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Structural and functional characterization of leukocidin peptide mediating adhesion between heparan sulfate and bacterial cell wall of Staphylococcus aureus

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Staphylococcus aureus necrotizing pneumonia is a severe disease caused by *S. aureus* strains carrying the Panton Valentine leukocidin (PVL) genes (*luk*S-PV & *luk*F-PV) encoded on various bacteriophages. Necrotizing pneumonia affects children and young adults (median age 15 years) and is characterized as a rapidly expansive pneumonia, associated with haemoptysis, leukopenia and pleural effusion, leading to high mortality rate (ca 65%). Lung tissues from staphylococcal necrotizing pneumonia patients revealed that respiratory epithelium ulcerations that extend from the larynx to the lobar bronchi were heavily colonized with abundant Gram-positive cocci. In addition to produce PVL, PVL+ clinical isolates display increased adhesion to extracellular matrix molecules (ECM) (collagen I, IV, laminin); this could play a central role during bacterial colonization to damaged airway epithelium.

To investigate the molecular basis of the observed increased adhesion of *S. aureus* PVL+ strains, we tested the ability of isogenic strains for PVL to attach to various matrix molecules. Cloning and expression of sub-fragment of PVL and the use of synthetic peptides showed that the LukS-PV signal peptide is efficient to significantly enhance the ability of *S. aureus* to attach to matrix components. Moreover we showed that adhesion to ECM components is efficiently inhibited by heparan sulfates (HS), a complex family of polysaccharides widely expressed on most cell surface and ubiquitous components of the ECM. This suggests that *in vivo*, HS could function as a molecular bridge between the matrix and *S. aureus* expressing the LukS-PV signal peptide. The structural analyses of the LukS-PV signal peptide by circular dichroism in various physico-chemical environments indicated that it could be released outside the cytoplasmic membrane after signal peptidase cleavage, which was confirmed by the use of antibodies directed against an histidine-tagged LukS-PV SP. Site directed mutagenesis of the LukS-PV signal peptide favors the hypothesis that the C-terminus of this signal peptide is associated to the bacterial cell wall, while its N-terminus, which is highly positively charged, binds to matrix-associated HS.

The aim of the present project is to further characterize the structure-function relationship of this atypical signal peptide by using multidisciplinary approaches (biochemical, biophysical, and microbiologic approaches).

To test our central hypothesis and accomplish the overall objective of this application, the five following specific aims are as follows (1) Structural characterization of the LukS-PV signal peptide: to examine how the biochemical properties of LukS-PV signal peptide could support this model, the tridimensional structure of different signal peptide will be performed by using nuclear magnetic resonance (NMR) in various membrane mimetic environments; (2) To demonstrate physically that the signal peptide is exposed at the bacterial surface: *S. aureus* strains expressing his-tagged signal peptide will be examined by microscopy or orbitrap mass spectrometry; (3) To identify the cell wall structure to which the signal peptide is bound: solid-state NMR or BIAcore assay and liquid state NMR will be used; (4) To characterize the interaction between the LukS-PV signal peptide N-term motif and the HS: different mutated signal peptides will be tested in a BIAcore assay, and recombinant bacteria expressing these peptide will be tested in a cell culture model (wild-type cells and knock out cells for HS production); (5) To demonstrate in an

animal model that increased adhesion play a role in virulence, the *in vivo* relevance of these observations will be investigated in a mice pneumonia model.

This project will be the first study to demonstrate the functions of a bacterial signal peptide beyond protein secretion. Our proposal addresses the mechanisms of the enhanced adhesion of PVL-positive strains to various ECM components *via* the LukS-PV signal peptide for which detailed molecular mechanistic understanding is still lacking. This project may thus help to understand the mechanism of pathogenesis of the staphylococcal necrotizing pneumonia, which is an infrequent but highly lethal disease and to pave the way for the development of novel strategies for combating staphylococcal diseases. Our objectives may reveal a new potential target for bacterial treatment, namely LukS-PV signal peptide (and perhaps others staphylococcal or bacterial signal peptide) that is involved in the bacterial pathogenesis, especially at the first stage of infection: adhesion. Since adhesion is one of the earliest stages of infection, interfering with this process should be beneficial to the host.
