Introduction:

*Mycobacterium tuberculosis* (*M.*tb) is the causative agent of tuberculosis; it continues to cause active disease in about 15 million cases globally at any given point of time (WHO report, 2010). The disease plays major havoc in developing and under-developed countries despite the availability of a vaccine and drugs. Long duration of the chemotherapy, increased incidence of HIV/AIDS and emergence of drug resistant strains results in poor control of the disease. Also, BCG vaccine, the only available vaccine against tuberculosis has been virtually ineffective to any protection against adult pulmonary tuberculosis—the most deadly form of the disease (Black et al., 2002). However, BCG will continue to be a part of the universal immunization regime as it has been found to protect against leprosy (Fine and Rodrigues, 1990) as well as against the childhood manifestations of TB (miliary or meningeal) (Rodrigues et al., 1993). Both *M.*tb and BCG induce an inefficient immune response that can only partially contain the infection, ultimately failing to prevent the development of disease (Cooper, 2009; Cooper and Khader, 2008; Fine, 1995; Fine, 1995a). Also, the efficacy of BCG vaccination has been thought to be affected by exposure to large quantities of environmental mycobacteria (Fine, 1995; Palmer and Long, 1966). All these factors necessitate the development of a more efficient vaccine than the existing BCG vaccine.

Although, one third of the world’s population is infected with *M.*tb, most of the infections are self-contained with only 5% to 10% disease manifestations suggesting that the immune system can normally control the *M.*tb infection but do not eliminate the pathogen. *M. tuberculosis* in the aerosol droplets exhaled by a pulmonary TB patient enters the alveolar passages of exposed humans and comes in contact with resident macrophages which they invade. The infected macrophages reside and persist within a granuloma which consists of macrophages and giant cells, T cells, B cells and fibroblasts. Consequently upon their infection with any pathogen other than *M.*tb, the infected macrophages mount a strong effector response against the invading pathogen, which
comprises of phagolysosome fusion, ROS generation, production of RNI via the iNOS-dependent cytotoxic pathway and mechanisms mediated by various cytokines such as TNFα, IL-12, IL-1, IL-6 and IL-10. Macrophages upon activation produce nitric oxide which kills bacteria. TNFα and IL-12 are proinflammatory cytokines which aid in driving Th1 response against *M. tb* infection. IL-6 is implicated in inflammation, haematopoiesis and T cell differentiation and IL-1 in acute phase response and containment of *M. tb* infection, whereas IL-10 is an anti-inflammatory cytokine with macrophage deactivating properties. These events occur through MAP kinase signaling pathways such as ERK1/2, p38 and JNK pathways. However *M. tb* on the other hand, has developed mechanisms for evading immune responses resulting in active pulmonary tuberculosis. One such mechanism is secretion of proteins in the host, though some proteins are highly immunogenic and can aid in mounting an efficient immune response against *M. tb*. For instance, proteins like ESAT-6 and CFP-10 (also known as MTSA-10) belonging to RD1 genetic locus has been implicated in *M. tb* virulence. Both the proteins are highly immunogenic and are found in the early culture filtrates of *M. tb* after short periods of growth and in the absence of obvious autolysis. Paradoxically, CFP-10 has also been shown to modulate macrophage functions (Trajkovic et al., 2002). Also, CFP-10, ESAT-6 and the CFP-10: ESAT-6 complex of *M. tb* have been shown to downregulate the signal transduction pathways responsible for immune activation of macrophages (Ganguly et al., 2007; Ganguly et al., 2008). More recently, ESAT-6 has been shown to downregulate MHC class II expression by inhibition of IFNγ-inducible CIITA expression (Kumar et al., 2011). Therefore, it will be interesting to study and characterize the secreted proteins of *M. tb* to understand their role in the pathogenesis of the tuberculosis.

