CHALLENGES FACED IN THE PLANNING, DEPLOYMENT, AND ADOPTION OF A LAYERED MEDICAL DEFENSE STRATEGY

Targeting The Cell Wall Of Francisella: Peptidoglycan Remodeling Enzymes Are Essential For Cell Morphology And Virulence, And Show Potential For Therapeutic Targeting

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Francisella tularensis is the causative agent of tularemia and represents a significant biothreat risk. Currently, there is no FDA-approved tularemia vaccine, and identification of novel therapeutic targets remains a critical gap in strategies for combating this pathogen. Cell wall synthesis and remodeling enzymes are attractive targets for therapeutics against diverse bacteria as they are often essential for cell survival. However, cell wall recycling and the enzymes required for this process are poorly understood in Francisella. Here, we investigate the roles of the lytic transglycosylase enzymes, Slt and MltA, in the biology and virulence of Francisella spp. The lytic transglycosylases are a class of peptidoglycan-modifying enzymes that cleave the glycosidic bond between disaccharide residues within the peptidoglycan layer, allowing cells to divide properly, respond to environmental signals, and incorporate surface structures into the cell envelope. Previous work in our lab showed that mutation of slt in the F. novicida surrogate strain leads to significant growth defects in acidic pH conditions, as well as cell morphology defects, including increased size, membrane protrusions, and cell fusion, which was partially restored by growth in neutral pH or genetic complementation. Furthermore, the F. novicida slt mutant was significantly attenuated during intranasal challenge of BALB/c mice, while virulence was restored in the complement strain. Notably, both the slt and mltA genes are essential in the more virulent F. tularensis Schu S4 and LVS strains, underscoring their value as therapeutic targets. Therefore, Slt and MltA recombinant proteins were designed and produced to characterize enzyme function and identify potential inhibitors. Recombinant Slt was shown to cleave pre-polymerized lipid II substrates, with a preference for gram-negative substrates, while MltA demonstrated positive enzymatic activity against a fluorogenic glycanase substrate. Current work is aimed at developing enzymatic assays in order to screen for inhibitors against these targets for new antibiotic development. The use of new inhibitors against such cell wall synthesis enzymes could be leveraged against tularemia in combination with existing antibiotics in a layered countermeasure defense strategy.