Tuberculosis (TB) is one of the three major infectious diseases along with HIV/AIDS and malaria, which accounts for maximum number of deaths (Franco-Paredes et al., 2006). According to the World Health Organization, TB is responsible for about two million deaths annually (2010). The causative agent of TB, *Mycobacterium tuberculosis* (*M. tb*), also infects nine million people each year (Parida and Kaufmann, 2010). It is estimated that one third of the global population is infected with *M. tb*. In the wake of its liaison with AIDS, emergence of multi-drug resistant strains and failure of BCG vaccine to control the disease; WHO has announced TB as a global emergency (Dye, 2006). Even though, the immune system controls the infection initially; but is highly incapable of a sterile eradication. Therefore, in most cases, the infection becomes latent and hence there is always an associated risk of reactivation and spreading of disease, which affects both the individual and the community (Kaufmann, 2010). Although drug regimen can cure TB, it also may pave way for the emergence of drug resistant strains of the bacteria (Koul et al., 2011; Russell et al., 2010). Therefore, vaccination strategies against TB could be the only rational way for its eradication (Gandhi et al., 2010).

The only available vaccine for the disease, *Bacillus Calmette-Guerin* (BCG), is estimated to have been administered to more than four billion people (Kaufmann, 2010; Kaufmann et al., 2010). Yet, the ever increasing number of TB cases each year is suggestive of the fact that BCG immunization would fall way short of achieving the target of the WHO STOP TB program, which aims to reduce the number of new TB cases to one per million (Lonnroth et al., 2010). Despite its wide usage, BCG has not adequately reduced the global TB burden. Notably, its efficacy is highly controversial with very poor in high *M. tb* burden countries (Andersen and Doherty, 2005; Fine, 1995; Kaufmann et al., 2010; Russell et al., 2010). Since TB-endemic areas account for most of the cases, preventing the disease in these regions is imperative to the progress in achieving the utopian goal of eradicating TB. This warrants serious
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attempts to improve or modify BCG, or to develop altogether novel vaccination strategies (Kaufmann, 2010).

Vaccination in TB-endemic areas - Quo Vadis.

Three major reasons could probably explain the failure of BCG in TB endemic population. First is the interference by non-TB mycobacteria (NTM) in the BCG-mediated immune response, second is the obstruction in antigen processing and presentation by mycobacteria, and third is skewing of BCG induced response by helminths (Andersen and Doherty, 2005; Rook et al., 2005; Verma and Grover, 2009). Currently, there are about twelve TB vaccine candidates in clinical trials (Kaufmann et al., 2010). Of these, many are whole cell based formulation like the recombinant BCG or attenuated \textit{M. tb}. Further, some of them employ viral vectors containing \textit{M. tb} antigens (Kaufmann et al., 2010). However, based on the insights obtained from the clinical trials and studies conducted on experimental animals conclude that the current candidate vaccines may also fail in TB endemic areas.

An aspect of great concern with the current TB vaccine candidates in trials is the extensive requirement of antigen processing and presentation. In TB-endemic areas, this could be a serious obstacle since many of the environmental species of NTMs have been shown to interfere in antigen processing and affect optimal priming of T cells (Andersen and Doherty, 2005; Flaherty et al., 2006; Noss et al., 2000; Rook et al., 2005; Soualhine et al., 2007; Tsuyuguchi et al., 1990; Weir et al., 2006; Young et al., 2007). Further, there are evidences for the pre-existing neutralizing antibodies for some of the viral vectors (O’Brien et al., 2009). This would prevent the vaccine from engendering long-lasting T cell memory. Importantly, since exposure to NTMs and helminths prevent efficient immunity upon BCG vaccination, they may also interfere in the protective immune response elicited by cell based vaccine (Rook et al., 2005; Verma and Grover, 2009). Taking the detrimental effects of pre and post
exposure to NTMs, helminths and viruses, a vaccine formulation that requires minimal antigen processing, formulated with strong adjuvants that elicit robust and enduring Th1 memory response, may eventually be quite successful in TB-endemic settings (Gowthaman et al., 2011).