*Mycobacterium tuberculosis* secretes a large number of proteins. Their functions and the mechanism(s) of their action(s) are largely unknown. The fact that only live mycobacterial vaccines provide protection against tuberculosis infection (Mustafa, 2002; Orme, 1988) indicates that secretory proteins of *M. tuberculosis* complex (MTC) play an important role in stimulating protective immune
response. This brings forth the need for investigating further the major secretory antigens of *M. tb*. One such secreted protein of *M. tuberculosis*, MPT 70 (Rv2875) and its identical homologue in *M. bovis*, MPB 70 are 163-residue polypeptides, secreted from mycobacterial cells following cleavage of a 30-residue signal peptide from the precursor peptide (Cole *et al.*, 1998; Hewinson *et al.*, 1993). MPB 70 of *M. bovis* is a major serodominant antigen, which stimulates a strong, delayed type hypersensitivity response and cellular immune responses during the infection in cattle (Billman-Jacobe, 1990; Pollock, 1994). Virulent *M. bovis* strains secrete high levels of MPB 70 when grown in culture, but the protein is produced at much lower levels by *M. tuberculosis* and by a number of *M. bovis* BCG strains such as Pasteur, Beijing, Copenhagen and Glaxo (Harboe *et al.*, 1998; Miura *et al.*, 1983; Harboe *et al.*, 1984; Hewinson *et al.*, 1996). Other members of Mycobacterium tuberculosis Complex, including *M. tb*, *M. africanum* and *M. microti*, are low producers of MPB/MPT 70. The *M. bovis* BCG strains BCG Tokyo, Moreau, Russia and Sweden are high producers of MPT 70 (Wiker, 2009). The difference in the expression level of MPB 70 between the two groups of BCG strains is due to a mutation in sigK, the gene encoding the sigma factor K responsible for transcription of mpt70 gene (Charlet *et al.*, 2005). Moreover, the difference in the expression level of MPB/MPT 70 between *M. bovis* and *M. tuberculosis* is due to mutations in a regulatory gene, Rv0444c encoding anti-sigK factor RskA (Said-Salim *et al.*, 2006). *M. bovis* lacks a functional anti-SigK which fails to repress the SigK resulting in constitutive expression of MPB/MPT 70. Though *M. bovis* and *M. tuberculosis* show significant difference in MPT 70 expression in culture, they both elicit a strong immune response directed to MPB 70 upon infection, suggesting that expression of the protein is up-regulated by *M. tuberculosis in vivo* (Roche *et al.*, 1994; Hewinson, 1996). Also, a study with DNA vaccine encoding MPB/MPT 70 has shown that it has a significant therapeutic effect on *M.tb* infected mice (Lowrie *et al.*, 1999). The structure of MPT 70 reveals a complex and novel bacterial fold, which has clear structural homology to the two C-terminal FAS1 domains of the cell adhesion protein fasciclin I (Carr *et al.*, 2003), known to be involved in mediating interactions
between cells and the extracellular matrix (Kim et al., 2000; Billings, 2002). The structure suggests that it contains two functional surfaces on opposite faces, which are probably involved in binding to host cell proteins (Carr et al., 2003). However, the function of the protein and its effect on the host cell response are not known yet.

The present work is focused towards studying the functional aspect of MPT 70 and identifying the host proteins with which it interacts and also modulation of the host cell-macrophage functioning and response. This study aids in characterization of MPT 70 as a pro-inflammatory protein which may help the host immune system to better recognize M.tb and therefore may pave a way for development of MPT 70 based vaccine for protection against tuberculosis.

**Aims and Objectives:**

The aim of this project was to study the interaction of MPT 70 with host cell proteins and determine its role in regulation/modulation of the host cell immune functions.

The objectives include:

- To clone and express MPT 70 in *E. coli* and generate antibodies against it in mice.
- To identify the proteins from host cells - macrophages which specifically interact with MPT 70 by using pull down assays and mass spectrometry.
- To clone MPT 70 in pBT vector and search for its potential interactors from human lung library through bacterial two hybrid system.
- To determine the effect of MPT 70 on the cytokine production by macrophages.
- To study the effect of MPT 70 on LPS- and IFNγ-inducible NO production in macrophages.
- To determine the effect of MPT 70 on the MAPK signal transduction pathways of the host cell involved in cytokine and NO production.