DISSERTATION SUMMARY

Staphylococcus aureus isolated from atopic dermatitis skin produces staphylococcal enterotoxin Y that predominantly induces TCR Vα-specific expansion of T cells

（アトピー性皮膚炎から分離された黄色ブドウ球菌が産生するエンテロトキシン SEY は特異的な TCR Ｖα レパートリーの Ｔ 細胞を活性化させる）

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Staphylococcus aureus is a ubiquitous Gram-positive bacteria, known worldwide to cause a variety of infectious diseases ranging from skin infections to fatal systemic infections in humans and animals. Staphylococcal enterotoxins (SEs) are members of superantigen family and to date more than 20 different SE were identified. They reported to sharing several biological activities and structural features. These bacterial proteins are known to have pyrogenic, superantigenic, and emetic activities. Some of these are implicated in toxic shock syndrome and are causative agents of staphylococcal food poisoning .

Many studies have shown significant associations of SEs with human diseases. The mechanisms of action of SEs in toxicicity of staphylococcal infections and exacerbation of *S. aureus* pathogenicity have been documented in the context of classical SEs such as SEA-SEE, SEH and toxic shock syndrome toxin (TSST). Most of the SEs interact with the specific variable regions of T cell receptor β chains (TCR Vβ), leading to cytokine production and mitogenic response in T cells that orchestrate the onset of inflammation. Superantigens contribute to skin disorders by skin homing of T cells and mediate regulation of the immune response. Superantigens trigger hosts to produce specific immunoglobulin E (IgE). IgE-relevant superantigens thus stimulate mast cell and basophil degranulation resulting in the release of mediators such as cytokines and chemokines eventually promoting skin rashes and itch manifestation in dermatitis patients.

While performing genomic characterization of *S. aureus* isolated from the skin of atopic dermatitis patients (AD) for unique virulence factor(s), we identified a novel ORF exhibiting structural similarity to superantigen. Concurrently, the ORF was identified by Ono et al. (2015) as a new enterotoxin-like protein from a bovine isolate of *S. aureus* designated as SE*l*Y_{bov}. Recombinant SE*l*Y_{bov} had superantigen activity in human peripheral blood mononuclear cells. They further demonstrated emetic activity in a primate animal model, proposed to rename SE*l*Y_{bov} to SEY_{bov}. Among SEs, SEY_{bov} was close to SET which share 32% amino acids sequence identity. Interestingly, this SEY_{bov} showed very weak superantigenic activity for mouse splenocytes even at 10μg/ml, unlike other enterotoxins such as SEA and SEB that induce mouse T cell proliferation dose dependently at nanogram concentration.

We herein investigated the prevalence of *sey* gene in human clinical isolates of diverse origins and demonstrated that *S. aureus* from AD or impetigo/SSSS possesses *sey* gene about 17~22%. In present study, 42 strains were positive for the *sey*, and the positive isolates were found in skin diseases AD and impetigo/SSSS. We characterized *sey* products

of the *S. aureus* isolates identified three SEY variants (SEY_1, SEY_2, SEY_3) are produced by isolates belonging to three independent clonal complex (CC121, CC20, CC59). Most sey^+ isolates produced SEY in broth culture.

In addition, during the surveillance of *S. aureus* from AD patients, we isolated an *Staphylococcus argenteus* and next generation sequencing (NGS) analysis identified a single gene encoding a toxin on its chromosome which the deduced amino acid sequence of this toxin has 98% sequence identity with SEY from *S. aureus*. As putative virulence factor in *S. argenteus*, *sey* mRNA was expressed and transcription level reached the maximum level at early stationary phase. A recombinant S_{arg}EY prepared in *Escherichia coli* showed immunological crossreactivity against anti-SEY serum. We determined its superantigen activity using human PBMCs. Similar to SEY variants, S_{arg}EY was able to induce proliferation of $CD4^+$ and $CD8^+$ T cells as well as production of TNF-α and IFN-γ. As potential food poisoning agent, S_{arg}EY exhibited emetic activity in monkey model. Unlike SEY_{bov}, the three recombinant SEY variants and S_{arg}EY exhibited stability to heat treatment but still susceptible to digestive enzymes. The S_{arg}EY character is very close to that of atopic *S. aureus* and this putative virulence factor may account for *S. argenteus* pathogenicity.

In present study, we employed a new approach, NGS of TCR Vα/β repertoires in human peripheral blood mononuclear cells following in vitro stimulation with staphylococcal enterotoxins to explore the TCR dependency of the uncharacterized SEs. To our surprise, SET, SEY and S_{arg}EY predominantly activated human T cells with a particular TCR Vα profile, a unique observation since most staphylococcal enterotoxins exert their superantigenic activities through activating T cells with specific TCR Vβ profiles. Remarkable enhancement of TCR Vα transcription in T cells following toxins stimulation was observed in SET for TRAV 13.2 and 29/DV5 genes, while SEY for TRAV 8.2 and 8.6 and S_{arg}EY for TRAV 8.2, 8.4 and 8.6. SEH was reported to stimulate human T cells in particular TRAV 27 by real-time RT-PCR method. Besides TRAV 27, TCR sequencing revealed the stimulation of TRAV 25, 30, 34 and 35 genes by SEH. In contrast, we confirmed SEB stimulation of Vβ subfamilies 3, 12, 14, 17 and 20 as described previously. In addition, TCR sequencing demonstrated enhanced transcriptions of Vβ 6 and 15, which were not analyzed by flow cytometry due to the unavailability of specific antibodies to those Vβ, while no particular Vα enhancement. In addition to SEH, our study contributed to define detailed TCR Vα activation profiles of newly identified staphylococcal superantigen. Further, TCR sequencing demonstrated other undescribed Vα repertoires induced by SEH. In addition to SEH, our study contributed to defining detailed TCR Vα activation profiles of newly identified staphylococcal superantigen. Further, TCR sequencing demonstrated other undescribed Vα repertoires induced by SEH. We showed TCR sequencing of whole RNA trancript is superior to TCR repertoire analyses based on flow cytometry or PCR in characterization of specific Vα and Vβ expansion following superantigen stimulation.

In conclusion, we demonstrated Staphylococcal from AD patient possesess SEY and S_{arg}EY. The SEY and S_{arg}EY may contribute in skin pathogenesis via activation of skin T-cells through TCR Vα manners.