CHAPTER V

Summary and Conclusions

No one should have to die of a disease that is treatable.

…Anonymous
Chapter V

SUMMARY AND CONCLUSIONS

*Mycobacterium tuberculosis* has the history of ruining civilizations and even today it is responsible for much despair and mortality. Every week, more than 160,000 individuals develop TB and ~27,000 human lives are lost globally due to this dreaded disease. The lethal liaison between TB and HIV infections and the emergence of various forms of drug resistant *M. tuberculosis* strains have made the situation even more precarious. It is estimated that one third of the world’s population is latently infected with *M. tuberculosis*. The current vaccine, BCG is effective in preventing severe forms of TB, mostly disseminating and meningeal forms in children. However, BCG is ineffective in providing consistent protection against the disease in adults and older people. Under the best of the circumstances, it has provided 80% protection, which generally has been to the tune of 40-60% on an average. Therefore, there is an urgent need to develop a superior TB vaccine than BCG. The purpose of an effective live vaccine would be best served if the vaccine strain is antigenically as similar as possible to the disease-causing pathogen in order for it to generate the host immune responses that mimic natural infection. Comparative genomic studies have revealed that BCG, in comparison to *M. tuberculosis*, lacks 16 defined regions (RD1-16) comprising of ~150 genes, some of which are known to encode potential antigenic determinants that could increase the immunogenicity of a vaccine. This makes the use of attenuated *M. tuberculosis* strains rather than BCG, for the generation of appropriate immune responses, an attractive idea.

The current TB therapy requires prolonged treatment schedule of 6 months for drug susceptible TB that may extend up to 30 months in the case of drug resistant TB. Non-compliance to such a prolonged regimen often leads to the emergence of MDR and XDR TB. Even if a patient successfully completes chemotherapy, due to inability of the current therapy to impart complete sterilization, there is a 5-10% lifetime risk of reactivation of the latent infection. This risk substantially increases to 10% annual risk in the immunocompromised individuals. Hence, alternate strategies are urgently required that can shorten the duration of chemotherapy and reduce reactivation of the disease.

With the larger objective of developing effective interventions against TB, in this study, we have evaluated different vaccination strategies namely, prophylactic and immunotherapeutic strategies for vaccination against TB. The prophylactic strategy deals with
the development of an *M.tuberculosis* mutant (Mtb∆mms) as a candidate vaccine against TB by deleting the function of three virulence genes, namely, *mptpA* (Rv2234), *mptpB* (Rv0153c) and *sapM* (Rv3310) and evaluating its protective efficacy in the guinea pig model of experimental tuberculosis. The selected three genes, namely, *mptpA*, *mptpB* and *sapM* encode for mycobacterial secretory phosphatases that are essential for the virulence of *M.tuberculosis*. SapM dephosphorylates phosphatidylinositol 3-phosphate (PI3P), a membrane trafficking regulatory lipid, resulting in the arrest of phagosome maturation. MptpA has been demonstrated to block phagosome-lysosome fusion by inhibiting V-ATPase trafficking to the mycobacterial phagosome. MptpB inhibits ERK ½, p38 signaling pathways and caspase 3 activity, thus subverting the host immune response to infection. Thus, all the three genes are involved in the host pathogen interactions and signal transduction. The immunotherapeutic strategy deals with the development of a mice model of latency and evaluation of immunotherapeutic potential of DNA vaccines expressing the antigens α-crystallin (Rv2031c) or superoxide dismutase (Rv3846) of *M.tuberculosis* as an adjunct to chemotherapy to shorten the duration of chemotherapy and to prevent the reactivation of latent TB infection.

**Development of a multigene mutant of Mycobacterium tuberculosis by deleting the function of virulence associated genes and evaluation of its protective efficacy in guinea pigs**

- In this study, we have generated a mutant of *M.tuberculosis* (Mtb∆mms) by disrupting 3 virulence genes encoding a mycobacterial secretory acid phosphatase (*sapM*) and two phosphotyrosine protein phosphatases (*mptpA* and *mptpB*) and have evaluated its protective efficacy in guinea pigs.

