Summary

Mycobacterium tuberculosis (Mtb) has developed remarkable mechanism of latency that helps it survive in a clinically undetectable stage by evading the host immune response. This latent phase of infection that may prolong for decades is achieved by major shifts in growth, metabolism and replication patterns of bacteria in response to immune activation of the host. Majority of the genes contributing to the induction of non-replicative state belongs to dormancy regulon (DosR) and are highly upregulated during dormancy. One of the most prominently upregulated gene of DosR is *Acr1* that codes for a heat shock protein HspX or commonly called 16 kDa. In the past, various studies have focused on myriad aspects of this protein involving its role as a vaccine candidate, drug resistance and as a chaperone. Moreover, its effect on *Mtb* survival has also been studied in mutants and a*cr1* knockout strains of *Mtb*. We were the first group to report that Acr1 when encounters with pre differentiated dendritic cells (preDCs), hampers their maturation and show a phenotype that is similar to tolerized DCs. Our study also indicated its role in probable interactions happening at host-pathogen interface. Keeping this in mind, we designed this current study where we wished to explore the effects of Acr1 on post differentiated DCs (post DCs) and at the same time study its protein-protein interaction with the human host using a multidisciplinary approach. We observed that Acr1 on encounter with pre differentiated DCs inhibited its maturation, accompanied with impairment in the DCs migratory, T cell stimulatory and skewing of T cell differentiation towards Th1 and Th17. While in contrast treatment of GM-CSF and IL-4

matured DCs with Acr1, promoted its potentiality to migrate, stimulate and skewing of T cell differentiation towards Th1 and Th17. Interestingly, to reveal the molecular interaction of Acr1 with the host, which could shed some insights into the mechanism of Acr1 induced dormancy, through the interplay with host proteins. On performing computational molecular docking of Acr1 with human proteins. We observed a high affinity binding with IFN-γ, this interaction was further validated by ELISA and pull down assays, thus establishing that Acr1 can interact with IFN-γ *in vitro*. Further, on looking for biological implication of this binding. We observed that Acr1 binding with IFN-γ potentiated its activity to induce NO secretion and also facilitated the killing of bacteria. Additionally, we also aimed to generate a recombinant non-pathogenic strain of mycobacteria (*Mycobaterium Smegmetis*) that can be used a vaccine against *Mtb*. Several reasons like failure of BCG to evoke long lasting memory and its variable efficacy in TB endemic regions and its ability to impair the maturation of phagolysome motivated us come up with a better and cost effective vaccination strategy. It is worth to mention here that different groups have already tried to clone recombinant strains of BCG and other non-pathogenic mycobacteria like *Mycobacterium indicus pranii*. However, their studies were targeted to improve the efficacy of vaccine rather than improving its memory generating capability which is cardinal for a successful vaccine design. To this end, we tried to clone memory generating cytokines IL-7 and IL-15 in *M. Smegmetis*. Although, we could successfully expressed IL-7 and IL-15 individually but failed to coexpress them together. We could detect the bioactive secretion of IL-7 in the culture supernatant but currently failed to detect the presence of

IL-15 in secretory form. We will be proceeding to evaluate the efficacy of rMSIL7 in boosting the immunity against *Mtb*.