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(Title of the Ph.D. Thesis)

Role of extracellular M. bovis glutamine synthetase in the survival of

M. bovis in host macrophages

Abstract of the Ph.D. Thesis:

Pathogenic strains of mycobacteria secrete copious amounts of glutamine synthetase in the culture medium. Expression of glnA1 in a non pathogenic strain M. smegmatis released the active enzyme in the supernatant and the secretion of glutamine synthetase is independent of lysis of the cells. Expression of glnA1 is regulated by two nitrogen response sequences in the glnA1 promoter depending on availability of nitrogen concentration. The enzyme activity is linked to synthesis of poly-L-glutamine in the cell walls. The M. smegmatis strain containing M. bovis glnA1 gene survived longer in the THP-1 cells compared to wild type strain and produced cell wall associated poly-L-glutamine. Inactivation of the glnA1 gene by allelic exchange method produced a glnA1 mutant of Mycobacterium bovis producing no extracellular glutamine synthetase in the medium. The mutant was auxotrophic for glutamine when grown in sauton's defined medium and was able to grow in enriched 7H9 medium without glutamine supplementation. The glnA1 strain contained no detectable poly-L-glutamine in the cell walls and showed marked sensitivity to different chemical and physical stresses like lysozyme, SDS, and sonication. Sensitivity of the mutant to two anti-tubercular drugs, rifampicin and D-cycloserine were also increased. The glnA1 strain infected THP-1 cells with reduced

efficiency and was also attenuated for growth in the macrophages. The M. bovis mutant was not able to replicate in the organs of balb/c mice and was cleared within 4-6 weeks of infection. Disruption of glnA1 gene adversely affected biofilm formation on polystyrene surface. The results of this study demonstrates the role of extracellular M. bovis glutamine synthetase in establishing infection in host macrophages by modulation of hostile environment and the pathogen's adaptability to nitrogen deprived condition in the phagosomes of host macrophages. Presence of extracellular glutamine synthetase helps in formation of a cell wall component, poly-L glutamine, which is unique to the pathogenic strains and which provides rigidity to the cell wall and might influence the host response to the pathogen. The results of this study strongly show that absence of extracellular glutamine synthetase not only attenuates the pathogen, but also affects cell surface properties by altering cell wall chemistry of the organism through synthesis of poly-L-glutamine. Poly-L-glutamine structures are unique to pathogenic mycobacterium species and further research on the genetic loci which are involved in the assembly of poly-L-glutamine structures will definitely yield novel targets for drug development.