ABSTRACT

Staphylococcus aureus (S. aureus) is a common pathogen that is well known to cause sepsis and septic arthritis in millions of people worldwide. To survive in host blood, or to colonize joints and induce disease symptoms, *S. aureus* needs to resist the onslaught of host innate immune response. Lysozyme, a 14.3 kDa cationic bacteriolytic enzyme which is a part of the innate immune response, is found in high concentrations in all the biological fluids, tissues, as well as secreted by macrophages and neutrophils. Lysozyme disrupts the bacterial cell wall by hydrolyzing the conserved ß-1,4 glycosidic bond between *N*-acetyl glucosamine (NAG) and *N*-acetyl muramic acid (NAM), that forms the peptidoglycan backbone of bacterial cell wall. Previous *in vitro* and *in silico* studies have reported that *S. aureus* is completely resistant to lysozyme due to its peptidoglycan modification by *O*-acetylation of NAM, caused by *O*-acetyl transferase (OatA) enzyme present in *S. aureus* cell membrane. However, the impact of *O*-acetylated peptidoglycan in the pathogenesis of *S. aureus* has never been examined *in vivo*. In this study, the role of *O*-acetylated peptidoglycan was investigated *in vitro* in human biological fluids and then in a *Drosophila melanogaster* infection model. Obtained results were further confirmed in murine model of sepsis and septic arthritis.

To understand the effect of lysozyme on OatA mutant (Δ*oat*A), lysozyme mediated lysis assays, intracellular survival and survival in human biological fluids were performed. The role of OatA in imparting immune susceptibility was studied in two different animal models. First, the survival rates and behavioral analysis of *Drosophila melanogaster* infected with Δ*oat*A mutant was compared to flies infected with wild type strain (SA113) of *S. aureus*. Second, using mouse models for sepsis and septic arthritis we compared the onset and progress of the disease. The disease progression in mice was assessed by assessing body weight, renal abscess formation, severity of arthritis, and functional disability of the affected limbs. Further, histopathological examinations were performed to confirm and quantify disease severity. *In vitro* results suggested that the Δ*oat*A *S. aureus* strain was sensitive towards lysozyme and its survival in human blood, synovial fluid and in macrophages was considerably reduced compared to wild type strains. In line with our *in vitro data, Drosophila melanogaster* infected with Δ*oat*A *S. aureus* strain showed significantly higher survival and functionality compared to flies infected with the wild type strain. Similarly, studies in murine model of sepsis showed significantly less severe disease

onset in terms of bacterial burden in organs and renal abscess formation in mice infected with Δ*oat*A when compared with SA113. Further, in *S. aureus* induced arthritis model, mice infected with Δ*oat*A strain developed milder disease compared to mice infected with the wild type SA113 strain. Radiological and histopathological data of infected knee joint revealed that Δ*oat*A infected mice had significantly lesser joint destruction, and was also accompanied by a reduced bacterial load. In this thesis, we conclude that peptidoglycan *O*-acetylation plays an important role in the pathogenesis of *S. aureus* mediated sepsis and septic arthritis. Using *in vivo* studies, we have tested and further confirmed the role of OatA as a fitness factor for *S. aureus* survival, inside the host via escaping lysozyme mediated mechanism of bacteriolysis.