Peptidoglycan amidation enzymes in *Mycobacterium tuberculosis* are new drug targets for Tuberculosis treatment and development of a novel TB vaccine

Abstract

Mycobacteria assemble a complex cell wall with cross-linked peptidoglycan (PG) playing an essential role for maintenance of cell wall integrity and tolerance of osmotic pressure. In previous work, it was demonstrated that various hydrolytic enzymes are required to remodel PG during essential processes such as cell elongation and septal hydrolysis. In this thesis work, we explore the chemistry associated with PG cross-linking, specifically the requirement for amidation of the D-glutamate residue found in PG precursors. In *Mycobacterium smegmatis*, an experimental surrogate for *Mycobacterium tuberculosis* (Mtb), we confirm the essentiality of D-glutamate amidation in PG cross-linking by labeling cells with synthetic fluorescent PG probes that require amidated side chains for stable incorporation. We also use CRISPRi targeted knockdown of genes encoding the MurT-GatD complex, previously implicated in D-glutamate amidation in other bacterial species, and demonstrate that these genes are essential for mycobacterial growth. We show that MurT-GFP co-localizes with mRFP-GatD at the cell poles and septum, which are the sites of cell wall synthesis in mycobacteria and that these enzymes interact to form the PG amidation complex. Furthermore, time-lapse microscopic analysis of MurT-GFP localization in fluorescent D-amino acid (FDAA) - labeled mycobacterial cells during growth demonstrated co-localization with maturing PG, suggestive of a role for PG amidation during cell wall remodeling and repair. Depletion of MurT and GatD caused reduced PG cross-linking and increased sensitivity to lysozyme and β-lactam antibiotics. Cell growth cessation was found to be the result of a shutdown of PG biosynthesis mediated by the serine/threonine protein kinase B (PknB) which senses uncross-linked PG. Collectively, the first component of this work in *M. smegmatis* demonstrates the importance of D-glutamate amidation in mycobacterial PG precursors. We further exploit this enzyme complex in the second part of the dissertation to develop a novel CRISPRi-based recombinant BCG (rBCG) vaccine named rBCG::iE-DAP. This rBCG vaccine is based on the depletion of MurT-GatD, which results in reduced PG side chain amidation. As amidation masks detection of PG by the NOD1 pathogen recognition receptor, we postulate that this novel recombinant BCG will facilitate enhanced

immunity against TB disease. As expected, the recombinant BCG induced increased immune activation and protection against Mtb infection in a mouse model of TB infection. This work highlights the MurT-GatD complex as a novel drug target and as a target for development of a next generation TB vaccine targeting innate immune responses against Mtb infection.