**Promiscuous T cell epitope based lipidated-peptide vaccines.**

An attractive option is to use peptide based vaccine against TB, especially in an Indian context (Gowthaman et al., 2011). Peptide vaccines have tremendous advantages that supersede the current TB vaccines that are in clinical trials. The risks associated with BCG and many of the vaccines in trials are pathogenic responses to the host, danger of autoimmunity, risk of genetic integration, conversion to virulence, and disease in immunocompromised individuals, which could be circumvented by peptide vaccines since they contain precisely defined epitopes (Purcell et al., 2007). Further, peptide vaccines offer tremendous flexibility in choosing the epitopes from diverse array of antigens including those that are not expressed by BCG or are expressed during TB latency (Siddiqui et al., 2011). As peptides do not require extensive processing, hence their performance will not be hindered by environmental mycobacteria. Therefore, peptide vaccines offer an attractive alternative vaccination strategy in TB-endemic areas.

Conventional peptide vaccines have been plagued by two problems. Firstly, peptides are poorly immunogenic. The concept of “danger signal” could explain this lack of immunogenicity in peptides (Matzinger, 2002). The immune system has evolved to discriminate between self and non-self. This discrimination is based on the sensors of immune cells that identify molecules associated with pathogenic organisms and also the inflammatory milieu which is followed by breaches in barrier immunity. Cells of the immune system are endowed with Pattern Recognition Receptors (PRRs) that recognize Pathogen Associated Molecular Patters (PAMPs). Toll Like Receptors (TLRs) are such PRRs that are expressed on
most immune cells, especially the APCs (Akira et al., 2006). Hence, when perceived out of context of the pathogen or whole antigen, peptides are insufficient to activate dendritic cells and other APCs. Thus, it is imperative to employ adjuvants to formulate peptides as vaccines. Most adjuvants alarm the immune system by activating TLRs or other PRRs like NOD Like Receptors (NLRs), RIG-1, etc. Hence, agonists of PRRs could potently work as adjuvants (Coffman et al., 2010). Due to the paucity of adjuvants for human use and also since the commonly used adjuvants such as alum, MF59, AS03 give a mixed Th1/Th2 response, an urgent need also exist to develop novel and cost effective adjuvants. TLR agonist based adjuvants have been used in a number of studies to generate successful long lasting immune responses (Jackson et al., 2004; Pulendran, 2004) It has been shown by Blander and Medzhitov that the TLR triggering moiety and the antigen should be physically associated to elicit antigen specific immune response (Blander and Medzhitov, 2004, 2006). Lipopeptide vaccines, which employ TLR-2 agonists conjugated to peptides, are being successfully used in a variety of disease models, including viral infections and cancer (Jackson et al., 2004).

Secondly, peptides may not work efficaciously in genetically out-bred populations. A necessary condition for peptides to work as vaccines is their binding to diverse form of HLA (Human Leukocyte Antigen) alleles (Gowthaman and Agrewala, 2009). The human MHC (HLA) is the most polymorphic and diverse system in the entire human genome. Therefore, a particular peptide may not bind to all HLA alleles. Indeed, most of the antigenic peptides derived from mycobacterial antigens are restricted to just one or two HLA alleles (Agrewala et al., 1997; Agrewala and Wilkinson, 1998, 1999). This is a serious limitation for a global vaccine but can be overcome by prudent selection of promiscuous peptides (Agrewala and Wilkinson, 1999; Gowthaman and Agrewala, 2009). Promiscuous peptides bind effectively to many different HLA alleles.
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Therefore, employing promiscuous peptides from antigens of \textit{M. tb}, would be an attractive proposition.

With this background in view, this study was aimed to develop a unique lipopeptide (L91) by covalently linking the promiscuous peptide (sequence 91-110) of 16 kDa secretory antigen of \textit{M. tb} to Pam2Cys to be used as a prospective vaccine against TB. During the current study, we therefore fulfilled following objectives:

1) To investigate the role of L91 in activating dendritic cells.
2) To examine immunogenicity of L91 in mice.
3) To evaluate the probability of L91 to generate enduring T cell memory response.
4) To assess the protective efficacy of the vaccine in mouse and Guinea pig model of TB.
5) To understand the prospective mechanism of protection in mice induced by L91.
6) Finally, whether L91 can activate the cells obtained from PPD+ human volunteers.

In addition, we also examined

1) The role of TLR-2 signaling on T cell exhaustion.
2) The efficacy of \textit{in silico} tools to predict T cell epitopes.