Department of Bacteriology

Yoshimitsu Mizunoe, *Professor* Hitomi Shinji, *Assistant Professor*

General Summary

Research projects of our department have focused on 1) the mechanism of inhibition of *Staphylococcus aureus* colonization by commensal *Staphylococcus epidermidis*; 2) fibronectin-mediated colonization via fibronectin-binding proteins (FnBPs) in *S. aureus* infection; 3) molecular mechanisms of bacterial ATP secretion; 4) the isolation and characterization of biofilm detachment factor from *S. aureus*.

Research Activities

Analysis with fnb-mutant strains of FnBP functions in in vivo and in vitro infections with S. aureus

FnBPA and FnBPB are important adhesins for *S. aureus* infection. We constructed mutant strains deficient in *fnbA* or *fnbB* or both from *S. aureus* SH1000, which has intact *rsbU*, and the role of these adhesins in a mouse model of systemic infection was studied. Intravenous infection with *fnbA*- or *fnbB*-mutants or with an *fnbA*-/*B*-mutant resulted in a marked decrease in *S. aureus* pathogenicity *in vivo*. However, only FnBPA showed an indispensable role in *in vitro* experiments: internalization into epithelial cells, endothelial cells, and fibroblasts and ingestion by macrophages. Activation of nuclear factor κB in fibroblasts and redistribution of integrin in macrophages were also induced by FnBPA. These results indicate that FnBPA is sufficient for interaction with host cells *in vitro*, whereas both FnBPA and FnBPB are needed for the progression of systemic infection *in vivo*.

S. epidermidis Esp inhibits S. aureus biofilm formation and nasal colonization

Commensal bacteria inhibit pathogen colonization; however, complex host-microbe and microbe-microbe interactions have so far prevented a detailed understanding of the mechanisms involved in the inhibition of colonization. Here, we show that the serine protease Esp secreted by a subset of the commensal bacterium *S. epidermidis* inhibits biofilm formation and nasal colonization by *S. aureus*, a pathogen of humans. Epidemiologic studies have demonstrated that the presence of Esp-secreting *S. epidermidis* in the nasal cavities of human volunteers correlates with the absence of *S. Aureus*. Both purified Esp and Esp-secreting *S. epidermidis* inhibit biofilm formation and destroy pre-existing *S. aureus* biofilms. Furthermore, Esp increases the susceptibility of *S. aureus* in biofilms to immune-system components. Studies in human subjects have shown that Esp-secreting *S. epidermidis* eliminates *S. aureus* nasal colonization. These findings indicate that Esp hinders *S. aureus* colonization *in vivo* via a novel mechanism of bacterial interference, which could lead to the development of novel treatments to prevent *S. aureus* colonization and infection.

Isolation and identification of ATP-secreting bacteria from mice and humans

A recent report has shown that ATP causes colitis in mice via the differentiation of T helper 17 cells. Although ATP has been suggested to be secreted by commensal bacteria in the murine intestine, such ATP-secreting bacteria had not been isolated or identified. In the present study, we isolated and identified ATP-secreting bacteria from mice and humans. In humans, assessment of the relationships between the ATP-secreting bacteria and colitis will facilitate the understanding of various aspects of colitis, including the pathology, development of treatments, prophylaxis, and prognosis. Because ATP is essential for the organisms, the extracellular secretion of ATP may indicate an unknown symbiotic relationship with a pathogen or commensal bacterium in the microbial flora of the gut.

Analysis of a biofilm detachment factor secreted by S. aureus

Biofilms are communities of microorganisms within a polymeric matrix that are attached to a surface. Bacterial biofilms are found on such diverse surfaces as rocks in the oceans, medical devices, and water pipes in the factory and home and cause contamination that is difficult to eliminate. The bacteria within the biofilm matrix are protected from the host immune system and from antibiotic attack. As a result, biofilm formation is a major determinant of virulence in chronic bacterial infections. Therefore, finding a substance that can disassemble biofilms might have wide-ranging medical and industrial applications to prevent or eradicate biofilms. *S. aureus* causes a wide range of acute and chronic human infections. The organism can attach to solid surfaces of native tissues or artificial devices, thereby forming a biofilm matrix that encases the bacteria. We found that *S. aureus* secretes a factor that detaches the *S. aureus* biofilm. The factor responsible for the detachment effect is a heat-stable, water-soluble molecule. When supernatants fractionated through filters of different molecular sizes were added to the biofilm, the biofilm was detached by the <1 kDa fraction. Ongoing studies focus on the identification of the factor.

Publications

Lindmark B, Rompikuntal PK, Vaitkevicius K, Song T, Mizunoe Y, Uhlin BE, Guerry P, Wai SN. Outer membrane vesicle-mediated release of cytolethal distending toxin (CDT) from Campylobacter jejuni. *BMC Microbiol* 2009; **9:** 220.

Sato F, Iwase T, Tajima A, Shinji H, Mizunoe Y. Biofilm Formation of Clinical Isolated *Staphylococcus* Species. *Bacterial Adherence Biofilm* 2009; **23:** 23–8.

Piao H, Minohara M, Kawamura N, Li W, Mizunoe Y, Umehara F, Goto Y, Kusunoki S, Matsushita T, Ikenaka K, Maejima T, Nabekura J, Yamasaki R, Kira J. Induction of paranodal myelin detachment and sodium channel loss in vivo by Campylobactor jejuni DNA-binding protein from starved cells (C-Dps) in myelinated nerve fibers. *J Neurol Sci* 2010; **288:** 54–62.