Chapter 3

Rationale of the Study and Objectives
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While a number of new candidate vaccines for tuberculosis have been proposed over the last decade (http://www.stoptb.org/wg/new_vaccines/) (Kaufmann, 2010), a vaccine more effective than BCG is yet to emerge. Therefore, we believe that some molecular modifications in BCG may potentially improve the efficacy of BCG and may be effective even in the regions where native BCG has failed to provide protection against adult pulmonary TB.

Virulent strains of *M. tb* modulate the host’s immune system to ensure their unhindered survival and replication within the hostile environment of hosts. In contrast, BCG vaccine strains stimulate the immune system leading to development of anti-tuberculosis immunity. Therefore, a good understanding of immune responses induced by virulent *M. tb* (H37Rv) and the vaccine strain BCG is crucial for introducing any molecular modifications in BCG. Immune responses in murine models of TB are well characterized, and largely reflect those seen in human disease. *M. tb* infects professional Antigen Presenting Cells (APCs). Immune responses are initiated and directed from these professional APCs, which, in turn, affects the outcome of the infection/disease. Therefore, our first aim is to “compare the immunogenicity of virulent strain H37Rv and vaccine strain BCG in mouse model”. These will enable us to identify the factors that are essential for projection of host resistance in TB. For mounting effective immune responses, APCs present not only antigenic peptide(s) bound with MHC molecules, but also provide co-stimulatory molecules, and the T-cell polarizing cytokines. Cytokines are mainly responsible for the differential Th cell polarization, and in turn host susceptibility or resistance to TB. Therefore, we would evaluate production of cytokines in the DCs infected with H37Rv and BCG.

RD (Region of Difference) regions are large portions of the *M. tb* genome that are deleted in most strains of *M. bovis* BCG including the one commonly used for vaccination. There are 16 such deletions and they encompass 129 ORFs (Mahairas et al., 1996; Skjot et al., 2000; Weldingh and Andersen, 1999). The RD1 region is found to be deleted in all the strains of *M. bovis* BCG and the RD1 region has not been detected in a majority of environmental non-pathogenic mycobacteria (Harboe et al., 1996; Skjot et al., 2000). Interestingly, RD1 mutant of *M. tb* somewhat resembles
BCG in vaccine properties while RD1 complemented recombinant BCG resembles H37Rv in virulence (Ganguly et al., 2008b). These observations underline the critical virulence factors inherent in RD1 region of Mtb. Thus, our second goal is to “compare immunogenicity of RD1 mutant H37Rv and RD1 recombinant BCG strains with their parental strains respectively in vitro and in vivo”. For this we proposed to determine cytokine production in infected DCs. Depending on the cytokines in the microenvironment, differentiation of Th cells will commence. Therefore, we will monitor Th cells activity in animals that are infected with BCG, H37Rv, RD1-deleted H37Rv, and RD1-incorporated BCG. It is important to mention here that balance of various Th cell activities dictate the outcome of the disease. It has been shown that Th1 and Th17 cells enhance protective response whereas Th2 and Treg cells hinder host resistance to tuberculosis (Chen et al., 2007; Goldsack and Kirman, 2007; Khader et al., 2007; Scott-Browne et al., 2007; Young et al., 2008).

Interestingly, a predominant and highly immunogenic RD1 secretory protein, ESAT-6, exhibits bipolar immune responses and it has been shown to bind to TLR2 (Pathak et al., 2007). It is important to note here that TLR-2 plays an important role in the host resistance against TB. Thus, TLR-2 deficient mice are highly susceptible to TB (Salgame, 2005). Therefore, our third goal is “to determine immunogenicity and the disease progression induced by BCG, H37Rv, RD1 mutant H37Rv, and RD1 recombinant BCG in TLR-2 knock out mice” and “to evaluate the role of ESAT-6 in modulation of Th cells responses in M. tb infection”.

Recent studies in our laboratory (Singh, Y. et al., unpublished data) indicated that M. tb controls miRNAs expression which can affect the host immunity by regulating various signalling pathways. Our miRNAs microarray analysis in mice model suggested that various miRNAs are modulated after M. tb infection. Our miRCURY LNA miRNAs microarray data revealed that the expressions of 10 miRNAs (miR712, 23a, 710, 881, 882, 877, 146a, 125a-5p, 99b and 222) are modulated in the M. tb infected DCs. We have further focussed on miR146a which is upregulated by M. tb infection as well as lipopolysaccharide (LPS) stimulation. miR146a is a negative regulator of innate immune components such as IL-6. Thus, our fourth aim is “to study the role of RD1 in the modulation of miR146a expression and to determine how this modulation affects different Th cells responses”. 