- We did not observe any difference in the growth characteristics of Mtb∆mms and the parental strain in MB7H9 medium, however, a significant difference was observed in the growth kinetics between these two strains in THP-1 macrophages. We observed that Mtb∆mms displayed a significantly reduced ability (~2.89 fold difference) to infect macrophages in comparison to the parental strain. Moreover, while *M.tuberculosis* continued to grow normally for 6 days, Mtb∆mms was highly attenuated for growth in human THP-1 macrophages.

- When guinea pigs were inoculated with Mtb∆mms, no bacilli were recovered from the lungs and spleens of guinea pigs after 10 weeks of inoculation. Although, the bacilli
Summary and Conclusions

were recovered from the spleens of the animals during the initial phase (4 weeks post inoculation), the bacillary load was only 1.4% of that observed in the case of *M. tuberculosis* infected animals (70 fold fewer bacilli in MtbΔmms inoculated animals)

Subsequently, when MtbΔmms was evaluated for its protective efficacy in guinea pigs, we observed that similar to BCG vaccination, MtbΔmms exhibited a significantly reduced CFU in the lungs of the animals, when compared with the unvaccinated animals at 4 weeks post infection. In addition, our observations, at 12 weeks post infection, demonstrated that MtbΔmms exhibited a more sustainable and superior protection in lungs as compared to BCG. However, the mutant failed to control the hematogenous spread as was evident from the fact that the splenic bacillary load between MtbΔmms vaccinated and sham immunized animals was not significantly different. The gross pathological observations and histopathological observations corroborated the bacillary loads recovered from the organs.

This study highlights the importance of *M. tuberculosis* mutants in imparting protection against pulmonary TB. We demonstrate that mutation of genes encoding the signal transduction associated phosphatases of *M. tuberculosis* provides optimism for the generation of novel potential vaccine candidates against TB. The MtbΔmms was not only significantly attenuated for growth in macrophages and guinea pigs, it also imparted an enhanced protection against the disease in lungs when compared with BCG. However, further modifications would be required in order for MtbΔmms to elicit more appropriate immune responses for imparting a superior protection including the control of hematogenous spread. Our future efforts would focus on addressing these issues.

Evaluation of the immunotherapeutic potential of adjunctive immunotherapy with DNA vaccines in a murine model of latent TB to shorten the duration of chemotherapy and to prevent the reactivation of latent infection

In this study, by employing modified Cornell model, we have evaluated the potential of adjunctive immunotherapy with DNA vaccines to shorten the TB chemotherapy period and reduce disease reactivation.

We show that adjunctive immunotherapy with DNA vaccine encoding mycobacterial latency antigen α-crystallin significantly shortens the duration of chemotherapy
resulting in a faster clearance of infection when compared with the chemotherapy alone thus imparting more efficacious therapeutic effect. α-crystallin based DNA vaccine (DNAacr) significantly reduced the chemotherapy period from 12 weeks to 8 weeks when compared with the chemotherapy alone. Immunotherapy with SodA based DNA vaccine (DNAsod) reduced the pulmonary bacilli but only as much as the DNA vector, pAK4 (DNAvec).

In the case of reactivation of latent infection, the adjunctive immunotherapy with DNAacr or DNAsod resulted in a significant reduction in the bacillary load in lungs when compared with the chemotherapy alone, suggesting that it can potentially delay the reactivation of the disease. However, since this reduction in bacillary load was not significantly different than in the case of treatment with the DNAvec, it suggested that the observed reduction in the bacillary load and the consequent delay in the reactivation of disease may be associated with the immune stimulation caused by the DNA vector alone and may not be attributable to the role of antigens.

Both DNA vaccines resulted in the production of significantly higher number of $T_{EM}$ cells than the chemotherapy alone, however, only in the case of DNAsod, this enhancement was significant over the DNAvec treatment.

Overall, our findings emphasize the immunotherapeutic potential of DNAacr in shortening the duration of TB chemotherapy.

In a previous study, we have reported the prophylactic potential of DNAacr vaccine as a booster to BCG or recombinant BCG expressing the same antigen, in a heterologous prime boost approach. In the present study, we demonstrate the immunotherapeutic potential of DNAacr along with chemotherapy to shorten the duration of chemotherapy. Hence, α-crystallin based DNA vaccine holds a significant promise for its use both as a prophylactic vaccine as shown by us earlier and as a partner in the therapeutic approach as shown in this study.