Abstract

to the PhD thesis titled

Structural Characterization of LpqH, an Immunomodulatory Lipoprotein of *Mycobacterium tuberculosis*, and its Interaction with the Host Cell Adhesion Molecule E-Cadherin

by

Shruti Chatterjee (17BS91R03)

Mycobacterium tuberculosis (*Mtb*) is an airborne pathogen that is transmitted via aerosols from infected individuals. *Mtb* possess a collection of pathogen-associated molecular patterns (PAMPs) on their surface which are known to help the pathogen recognize host cells during tuberculosis. A major group of these mycobacterial PAMPs includes the membrane-anchored lipoproteins, of which, one of the most studied lipoproteins is LpqH. These lipoproteins have been reported to be a crucial virulence factor, that interact with a group of pattern recognition receptors on the surface of alveolar macrophages, the first site of action of *Mtb* after they enter the alveolus. Apart from alveolar macrophages, these bacteria are also known to invade and proliferate within alveolar epithelial cells, which aids the pathogen in protecting itself from the hostile environment of the macrophages, thereby enhancing their chance of survival within the host. However, the exact mechanism of interaction of *Mtb* with these alveolar epithelial cells is yet to be elucidated. This work intends to fill in this gap by exploring the interaction between this vital *Mtb* lipoprotein LpqH and one of the major host adherens junction protein E-cadherin, which are expressed constitutively at a high level on the surface of the alveolar epithelial cells.

In the first objective of this thesis, the comprehensive structural characterization of the non-acylated variant of LpqH was performed. The crystal structure of LpqH, solved at a resolution of 1.26 Å, reveals that the protein possesses a unique fold. In addition to this, the functionality of the purified protein was demonstrated using flow cytometry-based cell binding and apoptosis experiments. Moreover, comparing the sequences of LpqH from various *Mycobacterium* species revealed a patch of conserved residues on the protein's surface that is thought to play a key role in binding to its cognate partner, thereby facilitating the host-pathogen interaction.

The second objective aims to explore a novel heterophilic interaction between LpqH and E-cadherin, the major adherens junction protein, expressed on the alveolar epithelial cell surface. Surface plasmon resonance studies demonstrate that the membrane-distal N-terminal ectodomain of E-cadherin can recognize and bind to non-acylated LpqH. In addition to this, mutagenesis experiments further helped us acquire insights into this novel heterophilic interaction. It has been previously reported that the second tryptophan residue (W2) present at the N-terminus of E-cadherin plays a very crucial role in facilitating the homophilic interactions mediated by the protein. In this objective, site-directed mutagenesis studies showed that this very tryptophan is also critical for the LpqH:E-cadherin interaction and a point mutation at this site (W2G) severely hinders its recognition by LpqH.

In the third objective, this novel molecular crosstalk was analyzed in a physiological context. Flow cytometry-based cell-binding studies were first performed using a mammalian heterologous expression system (CHO-K1), where E-cadherin was transiently expressed on their surface along with a C-terminal EGFP tag. The study revealed that fluorophore-conjugated LpqH binds only to those CHO-K1 cells that express E-cadherin on their surface, thus demonstrating that, the binding of LpqH to CHO-K1 was mediated by E-cadherin. Following this, three different epithelial cell lines which inherently express

varying levels of E-cadherin on their surface, were chosen to perform similar cell-binding studies. This study revealed that the level of LpqH binding to these epithelial cells is proportional to their surface expression of E-cadherin, thus suggesting the fact that E-cadherin is the primary mediator that can promote *Mtb* colonization on the surface of host alveolar epithelial cells.

Although it has long been known that LpqH interacts with TLRs on the surface of alveolar macrophages, the current study for the first time reports that LpqH can also recognize E-cadherin, the prominent cell adhesion molecules present on the surface of alveolar epithelial cells. This, in turn, opens a new avenue of research in the field of *Mtb* infection, which can further pave the way to the development of novel therapeutics in future for the treatment of tuberculosis.

Keywords: Mycobacterial lipoprotein LpqH, E-cadherin, cell adhesion molecules, protein-protein interaction, surface plasmon resonance (SPR), flow cytometry, X-ray crystallography.