimulus due to low expression TLR2.

- AD and control strains of *S. aureus* induce different T cell responses via LC.
- *S. aureus* TF3378, AD strain, triggers increased IL-2 and less IFN- γ production.
- Altered T cell responses could be due to differences in cell wall proteins of *S. aureus*.

Acknowledgment

This work was supported by JSPS KAKENHI Grant Number 15H06428.