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Identification of TLR2-activating lipoproteins in *Staphylococcus aureus*

Kazuki TAWARATSUMIDA

1. Introduction

S. aureus is gram-positive bacteria, and has high pathogenicity as a causative factor of inflammatory disease. Because *S. aureus,* including drug-resistant strain MRSA, is also famous as a pathogen causing hospital-acquired infection, studies for clarification of its pathogenic factor has been done vigorously. It is reported that *S. aureus* activate immune cells through TLR2 and trigger inflammation. Previously, lipoteichoic acid (LTA) fraction derived from *S. aureus* was found to activate cells via TLR2, and these components were thought to be the stimuli. However, recent studies showed that highly purified LTA didn't activate TLR2, indicating that LTA fraction contains TLR2-activating contaminants. In this study, I separated TLR2-activating lipoproteins expressed in *S. aureus* and elucidated the TLR2-activating structure.

2. Results and Discussions

The lipoprotein fraction of *S. aureus* was prepared by glass beads disruption followed by Triton X-114 phase partitioning. The TLR2-activating molecules were mainly detected in the mass range of 30-35 kDa. Seven lipoproteins were identified by the mass spectra of their tryptic digests. Among them, three lipoproteins were separated by preparative SDS-PAGE and proved to activate TLR2. After digestion with trypsin in the presence of sodium deoxycholate, the N-terminus of the lipopeptide was isolated from lipoprotein SAOUHSC_02699 by normal phase HPLC and characterized as a S-(diacyloxypropyl)cystein-containing peptide using tandem mass spectra. Synthetic lipopeptide counterpart also stimulated cell via TLR2.

In this study, I identified the N-terminal structure of *S. aureus* lipoprotein SAOUHSC_02699 as diacylated lipoprotein. We also confirmed its activity using synthetic counterpart. In several bacteria, but not all, the N-terminus of the diacylglyceryl-modified cysteine residue is fatty acylated by a lipoprotein N-acyltransferase (lnt). Stoll, H. et al. screened the published genome sequences of *S. aureus* strains for a gene encoding an lnt homolog and found no such protein. This data corresponds to our data that the lipoproteins in *S. aureus* are diacylated.

3. Conclusions

In conclusion, I identified TLR2-activating lipoproteins from *S. aureus* cells and characterized N-terminal lipopeptide structure of a lipoprotein SAUOHSC_02699 as diacylated. Since these lipoproteins are considered to contribute to the virulence of *S. aureus,* these findings will help the prevention and treatment against *S. aureus* infection.

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University of Michigan Medical School