3. SUMMARY

The immune system has been robustly evolved to control the infection of myriad of pathogenic organisms. However, some pathogens can overcome host defense barriers and evade and subvert the immune response to establish a chronic infection. *Mtb* is one such successful pathogen. On infection, *Mtb* resides in the hostile environment of macrophages with the ability to persist for longer duration and establish latent infection. The latent infection is asymptomatic and serves as a larger reservoir of the bacterium. *Mtb* under stress conditions such as hypoxia, nutrient starvation and oxygen radical generation resembles the conditions that mycobacteria encounter *in situ* during latent infection. It has been suggested that the mycobacteria enter the non-growth or stationary phase during such stress conditions. During latency, *Mtb* might secrete some molecules/proteins, which may help the bacterium to tune and tame the immune response and survive successfully. It was observed that there are many proteins which are specifically upregulated at the time of latency. One of the predominant proteins at the time of latency is shsp 16 kDa (HSPX). 16 kDa not only promotes the survival of *Mtb* in the extreme conditions by stabilizing proteins, but is also immunogenic and can elicit both B cell and T cell responses. Not much has been studied on the role 16 kDa antigen on DCs.

In the present study, we have studied the role of 16 kDa of Mtb in influencing the activity of DCs. We observed that 16 kDa antigen of *Mtb* interferes in the differentiation of DCs. These DCs exhibit phenotype with low allostimulatory and reduced antigen uptake ability. Surprisingly, 16 kDa induces activation in terms of expression of costimulatory molecules and release of cytokines when incubated with immature DCs. We also observed that 16 kDa binds to TLR-2. Downstream signalling events involved in the differentiation of DCs were also examined in the presence of 16 kDa antigen. TLR stimulation upregulates the expression of SOCS molecules.

Surprisingly we observed that 16 kDa upregulates SOCS-3 but downregulates SOCS-2. Further, differential phosphorylation of STAT-6 and STAT-3 was also observed. Decreased phosphorylation of STAT-6 might be involved in inducing latency. However, activation of STAT-3 may play a crucial role in inducing tolerance. We also observed decrease in the nuclear translocation of NF-κβ and thus we conclude that 16 kDa suppress the immune response by impairing the differentiation of DCs.

Besides immunomodulatory role of 16 kDa antigen, we also checked its vaccine potential. BCG is the only vaccine available for human use. There are various limitations associated with it like variable efficacy in adults and inability to take care of latent TB. The ineffectiveness of BCG for latent TB is because it does not optimally express latency antigens. Further BCG protective efficacy wanes in adults, thereby doubting its efficiency in the generation of long-lasting memory T cells. Liposomes can successfully augment memory T cell response (Acharya and Murthy, 2011; Steers et al., 2009). It may be considered that in BCG vaccinated individuals, immune response may not be generated against latency antigen.

Therefore, in the current study, 16 kDa was entrapped in fusogenic liposomes (L16) prepared from yeast lipids, and this formulation was used to bolster the immunity of animals that were vaccinated with BCG. This prime-boost strategy, using BCG and L16 (BCG-L16) evoked better Th1 memory response than BCG alone. Large number of multifunctional CD4 and CD8 T cells that were producing both IFN-γ and TNF-α were generated in the lungs as well as secondary lymphoid organs in the animals vaccinated with BCG-L16. Interestingly, substantial decline in the level of IL-4 was also seen. Finally, the protective efficacy of BCG-L16 vaccination was established by substantial reduction in the bacterial burden in the lungs. This was further substantiated by reduced number of granuloma formation and lung pathology.