Summary:

*Mycobacterium tuberculosis* (*M.tb*) is one of the most ancient human pathogens known to have co-evolved along with the human immune system to successfully persist within its host. *M. tuberculosis*, the etiological agent causing tuberculosis infects one third of the world's population claiming 1.5 million lives annually. *M.tb* though capable of infecting almost any cell type, primarily infects and multiplies in the macrophages, the phagocytic antigen presenting cells of immune system. Normally, during the course of an infection, macrophages after coming in contact with the microbial pathogen, phagocytose it and process its antigens to present them in association with class II MHC molecules to T cells, thus initiating the adaptive immune response. During this process, activated macrophages also produce antimicrobial molecules such as nitric oxide, ROS and proinflammatory cytokines viz TNFα, IL-12, IL-6 and IL-1 etc. These molecules along with MHC class II presented antigens, activate CD4+ T cells to produce IFNγ and drive Th1 helper response. However, *M.tb* has evolved mechanisms to evade this normal course of immune response. One such mechanism is to modulate host cell response through production of secreted proteins. *M.tb* genome encodes for nearly 4000 proteins; functions and mechanism of action of many of them are not known. The secreted proteins of *M.tb* have been shown to play an important role in disease pathogenesis with some even acting as virulence factors. Secretory proteins like CFP-10 and ESAT-6 modulate the signal transduction pathways responsible for immune activation of the macrophages so as to downregulate the macrophage effector functions and MHC class II antigen presentation, thereby aiding in evasion of the immune surveillance. However, certain proteins of *M.tb* are highly immunogenic and hence can be exploited as potential vaccine candidates against *M.tb* infection. The present study deals with a protein from similar genre, known as MPT 70 or MPB 70 if expressed from *M. bovis*. MPT 70 encoded by *M.tb* gene *Rv2875*, is a 163-residue polypeptide, secreted from mycobacterial cells following cleavage of a 30-residue signal peptide from the precursor polypeptide. The gene for MPT 70 is essentially limited to members of the *Mycobacterium tuberculosis* complex (MTC) and is completely absent from
environmental mycobacteria. It is expressed in high amounts in virulent *M. bovis* culture but in very low amounts by *M. tb*. *M. bovis* BCG strains Tokyo, Moreau, Russia and Sweden are high producers of MPT 70 and BCG strains Pasteur, Beijing, Copenhagen and Glaxo are high producers of MPT/MPB 70. MPB70 of *M. bovis* being a major serodominant antigen stimulates a strong, delayed type hypersensitivity response and cellular immune responses upon infection in cattle. Also a DNA vaccine candidate encoding MPT 70 has been shown to provide protection against *M. tb* infection in mice.

In the light of these facts, the current study aimed at deciphering the function of MPT 70 in modulation of the host cell response. The present study focussed at generation of nitric oxide and cytokine secretion by MPT 70 primed macrophages and the signaling pathways involved in these macrophage effector functions. The outcome of the present study can be summarized as follows:

1. MPT 70 binds to the surface of macrophages to modulate its functions.

2. MPT 70 significantly increased the production of proinflammatory cytokines IL-12, IL-1β, IL-6 and TNFα, the cytokines implicated in protective immune responses against *M. tuberculosis* infection, in a dose dependent manner; in contrast, production of anti-inflammatory cytokine IL-10 was not detected.

3. Pre-treatment of macrophages with MPT 70 resulted in increased NO release upon subsequent exposure to *M. tuberculosis* whole cell lysate or LPS, a potent inducible NO synthase (iNOS) activator suggesting that MPT 70 sensitzes macrophages for LPS-induced NO production.

4. MPT 70 increased IFNγ induced NO production in dose dependent manner in murine macrophages when both the stimuli were applied simultaneously suggesting that MPT 70 synergises with IFNγ for NO release, perhaps, aiding in NO mediated killing of *M.tb*.

5. TNFα released by MPT 70 and MPT 70- IFNγ- treated macrophages as well as NO induced by pre- MPT 70- LPS- treated and MPT 70- IFNγ-
treated macrophages, are partially dependent on both p38 and ERK1/2 pathways.

6. MPT 70 increased p-ERK1/2 and p-p38 expression in macrophages. Also, p-ERK1/2 and p-p38 expression in MPT 70 pre-treated macrophages was further increased after LPS stimulation suggesting that MPT 70 heightens LPS- induced ERK1/2 and p38 activation and thus magnifies LPS effects.

Hence, the current study showed that MPT 70 binds to the macrophage surface receptors and activate macrophage to produce anti-mycobacterial NO and proinflammatory cytokines TNFα, IL-12, IL-1, IL-6 via activation through ERK1/2 and p38 MAP Kinase pathways. MPT 70 even magnifies the LPS and IFNγ-induced macrophage activation.

A clear understanding of functions of *M.tb* secretory protein in modulation of the host immune responses will help us design better vaccine candidates against *M.tb* infection.