# Department of Bacteriology

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## General Summary

Research projects of our department have focused on: 1) the mechanism of inhibition of *Staphylococcus aureus* colonization by commensal *Staphylococcus epidermidis*; 2) the role of beta-hemolysin in the inhibition of interleukin (IL)-8 production by human umbilical endothelial cells; 3) the fibronectin-mediated colonization via fibronectin-binding protein (FnBP) A in *S. aureus* infection; 4) the induction of apoptosis of fibroblasts by intracellular *S. aureus*; 5) the mechanism of bacterial biofilm formation; and 6) the molecular analysis of viable but nonculturable bacteria.

## Research Activities

*A biofilm destruction factor secreted by an indigenous bacterium inhibits pathogenic bacterial colonization*

Although indigenous bacteria are generally considered to inhibit pathogen colonization, complex host-microbe and microbe-microbe interactions have made it difficult to understand in detail the mechanisms that might be involved. In this study, we demonstrate that Esp, a protein belonging to the serine protease family which is secreted by the indigenous bacterium *S. epidermidis,* inhibits *S. aureus* nasal colonization via biofilm destruction. *S. aureus* is the main pathogen colonizing the nasal cavity in humans. Both in vitro and epidemiological studies have demonstrated that *S. epidermidis* strains can be categorized into inhibitory and noninhibitory types on the basis of their inhibition of *S. aureus* colonization. Furthermore, *S. aureus* was significantly less prevalent when the inhibitory *S. epidermidis* type was present within the nasal cavity. The molecule responsible for the inhibitory activity was Esp. This molecule destroyed the biofilms of *S. aureus,* including those of methicillin- and vancomycin-resistant strains, by disrupting the intercellular matrix but not by bactericidal activity.

*Inhibition of endothelial IL-8 production and neutrophil transmigration by S. aureus β-hemolysin*

The innate immune system plays a crucial role in the host response to infection with *S. aureus,* and leukocyte migration is a key event in host defense against bacterial infection. The endothelium plays an important role in neutrophil recruitment through modulation of the expression of cell adhesion molecules and cytokines, such as IL-8, which is a potent chemoattractant and activator of neutrophils.

We have previously reported that *S. aureus* secretes a factor that suppresses IL-8 production by human endothelial cells. Here we isolated the factor inhibiting IL-8 production from the supernatant and identified it as staphylococcal β-hemolysin. This

protein is an enzyme that specifically cleaves sphingomyelin, the major sphingolipid in membranes, and is highly hemolytic for sheep erythrocytes. However, little is known about the action of β-hemolysin on host cells.

β-hemolysin reduced IL-8 production without cyotoxicity to endothelial cells. Pretreatment with β-hemolysin decreased the expression of both IL-8 mRNA and protein induced by tumor necrosis (TNF)-α. The migration of neutrophils across TNF-α — activated endothelium was also inhibited by β-hemolysin. β-hemolysin reduced VCAM-1 expression but not ICAM-1 expression in activated endothelial cells. β-hemolysin did not inhibit activation of nuclear factor κB but did inhibit activation of extracellular signal — regulated kinase. These results show that β-hemolysin produced by *S. aureus* interferes with inflammatory signaling in endothelial cells and may help *S. aureus* to evade host immune responses.

*Interaction between fibroblasts and S. aureus at different growth phases*

A clinical isolate *S. aureus* OK11 was used. Staphylococci grown in brain heart infusion broth at 37°C for 2 hours (exponential phase) and 18 hours (stationary phase) were treated with purified fibronectin to examine the participation of fibronectin in the ingestion of bacteria by L929 fibroblasts. The bacterial numbers in each L929 fibroblast were counted under a light microscope. We have shown that the exponential phase staphylococci expressed a large amount of FnBP on their surfaces, whereas stationary phase cells did not. L929 fibroblasts that had a network of fibronectin on their surfaces ingested a large number of bacteria in the exponential phase but ingested fewer bacteria after they had been treated with fibronectin. This result suggests that fibronectin is an adhesin. Because L929 fibroblasts with fibronectin could ingest more staphylococci in the stationary phase than could L929 fibroblasts without fibronectin, fibroblasts might have other adhesins involved with bacterial ingestion.

*Contribution of FnBPB to fibronectin-mediated colonization in S. aureus infection*

To colonize host tissues and organs *S. aureus* has a variety of adhesions that bind to extracellular matrix proteins or plasma proteins. Of these adhesions, FnBPs are thought to be the most important for interacting with host cells, such as endothelial cells, epithelial cells, fibroblasts, and macrophages. As we have already reported using the *fnbA* mutant strain derived from the parental SH1000 strain, FnBPA is important for infection by *S. aureus* both *in vitro* and *in vivo* because it allows the effective colonization of host tissues.

On the other hand, the role of FnBPB in infection is poorly understood. The DNA sequence of *fnb*B suggests that FnBPB lacks 1 or 2 of the fibronectin-binding domains and a fibrinogen-binding domain. Therefore, FnBPB might be less important for the establishment of infection than is FnBPA. However, our results described above showed the complete reduction of colonization in both *in vitro* and *in vivo* experiments. These results suggest that FnBPB has no importance in infection, although it is expressed at levels equal to those of FnBPA, and that FnBPA and B cooperate with one another for exhibiting their functions. To determine whether these possibilities are true, we are planning to obtain an *fnb*B-deficient mutant and *fnb*A/*fnb*B double-deficient mutant

from the SH1000 strain.

## Publications

***Seki K.*** Interaction between *Staphylococcus aureus* and host cells (in Japanese). *Tokyo Jikeikai Ikadaigaku Zasshi (Tokyo Jikeikai Med J)* 2007; **122:** 123–35.

***Iwase T, Hoshina S, Seki K, Shinji H, Masuda S, Mizunoe Y.*** Rapid identification and specific quantification of *Staphylococcus epidermidis* by 5′ nuclease real-time polymerase chain reaction with a minor groove binder probe. *Diagn Microbiol Infect Dis* 2008; **60:** 217–9.

***Iwase T, Seki K, Shinji H, Mizunoe Y, Masuda S.*** Development of a real-time PCR assay for the detection and identification of *Staphylococcus capitis*, *Staphylococcus haemolyticus* and *Staphylococcus warneri*. *J Med Microbiol* 2007; **56:** 1346–9.

***Iwase T, Uehara Y, Seki K, Shinji H, Tajima A, Mizunoe Y.*** Inhibition of *Staphylococcus aureus* colonization by *Staphylococcus epidermidis*. *Bacterial Adherence Biofilm* 2007; **21:** 59–62.

## Reviews and Books

***Mizunoe Y.*** Vibrio cholera (in Japanese). *Toda Shin-Saikingaku* 2007; **33**: 563–72.