Introduction

More than one hundred and twenty nine years ago, on March 24, 1882, Robert Koch postulated his discovery of *Mycobacterium tuberculosis* (*M. tb*) being the causative organism of ‘TUBERCULOSIS’ (TB) that then killed over 40% of the working class in Germany (Koch, 1882). At that time similar figures were recorded for all major cities in Europe and the USA. Unfortunately, even today TB remains an enormous global health problem. According to WHO (WHO, 2008), in 2006 there were an estimated 9.2 million new cases of active TB in the world and nearly 1.7 million people died of active TB. It is the primary reason of death among the infectious diseases category, despite longstanding efforts to control its spread. The emergence of multi-drug resistance (MDR) strains of *M. tb* and dramatically increased susceptibility of patients infected with human immunodeficiency virus (HIV) to tuberculosis have fuelled the spread of the disease (Chintu and Mwinga, 1999) that and therefore cannot be treated adequately.

The genus *Mycobacterium* comprises of mostly soil dwelling saprophytes, and only a few members of the genus have evolved to adopt a pathogenic lifestyle, causing diseases of diverse nature and varying severity (Cosma et al., 2003). Tuberculosis is caused by members of the *M. tuberculosis* complex that consists of *M. tuberculosis*, *M. bovis*, *M. africanum*, *M. canettii* and *M. microti*. The mycobacteria grouped in the complex are characterized by 99.9% similarity at the nucleotide level and identical 16S rRNA sequences (Boddinghaus et al., 1990; Sreevatsan et al., 1997), but differ widely in terms of their host tropisms, phenotypes, and pathogenicity. Some are exclusively human pathogens (*M. tuberculosis*, *M. africanum*, *M. canettii*) or rodent *M. microti* whereas *M. bovis* have a wide host spectrum (Cosma et al., 2003). Prior to the introduction of pasteurization of milk, *Mycobacterium bovis* was responsible for approximately 6% of total TB deaths in humans in Europe (Cole, 2002). All members of the complex are slow-growing, with generation time ranging from 12 to 24 hours depending on environmental and microbial variables.

**Mycobacterial infection**

*M. tuberculosis* is a facultative, aerobic, intracellular pathogen, which has a predilection for the lung tissue rich in oxygen. TB occurs almost exclusively from inhalation of aerosol droplet containing *M. tuberculosis* expelled by an individual with
active pulmonary TB through coughing, spitting, singing and other forced respiratory maneuvers. Usually, repeated exposure to a TB patient is necessary for infection to take place. Inhaled droplets are deposited in the alveolar spaces, where the bacteria are taken up by phagocytic cells, mainly alveolar macrophages (Schluger and Rome, 1998) an event which induces a rapid inflammatory response and accumulation of cells.

