Introduction
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Tuberculosis (TB) is a major cause of illness and death worldwide, especially in Asia and Africa. Globally, 9.2 million new cases and 1.7 million deaths from TB occurred in 2006, of which 0.7 million cases and 0.2 million deaths were in HIV-positive people (WHO report, 2008). Amongst the aspects considered responsible for hampering tuberculosis control are mycobacteria co-infection with HIV, emergence of multiple and extensive drug resistant strains, massive migrations and BCG’s limited effectiveness (WHO report, 2008). *Mycobacterium tuberculosis*, a facultative intracellular parasite, is the main etiological agent of tuberculosis and has evolved successful strategies to invade and persist within the macrophages. But the disease can also be caused by *M. bovis*, *M. africanum*, *M. microti* and *M. canettii*, the members of *M. tuberculosis* complex (Tiruviluamala et al., 2002). Biochemically, it is difficult to distinguish *M. microti* from other members of *M. tuberculosis* complex whereas antigenically it is closely related to *M. tuberculosis* (Tsukamura et al., 1979). Keeping in view the failure of BCG in different regions and high degree of genomic similarity of *M. microti* to *M. tuberculosis*, it is important to have detailed analysis of the antigenic profile of *M. microti* (Manabe et al., 2002, Frota et al., 2004).

The envelope of mycobacteria consists of the plasma membrane, surrounded by a complex cell wall of carbohydrates and lipid, which is in turn surrounded by a capsule of majorly polysaccharide with relatively less quantity of protein. The success of this intracellular pathogen depends on intimate pathogen-macrophage interactions that are dictated in part by the chemical nature and the biologic functions of the outermost constituents of the bacillus (Hoft, 2008). This has led to intense research on the proteins located on the cell envelope and their respective functions (Plaza et al., 2007, Patarroyo et al., 2008). The cell wall is chemically composed of highly cross-linked peptidoglycan, arabinogalactan (AG) and mycolic acid. However, the main components of the capsule with respect to the polysaccharides are neutral and lipid-free D-glucan, D-arabino-D-mannan and D-mannan (Ortalo-Magne et al., 1995). This protective capsule not only controls access from the medium to the inside of the mycobacterial cell but also determines what components come into contact with host cells and tissues. Also, the ratio of polysaccharide to protein in the capsule is much higher in virulent species of mycobacteria (Ortalo-Magne et al., 1995, Lemassu et al., 1996). Mycobacteria utilize its capsular polysaccharides like glucans and mannans as ligands for receptor mediated entry in to host cell. The *M. tuberculosis* invades macrophages via variety of receptor molecules including complement receptors, mannose receptor and Fc receptors (Ernst, 1998, Greenberg, 1999, Cambi et
It has also been demonstrated that anti-polysaccharide e.g. arabinomannam (AM) antibodies prolongs the survival of mice infected with a lethal dose of *M. tuberculosis* (*Teitelbaum et al.*, 1998, *Glatman-Freedman et al.*, 2000).

As regards to the cell wall proteins, *M. tuberculosis* genome encodes nearly 800 putative membrane proteins (*Tekaia et al.*, 1999), which include 19 and 38 kDa lipoproteins, the 30/31 kDa fibronectin-binding proteins and the 40 kDa L-alanine dehydrogenase, besides the culture filtrate 24 kDa (MPB/T64) (*Lemassu and daffe*, 1994) and 45-47 kDa antigen complex (*Lagueyrerie et al.*, 1995). Mycobacterial cell envelope lipoproteins are among the most immunoreactive antigens capable of inducing both humoral and cell mediated immunological responses (*Bastian et al.*, 2008). The complete sequence of the *M. tuberculosis* genome (*Cole et al.*, 1998) has revealed about one hundred lipoprotein genes representing roughly 2.5% of *M. tuberculosis* open-reading frames, many of them being hypothetical unknown proteins, specific to Mycobacteria genus. The identification and characterization of such individual hypothetical lipoproteins is essential not only towards the understanding of the pathogenic mechanisms of mycobacteria but also towards their possible exploitation as protective antigens like lprA, lprG, *lpqH* (*Gehring et al.*, 2004, *Stewart et al.*, 2005, *Pecora et al.*, 2006). Also, the sensitive analysis *M. tuberculosis* cell envelope proteome has provided valuable information about cell envelope components and firm evidence supporting the existence of hypothetical proteins predicted by the genome sequence, showing no similarity with known proteins in other organisms (*Jungblut et al.*, 1999, *Betts et al.*, 2000). These proteins could be useful for developing highly specific immune-diagnostic tests (*Sinha et al.*, 2005) and new vaccines against tuberculosis.

Studies from this lab have primarily focused on identification and characterization of mycobacteria cell envelope components and those specifically present on infected macrophages. Using polyclonal antibodies, generated by immunizations with mycobacteria and infected macrophage membranes, lab studies first demonstrated the antigenic changes on the mycobacteria infected macrophage surface (*Majumdar et al.*, 2000). In *M. microti* as a model system, monoclonal antibodies showed localisation of antigenic determinants on the surface of mycobacteria infected cell (*Hardeep*, 2000). In a recent study, the gene encoding cutinase CutSB of *M. tuberculosis* Rv was cloned and overexpressed in *E. coli*. Using antibodies raised against this recombinant protein, the localization of Cut5 protein was demonstrated on cell wall of different mycobacterial species (*Gambhir*, 2007).
In continuation to these efforts, the objectives of this study were as follows:

- Generation of monoclonal antibody(ies) [mAb(s)] against mycobacterial cell envelope components (polysaccharides and/or proteins).
- Selection of mAb(s) based on its antigen specificity and/or inhibitory potential (towards host cell invasion and intracellular growth).
- Identification and characterisation of the components recognised by selected mAb. For this, attempts may also be made to generate and utilize the database.
- Antigenic/functional role, if any, of above selected molecule(s).