Chapter 1

Introduction
1. Introduction

Tuberculosis (TB) remains a major health problem, with an estimated one third of the world's population infected with *Mycobacterium tuberculosis* (*M. tb*), the causative agent of TB, resulting in 1.7 million deaths annually (WHO, 2010). Bacillus Calmette-Guérin (BCG), the only TB vaccine presently used in humans, has been widely used throughout the world since its inception in 1921, and an estimated 3 billion people have received it (Gupta et al., 2007). However, its efficacy against pulmonary TB in adults is highly variable (0-80%) (Colditz et al., 1994) and depends on ethnicity and geographical location (Brewer, 2000; Fine, 1989; Fine, 1995). The antigenic component(s) that is absent in BCG to elicit critical protective immune responses against TB has been an area of intense research (Fine, 1989; Fine, 1995). Early secreted antigenic target protein 6 (ESAT-6) is one of the most prominent antigens expressed by *M. tb*, but not by BCG (Brodin et al., 2004; Simeone et al., 2009). ESAT-6-specific T cells are frequently found in TB patients as well as in infected animals (Brandt et al., 1996; Ravn et al., 1999; Ulrichs et al., 1998). Thus, ESAT-6 is being extensively studied for its potential activity as a subunit vaccine (Dietrich et al., 2006). T cell receptor (TCR) transgenic T cells specific for ESAT-6 exhibit significant protection against TB (Gallegos et al., 2008). Consistent with this, a recombinant BCG strain that contains region of difference 1 (RD1), which includes ESAT-6, exhibited improved protection against TB (Pym et al., 2003). However, the basis of this improved protection remains elusive. Furthermore, the mechanism by which ESAT-6 vaccination induces protective immune responses against TB remains to be investigated.

Deletion mutants of virulent *M. tb* strains for RD1 or ESAT-6 (a protein product of the RD1 region) resemble BCG in their infectivity and attenuation (Lewis et al., 2003). Therefore, these bacterial strains provide insight into the rational selection and design of suitable candidate vaccines for *M. tb* infection. It is clear that vaccination with BCG produces T helper (Th)1 cell-mediated immune responses, and this is moderately effective in protecting against disseminated TB and against meningitis in children (Perera et al., 2009). However, immune responses that are critical for protection against adult pulmonary TB remain incompletely understood. Recently, it has been shown that Th17 cell responses play an important role in
establishing protective immune responses against TB (Khader et al., 2007). However, Th17 cells do not contribute to the primary immune responses in tuberculosis infection (Khader and Cooper, 2008). The antigen-specificity of protective Th17 cell responses in *M. tb* vaccination has not been reported. The differentiation of Th17 cells involves the cytokines interleukin (IL)-6 and Transforming Growth Factor (TGF)-β (Bettelli et al., 2006; Mangan et al., 2006). Earlier studies indicated that IL-6 production in Dendritic Cells (DCs) is regulated by microRNA-146a (miR146a) expression, which acts as a negative feedback regulator in Toll-Like Receptor (TLR) signalling by targeting IL-1 receptor associated kinase (IRAK)-1 and TNF receptor-associated factor (TRAF)6 (Starczynowski et al., 2010; Taganov et al., 2006). miR146a inhibits the expression of IRAK-1 and TRAF6 and impairs Nuclear Factor kappa-light-chain-enhancer of activated B cells (NF-κB) activity, which results in suppression of IL-6, IL-1β and Tumour necrosis factor (TNF)-α expression (Nahid et al., 2009; Taganov et al., 2006). Recently, it has been shown that expression of miR146a is also upregulated in viral and bacterial diseases to modulate immune responses (Liu et al., 2010; Lu and Liston, 2009). Therefore, we hypothesised that miR146a might have a key role in *M. tb* infection by regulating IL-6 production.

We showed that H37Rv and recombinant BCG containing the RD1 region (BCG::RD1) induce improved vaccine efficacy compared with BCG and H37Rv deletion mutants for RD1 (H37RvΔRD1). The virulent strain H37Rv and BCG::RD1 induced both Th1 and Th17 cell responses, whereas BCG and H37RvΔRD1 induced only Th1 cell responses. Inhibition of IL-17 by neutralizing antibodies dramatically reduced the vaccine efficacy of H37Rv and BCG::RD1. H37Rv and BCG::RD1 induced IL-6 and TGF-β in DCs, which generated a microenvironment conducive to the differentiation of Th17 cells. In contrast, BCG and H37RvΔRD1 induced dramatically lower levels of IL-6 and TGF-β. Interestingly, production of both IL-6 and TGF-β in DCs induced by H37Rv and BCG::RD1 was dependent on the TLR-2/Myeloid differentiation protein (MyD)88 signalling pathway. Furthermore, DCs infected with H37Rv or BCG::RD1 expressed lower levels of miR146a compared with BCG and H37RvΔRD1, which differentially affected IL-6 production in infected DCs. Consistent with this, ESAT-6-treated DCs produced IL-6 and TGF-β in a TLR-2/MyD88-dependent manner, and facilitated the polarization of Th17 cell responses. miR146a expression in DCs was unaffected by ESAT-6 treatment and comparable to
uninfected DCs, and ESAT-6 dramatically inhibited miR146a upregulation in BCG-infected or LPS-treated DCs. Therefore, these results indicate that interaction of ESAT-6 with TLR-2 generates a cytokine environment that facilitates the differentiation of Th17 cells, which in turn contributes to protection against TB.