論 文 内 容 要 旨

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 V α レパートリーの T 細胞を活性化させる）

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ABSTRACT

Staphylococcal enterotoxins (SEs) are members of a protein family of more than 20 different staphylococcal exotoxins, sharing several biological activities and structural feature. 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. While investigating the virulence trait of *Staphylococcus aureus* adhering to skin of atopic dermatitis (AD) patients, we identified a novel ORF with structural similarity to superantigen from genome sequence data of an isolate from AD skin. Concurrently, the same ORF was identified in a bovine isolate of *S. aureus* and designated as SEY. Recombinant SEY_{bov} previously reported had superantigen activity in human peripheral blood mononuclear cells (PBMCs) and showed emetic activity in a marmoset monkey model. We herein investigated the prevalence of the *sey* gene in 270 human clinical isolates of various origins in Japan. Forty-two strains were positive for the *sey* gene, and the positive isolates were from skin diseases atopic dermatitis and impetigo/SSSS with an average detection rate of 17~22%. There were three variants of SEY (SEY_1, SEY_2, and SEY_3), and the isolates producing SEY variants formed three distinct clusters corresponding to clonal complexes (CCs) 121, 59, and 20, respectively, suggesting clonal distribution of SEY variants in human isolates of *S. aureus*. Most sey^+ isolates produced SEY in broth culture.

During the surveillance of *S. aureus* from lesions of atopic dermatitis patients, we isolated a *Staphylococcus argenteus*, a new member of genus *Staphylococcus* registered in 2011 and previously considered as *Staphylococcus aureus* lineage. Complete genome sequencing and search for potential virulence genes identified a

single gene encoding possible superantigen on its chromosome. The deduced amino acid sequence of the *orf* showed 98% sequence identity to SEY from *S. aureus*. The *orf* mRNA was expressed in culture and transcription level reached the maximum at early stationary phase. The ORF protein expressed in *Escherichia coli* showed immunological crossreactivity with anti-SEY serum. We investigated its superantigen activity using human PBMCs, and the ORF protein was able to induce proliferation of $CD4^+$ and $CD8^+$ T cells as well as production of TNF-α and IFN-γ. We designated the ORF as S_{arg}EY and it exhibited emetic activity in marmoset monkey model. Like SEY_{bov}, the three recombinant SEY variants and S_{arg}EY exhibited similar biological activities and stability to heating and digestive enzymes. Comparison of genome sequences of the *S. argenteus* producing S_{arg}EY and representative *S. aureus* clinical strains from various origins indicated that the *S. argenteus* genome is very close to that of *S. aureus* from atopic dermatitis.

Our study contributed to defining detailed TCR Vα/β activation profiles of uncharacterized staphylococcal superantigen using a new approach, TCR sequencing. 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 SEs exert their superantigenic activities through activating T cells with specific TCR Vβ profiles. In addition to SEH, TCR sequencing demonstrated other undescribed Vα repertoires induced by SEH. We demonstrated *S. aureus* and *S. argenteus* human clinical isolates which are common in skin pathophysiology relevant to the loss of barrier function produces SEY and S_{arg}EY respectively. Both bacteria are commonly adherent to the lesion and prolonged infection may exacerbate the clinical condition. SEY in situ may contribute to the activation of skin resident T cells via TCR Vα interaction.