Introduction
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*Mycobacterium tuberculosis* (*M. tuberculosis*) 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. tuberculosis* 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. tuberculosis*, most of the infections are self-contained with only 5% to 10% disease manifestations suggesting that the immune system can normally control the *M. tuberculosis* 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 \textit{M. tuberculosis}, 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 TNF\(\alpha\), IL-12, IL-1, IL-6 and IL-10. Macrophages upon activation produce nitric oxide which kills bacteria. TNF\(\alpha\) and IL-12 are proinflammatory cytokines which aid in driving Th1 response against \textit{M. tuberculosis} infection. IL-6 is implicated in inflammation, haematopoiesis and T cell differentiation and IL-1 in acute phase response and containment of \textit{M. tuberculosis} 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 \textit{M. tuberculosis} 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 \textit{M. tuberculosis}. For instance, proteins like ESAT-6 and CFP-10 (also known as MTSA-10) belonging to RD1 genetic locus has been implicated in \textit{M. tuberculosis} virulence. Both the proteins are highly immunogenic and are found in the early culture filtrates of \textit{M. tuberculosis} after short periods of growth and in the absence of obvious autolysis. Paradoxically, CFP-10 has also been shown to modulate macrophage functions (Trajkovic \textit{et al.}, 2002). Also, CFP-10, ESAT-6 and the CFP-10: ESAT-6 complex of \textit{M. tuberculosis} have been shown to downregulate the signal transduction pathways responsible for immune activation of macrophages (Ganguly \textit{et al.}, 2007; Ganguly \textit{et al.}, 2008). More recently, ESAT-6 has been shown to downregulate MHC class II expression by inhibition of IFN\(\gamma\)- inducible CIITA expression (Kumar \textit{et al.}, 2012). Therefore, it will be interesting to study and characterize the secreted proteins of \textit{M. tuberculosis} to understand their role in the pathogenesis of the tuberculosis.
Decades of work have focused on the interaction of this pathogen with its established cellular host, the macrophage, but the identification and regulation of exact process remains to be fully worked out. While the macrophage is clearly important, many evidences suggest that understanding the role of dendritic cells, which are key regulators of immunity, is also a crucial step in identifying new means of controlling this disease. It is imperative to dissect the complex dynamic relationships between host cells and mycobacteria to highlight the new areas of intervention that have not been previously explored. A more complete understanding of the roles played by each component of immune system in protection or exacerbation of tuberculosis, as well as of the bacterium’s weapons to evade those components, will enhance the development of preventive and therapeutic strategies against this enormously successful pathogen.

Between infection and the appearance of first symptoms of the disease, bacteria interact with different microenvironments within the host. The outcome of such host–pathogen interactions are in large part due to selective gene expression at different phases of infection (Krinos et al., 2001). Consequently, understanding bacterial gene expression in vivo is central to our understanding of how bacteria colonize, invade, and interact with or disrupt the normal host cell functions and eventually produce disease. A clear understanding of the molecular events responsible for establishing and maintaining tuberculosis will likely lead to improved drug and vaccine design.

With respect to latent tuberculosis, an important area of research is to identify the factors and mechanisms by which M. tuberculosis evades host antimicrobial defenses and survive in the face of a strong immune response. Towards this aspect, many different mechanisms have already been explored which are using by bacteria is to reside in host cells. For example, pathogenic mycobacterial species survive inside macrophages by arresting the normal maturation of their phagosome, thereby restricting its acidification to pH 6.4 and limiting fusion with pre-formed lysosomes (Deretic et al., 2006; Russell 2001; Russell et al., 2005). Various bacterial effector molecules have been found to play a role in arresting phagosome maturation. These include a lipid phosphatase (SapM), a
tyrosine phosphatase (PtpA) (Bach et al., 2008), a serine/threonine kinase (PknG) (Walburger et al., 2004), a lipoamide dehydrogenase (LpdC) (Deghmane et al., 2007) etc. Survival of *M. tuberculosis* is not only facilitated by the manipulation of its primary host cell, but also due to resistance against stresses that the bacteria encounter in immunologically activated macrophages. Different genes and mechanisms help *M. tuberculosis* to specifically withstand the acidic, nitro-oxidative stresses of the host and help it to establish its own niche. Thus, a wide range of molecules and mechanism exists that help the bacteria to survive inside the hostile environment presented by the host.

A recent report has identified genes in human macrophages that regulate the survival of *M. tuberculosis* wherein inhibiting xenophagic pathways and the formation of autophagosomes as an increase in the levels of LC3 II upon the silencing of some target genes was observed (Kumar et al., 2010). Therefore, the present work is focused towards studying the role of host system when bacteria infect it. Is really host system is trying to evade the bacteria or somewhere it is helping bacteria to survive within.

To study this aspect we use RNA interference tool to carryout large-scale functional studies against host proteins in dendritic cells that were first infected with a virulent strain of *M. tuberculosis*. The use of siRNA libraries against a specific pathway is a powerful technique to study the effect of that pathway on the function of a set of related genes inside a cell (Ganesan et al., 2008; Luo et al., 2009; Cullen et al., 2005). There are various mechanisms and pathways studied which are targeted by *M. tuberculosis* to survive in DCs but we chose two key pathways- Calcium Calmodulin pathway and Cysteine Protease Pathway. These key pathways are targeted by *M. tuberculosis* in both DCs and macrophages. The calcium pathway that affects the survival and pro-inflammatory response generation from DCs (Noble et al., 2000; Malik et al., 2003), and the cysteine protease pathway that largely effect antigen processing and presentation to T cells, thereby modulating priming of T cells early on in the infection process (Hsing et al., 2005). Previously our own work has also highlighted the role of calcium homeostasis in regulating the survival of *M. tuberculosis* both in vitro and in vivo (Gupta et al., 2009).
Therefore, in the light of the above, in this study we elucidated the role of genes of these two pathways in modulating DC function with respect to *M. tuberculosis* infection by employing pathway specific siRNA libraries. Our results identify a set of as yet unreported genes whose expression levels proved critical for maintenance of the intracellular pathogen. These genes are targeted by *M. tuberculosis* in modulating the activation and function of DCs with respect to cytokine secretion and pro-inflammatory T cell responses, anti-defense mechanisms such as autophagy and reactive oxygen species generation.