Department of Microbiology (II)

Shogo Masuda, *Professor*
Keiko Seki, *Associate Professor*
Hitomi Shinji, *Lecturer*

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 of human umbilical endothelial cells; 3) fibronectin-mediated colonization via fibronectin-binding protein (FnBP) A in *S. aureus* infection; 4) induction of apoptosis in fibroblasts by intracellular *S. aureus*; and 5) the mechanism of bacterial biofilm formation.

Research Activities

Inhibition of S. aureus colonization by S. epidermidis

S. aureus has a marked affinity for human tissue. However, compared with indigenous staphylococci, *S. aureus* has a low detection rate in humans. Although this low detection rate is thought to be the result of some defense mechanism, the details remain poorly understood. We hypothesized that this phenomenon is attributable to inhibition of *S. aureus* colonization by *S. epidermidis*, the dominant indigenous staphylococci in the nasal cavities. To test this hypothesis, we performed an epidemiological survey. In addition, we performed *in vitro* experiments to explain the epidemiological findings. Two types of *S. epidermidis* were identified: 1 type inhibits *S. aureus* colonization and the other type does not. The detection rate of *S. aureus* was lower when the inhibitory *S. epidermidis* strain was present in the nasal cavities. *In vitro* studies showed that the *S. epidermidis* strain effectively inhibited *S. aureus* colonization and that the effect was directly proportional to the number of *S. epidermidis* organisms. Moreover, nasal administration of inhibitory *S. epidermidis* to volunteers significantly reduced *S. aureus* colonization. These findings suggest that the low detection rate of *S. aureus* in adult human nasal cavities is due to *S. epidermidis* that inhibits *S. aureus* colonization.

Beta-hemolysin from S. aureus inhibits the production of IL-8 and the transmigration of neutrophils through activated endothelium

S. aureus can cause a broad range of infections from superficial infections to severe invasive infections, such as endocarditis. Treatment of infections with antibiotics has become more difficult owing to the recent increase in *S. aureus* resistant to multiple antibiotics. 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. Previously, we reported that culture supernatant from *S. aureus* inhibits IL-8

production by human umbilical endothelial cells (HUVECs). The inhibitory factor was isolated and identified as β-hemolysin (β-toxin, sphingomyelinase C). 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. We showed that β-hemolysin inhibited IL-8 production in HUVECs and decreased neutrophil transendothelial migration. Furthermore, VCAM-1 expression was inhibited by β-hemolysin. The electrophoretic mobility-shift assay revealed that β-hemolysin did not inhibit activation of NF-κB, indicating that the other pathway is involved.

Apoptosis of fibroblasts induced by intracellular S. aureus

Two clinical isolates of *S. aureus*, OK1 and OK11, grown in brain-heart infusion broth at 37°C for 2 hours (exponential phase) or 18 hours (stationary phase) were used. To examine the interaction between *S. aureus* cells and L929 fibroblasts, each bacterial suspension was added to the fibroblasts adhering to the culture dish and incubated for 30 minutes. After washing, incubation was continued for an additional 3 hours in freshly prepared medium containing lysostaphin. The L929 fibroblasts ingested more OK11 cells during the exponential phase than in other phases and ingested many bacterial cells in spite of being a nonphagocytic cell. Intracellular bacteria of the exponential phase markedly induced caspase 3 activity. Cells infected with OK1 showed condensed chromatin with large clumps, which is characteristic of apoptosis. In contrast, intracellular organelles of some OK11-infected cells were severely damaged and showed small, condensed chromatin clumps beneath the nucleus membrane. More OK11 than OK1 cells could attach to the mouse kidneys during the exponential phase.

Fibronectin-mediated colonization via FnBPA is important in the infection of S. aureus

S. aureus has a variety of adhesins that bind to extracellular matrix proteins or plasma proteins and facilitate the colonization of host tissues and organs. Among these adhesins, FnBPs are thought to be the most important for interacting with host cells, such as endothelial cells, epithelial cells, fibroblasts, and macrophages. To determine whether FnBPs are responsible for the in vivo infection of *S. aureus*, the *fnbA* mutant strain was compared with the parental SH1000 strain. Female BALB/c mice (6 to 8 weeks old) were intravenously infected with SH1000 or the mutant strain, after which their survival was studied. Most of the mice infected with the parental strain died within 1 week after infection, whereas mice infected with the mutant strain survived for more than 20 days. Furthermore, many abscesses were found in the kidneys of mice infected with the parental strain, but the kidneys of mice infected with the mutant strain appeared normal. These results indicate the importance of FnBPA in effective colonization of host tissues in *S. aureus* infection.

Curli fibers are required for development of biofilm architecture in K-12 Escherichia coli and enhance bacterial adherence to human uroepithelial cells

Sessile bacteria show phenotypical, biochemical, and morphological differences from their planktonic counterparts. Curli, extracellular structures important for biofilm

formation, are only produced at temperatures less than 30°C in *E. coli* K-12 strains. We show that *E. coli* K-12 can produce curli at 37°C when grown as a biofilm community. Curli are required for the formation of a 3-dimensional mature biofilm, with characteristic water channels and pillars of bacteria. A wild-type curli-expressing *E. coli* strain adhered to several lines of human uroepithelial cells more readily than did an isogenic curli-deficient strain. The finding that curli are expressed at 37°C in biofilm and enhance bacterial adherence to mammalian host cells suggests an important role for curli in pathogenesis.

Publications

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