**Immune Evasive Mechanism**

*M. tuberculosis* invades and replicates in macrophages, cells of the host innate defense system designed to eliminate pathogenic microorganisms, through a variety of immune evasion strategies. The use of non-activating complement receptors (CR) to enter into macrophages may be advantageous for the bacterium, since engagement of these receptors does not induce the release of cytotoxic reactive oxygen intermediates (ROI) (Wright and Siverstein, 1983). The ability of pathogenic mycobacteria to adapt to the hostile environment of macrophages has been instrumental in its success as a pathogen. Mycobacteria interfere with host trafficking pathways by modulating events in the endosomal/phagosomal maturation pathway to create a protective niche, the mycobacterial phagosome (Houben *et al.*, 2006). The mycobacteria containing phagosome, while connected to the endocytic pathway, does not fuse with lysosomes or mature into phagolysosomes (Armstrong and Hart, 1975; Nguyen and Pieters, 2005). By blocking its delivery to lysosomes, the mycobacterium is able to avoid the acidic proteases of lysosomes; avoid exposure to the bactericidal mechanisms within lysosomes; prevent degradation and hence processing and presentation of mycobacterial antigens to the immune system (Pieters, 2001). Another mechanism by which mycobacteria could interfere with phagolysosomal fusion is by retention of an important host protein termed (Tryptophan Aspartate containing Coat protein (TACO), also known as coronin 1 on the mycobacterial phagosomal (Ferrari *et al.*, 1999; Russell, 2001). TACO represents a component of the phagosome coat, and retention of TACO prevents phagosomes from fusing with lysosomes, thereby contributing to the long-term survival of bacilli within the phagosome (Ferrari *et al.*, 1999). *M. tuberculosis* also blocks progressive acidification of phagosome by exclusion of proton-ATPase pump (Sturgill-Koszycki *et al*, 1994). *M. tuberculosis* infected macrophages become severely impaired to process and present antigens to T cells (Gercken *et al.*, 1994; Noss *et al.*, 2000). There is downregulation of surface costimulatory molecules and major histocompatibility molecules (Pai *et al.*, 2003) or ability to mount an antibacterial
response like production of nitric oxide (Cooper et al., 2002a). In 10% of the infected individuals, bacteria may resurface from preexisting granulomas as a result of a weakened immune response from preexisting granulomas (Manabe and Bishai, 2000). Currently there is not much information available on how *M. tb* survives against such a vigorous immune response- probable explanations could be the presence of a lipid rich cell envelope, constituted by an inner peptidoglycan layer. Covalently bound to the peptidoglycan is a branched polysaccharide, the arabinogalactan, whose outer ends are esterified with high molecular weight fatty acids, called mycolic acids (Barrera, 2007). The outer layer of the cell wall presents an array of free lipids such as phthiocerol dimycoserosates (PDIM), phenolic glycolipids (PGL), trehalose-containing glycolipids and sulfolipids. Traversing the whole envelope, some glycolipids such as the phosphatidyl-myoinositol mannosides, lipomannan (LM) and lipoarabinomannan (LAM) are anchored to the plasma membrane and extend to the exterior of the cell wall (Barrera, 2007); however *M. tb* might employ more than one strategy to survive such as manipulating host signaling pathway by secretory proteins. Therefore, bacteria may be able to trigger specific signals within host cell that interfere with its normal functioning. The secretory proteins of *M. tuberculosis* have gained attention in recent years both as vaccine candidates (Pym et al., 2003) and diagnostic tools because of their recognition by the sera and immune cells of patients. In this direction one of the important findings is the role of antigens encoded by the RD1 region of *M.tb* genome. RD1 region or region of difference-1 is a 9.5 kb region present in all the virulent strains of M. tb and *M. bovis* but is absent from all vaccine strains of BCG (Mahairas et al., 1996). RD1 region is a genetic locus encoding 9 proteins named Rv3871-Rv3879. There is strong experimental evidences to indicate that RD1 contributes significantly to the virulence of members of *M.tb* complex. Deletion of RD1 from M. tb results in decreased virulence that is similar to BCG (Lewis et al., 2003). Secretory proteins are also targets of the immune system and apart from triggering a protective response may also be involved in the clinical symptoms of the disease (Guinn et al., 2004). This idea is supported by the fact that only live but not dead mycobacteria can downregulate the macrophage immune function by blocking calcium mediated signaling (Malik et al., 2003). But despite a wealth of information available on these antigens, their role at the site of infection is yet to be ascertained.

In addition, persistence of the pathogenic mycobacteria inside the macrophage occurs through modulation of host cell signaling which allows them, unlike the other non-
pathogenic species to survive inside the host. Modulation of host cell-signaling is a dynamic process involving the interference of signaling pathways by bacterial molecules.

Several bacterial pathogens secrete virulent mediator molecules that modulate the host cell signaling (Koul et al., 2004). Macrophages are a common target for these pathogens that benefit from avoiding an encounter with the immune system, as well as for those that are aiming to secure a systemic spread (Rosenberger and Finley, 2003). The interaction between *Mycobacterium tuberculosis* (*M.tbc*) and the macrophage is a critical step in the pathogenesis of early infection. The macrophage is a key effector cell in activating innate immune responses and initiating the acquired immune response. Innate immunity likely provides an important early defense against *M.tbc* and could be a critical determinant of the ultimate clinical outcome. Knowledge of the *M.tbc*-macrophage interaction will contribute to the understanding of tuberculosis pathogenesis, identifying novel drug targets and better drugs. The *M.tbc*-macrophage interaction system also represents an ideal paradigm for unraveling microbiological and host cell biological mechanisms.

The series of events that allow pathogenic mycobacteria to alter the normal progression of events within mononuclear phagocytes is poorly understood, and moreover to circumvent the host immune responses, during the host pathogen interaction pathogenic mycobacteria involves multiple factors, including the alteration of phagocyte gene expression patterns. In this context we have carried out this study with the following aims and objectives:

1) In order to broaden the range of proteins under investigation, we have employed "Quantitative proteomics" strategy-“isobaric tagging for relative and absolute quantification” (iTRAQ)- that relies on the labeling of primary amino groups in proteolytically digested proteins over different time points of infection to investigate the global proteome modulation of infected cells.

2) Transcriptional profiling using microarrays provide a unique opportunity to decipher host pathogen cross-talk on the global level.

3) Finally, we have carried out intensive analysis of the host cell expression profiles in terms of its proteome as well as transcriptional status, in order to correlate the mRNA and protein expression profiles with respect to the significant molecules as identified by the patterns of gene expression at different times points in both experimental approaches.