

論文審査の結果の要旨

博士の専攻分野の名称	博士（ 学術 ）	氏名	FATKHANUDDIN AZIZ
学位授与の条件	学位規則第4条第①・2項該当		
<p>論文題目</p> <p><i>Staphylococcus aureus</i> 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レパトリーの T 細胞を活性化させる)</p>			
<p>論文審査担当者</p> <p>主 査 教 授 小松澤 均 印</p> <p>審査委員 教 授 坂口 剛正</p> <p>審査委員 教 授 太田 耕司</p>			
<p>〔論文審査の結果の要旨〕</p> <p><i>Staphylococcus aureus</i> is a ubiquitous Gram-positive bacteria, recognized worldwide causing a variety of infectious diseases ranging from skin infection to fatal systemic infections in human and animal. Staphylococcal enterotoxins (SEs) belongs to a family among more than 20 different staphylococcal exotoxins, sharing several biological activities and structural features. These bacterial proteins are shown to have pyrogenic, superantigenic, and emetic activities, and are causative agents of staphylococcal food poisoning. Some of these are implicated in toxic shock syndrome.</p> <p>Many studies have shown significant associations of SEs with human diseases. The mechanisms on how SEs are involved in toxic effects of staphylococcal infections or exacerbation of <i>S. aureus</i> pathogenicity have been documented in classical SEs (SEA-SEE), SEH and toxic shock syndrome toxin (TSST). 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. Previously, Ono et al. (2015) identified and characterized a new type of enterotoxin-like protein. The protein was similar to SET and was designated as SE/Y. This toxin was renamed from SE/Y to SEY after demonstrated emetic activity in marmoset monkey. Interestingly, SEY cloned from bovine isolate (SEY_{bov}) showed no superantigenic activity for mouse splenocytes, unlike other enterotoxins such as SEA and SEB which can strongly induce mouse T cell proliferation.</p> <p>SEY was primarily identified from strains isolated from several cases of food poisoning, a human nasal swab, a case of bovine mastitis and cases of <i>S. aureus</i> infection. However, systemic epidemiological analysis of the prevalence of the <i>sey</i> gene in human clinical isolates have never been performed. The study to clarify distributions among clinical isolates and pathophysiology role by identified SE in association to human diseases is essential.</p> <p>The student investigated the prevalence of the <i>sey</i> in 270 <i>S. aureus</i> human clinical isolates of various origins in Japan. He found that 42 strains were positive for the <i>sey</i>, and</p>			

the positive isolates were identified in skin diseases atopic dermatitis (AD) and impetigo/SSSS with an average detection rate of 17~22%. Moreover, there were three variants of SEY (SEY1, SEY2, and SEY3), and isolates producing SEY variants corresponding to certain clonal complexes (CCs) 121, 59, and 20, respectively. His research also demonstrated most *sey*⁺ isolates produced SEY in broth culture by developed ELISA with the variation of production from 3 to 178 ng/ml.

In addition, during the surveillance of *S. aureus* from AD patients, he isolated an *Staphylococcus argenteus* strain and next generation sequencing (NGS) analysis identified a single gene which the deduced amino acid sequence has 98% identity to SEY from *S. aureus*. A recombinant S_{arg}EY showed immunological cross reactivity against anti-SEY serum. He determined its superantigen activity using human PBMCs. Similar to SEY variants, S_{arg}EY was able to induce proliferation of human CD4⁺ and CD8⁺ T cells as well as production of TNF- α and IFN- γ . Although the effects on human lymphocytes did not differ among the SEY variants and S_{arg}EY, those of any SEY were not as strong as those of SEB which is known to act as a superantigen in both mice and humans. As potential food poisoning agent, S_{arg}EY exhibited emetic activity in monkey model. Unlike SEY_{bov}, the 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, he employed a new approach, NGS of TCR V α / β in determining TCR repertoires following T-cell stimulation with such uncharacterized SEs. Surprisingly, SEY and S_{arg}EY predominantly activated human T cells with a particular TCR V α profile. Its previously reported flow cytometry and PCR method were not able to determine SET character on T cells. In present study, he clarified that SET also employed TCR V α for T-cell activation. These result showed a unique observation since most staphylococcal enterotoxins exert their superantigenic activities through activating T cells with specific TCR V β profiles. Remarkable enhancement of TCR V α transcription was observed for each of these toxins: TRAV 13.2 and 29/DV5 genes for SET; TRAV 8.2 and 8.6 for SEY; and TRAV 8.2, 8.4 and 8.6 for S_{arg}EY. In addition to enhanced TRAV 27 transcription which has been detected by real-time RT-PCR method, TCR sequencing revealed expansion of TRAV 25, 30, 34 and 35 gene transcripts in SEH-stimulated T cells.

In addition to SEH, his study contributed to defining detailed TCR V α activation profiles of newly identified staphylococcal superantigen. Further, TCR sequencing demonstrated other undescribed V α repertoires induced by SEH. He showed TCR sequencing of whole RNA transcript is superior to TCR repertoire analyses based on flow cytometry or PCR in characterization of specific V α and V β expansion following superantigen stimulation. In conclusion, his study demonstrated *S. aureus* and *S. argenteus* human clinical isolates which are common in skin pathophysiology relevant to the loss of barrier function possesses SEY and S_{arg}EY respectively. The SEY and S_{arg}EY may contribute in skin pathogenesis via activation of skin T-cells through TCR V α manners.

Based on these results, this dissertation greatly contributes to elucidating the mechanism of pathogenesis in staphylococcal infections by clarifying the function of SEY, a virulence factor of *S. aureus* and *S. argenteus*.

Therefore, all the committee members admitted that this dissertation is of sufficient value to confer the Doctor of Philosophy to Fatkhanuddin Aziz.