Introduction
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*Mycobacterium tuberculosis,* the causative agent of tuberculosis infects 8 million people annually worldwide and causes the death of 2-3 million people per year (Raviglione, 2003). The emergence of multi-drug resistance (MDR) strains of *M. tuberculosis* and susceptibility of patients infected with human immunodeficiency virus to tuberculosis have fuelled the spread of the disease (Elliot *et al,* 1995; Chintu and Mwinga, 1999).

The primary route of infection for the tubercle bacillus is through the respiratory tract. The bacillus can be inhaled through air-borne fine droplets formed by coughing, sneezing etc. of patients with tuberculosis. In lungs alveolar macrophages are equipped to destroy majority of the pathogens, but the tubercle bacillus has an extraordinary ability to persist and replicate in this extremely hostile environment.

*M. tuberculosis* are small, slightly curved rod-shaped cells. The mycobacterial cell wall is highly complex and lipid-rich containing mainly mycolic acid (Honer zu Bentrap and Russell, 2001). *M. tuberculosis* can survive and replicate intracellularly inside the macrophage where most pathogens perish. Obviously *M. tuberculosis* have evolved effective mechanisms to survive most macrophage effector functions. One such mechanism is the inhibition of phagosome-lysosome fusion (Armstrong and Hart, 1975), normally phagosomes containing bacteria fuse with the lysosome where the pathogen is degraded but *M. tuberculosis* containing phagosomes fail to fuse with the lysosome arguably due to retention of a host protein TACO (tryptophan-aspartate containing coat
protein) on the phagosomal membrane (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 antigen 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 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 et al, 2003). The secretory proteins of *M. tuberculosis* have gained attention in recent years both as vaccine candidates (Harboe et al, 1996; Pym et al, 2003) and diagnostic tools. 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 (Colangeli et al, 2000; Guinn et al, 2004). This idea is supported by the fact that only live but not dead mycobacteria can downregulate the macrophage immune function (Malik et al, 2001).
The region of difference (RD)-1 is a 9.5 kilobase (kb) segment of \( M. \) \( \text{tuberculosis} \) genome that is absent from all the strains of BCG (Mahairas \textit{et al}, 1996). Two open reading frames of RD-1 region, Rv3874 and Rv3875 encode 10-kDa culture filtrate protein and 6-kDa early secreted antigenic target respectively (Cole \textit{et al}, 1998) and are cotranscribed. Therefore, CFP-10 and ESAT-6 are \( M. \) \( \text{tuberculosis} \) specific proteins. Recent studies have implicated a role of these secretory proteins in virulence of \( M. \) \( \text{bovis} \) (Wards \textit{et al}, 2000). The deletion of RD-1 locus from the genome attenuates the organism and, conversely, introduction of RD-1 region of \( M. \) \( \text{tuberculosis} \) into the genome of \( M. \) \( \text{bovis} \) BCG increases latter’s virulence and immunogenicity (Pym \textit{et al}, 2002; Lewis \textit{et al}, 2003; Pym \textit{et al}, 2003).
A secretory apparatus recently described in M. tuberculosis for the secretion of the proteins CFP-10 and ESAT-6 which lack any secretory signal sequence, out of the cell. Mutations in this system considerably attenuated the virulence of M. tuberculosis in a mouse model (Stanley et al, 2003). This attenuation of M. tuberculosis virulence during in-vivo infection in a mouse could be attributed to the inability of bacterium to downregulate the macrophage function, which makes CFP-10 and ESAT-6 two important factors in the modulation of macrophage response. CFP-10 and ESAT-6 are encoded by the genes esxA and esxB and are cotranscribed. In the above context, this study was undertaken with the following objectives:

1) Cloning, Expression and Purification of ESAT-6.

2) To identify the signaling pathways that are modulated by ESAT-6.

3) To compare the modulation of macrophage signaling by ESAT-6, CFP-10 and CFP-10 : ESAT-6 complex.