Vijender Singh (2010). ***Induction of generation of Enduring T Cell Memory Response by Vaccine Engendered from Live Mycobacterium Tuberculosis Infected Macrophages.*** Ph.D. Thesis. CSIR-IMTECH, Chandigarh/ Jawaharlal Nehru University, New Delhi: India.

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SUMMARY OF THE THESIS

Tuberculosis (TB) has emerged as a single major cause of very high morbidity and mortality. The etiological agent of the disease *Mycobacterium tuberculosis* (*M. tuberculosis*), infects one third of the world's population, particularly the developing countries. India alone contributing to 30% of the world's burden of tuberculosis(WHO, 2009). The current available vaccine against tuberculosis, bacille Calmette-Guérin (BCG), protects against severe childhood forms of the disease, but fails to protect against adult pulmonary TB in endemic countries (Fine, 2001). But unexpectedly, for more than 80 years, no new TB vaccine has successfully been developed. For successful control and eradication of TB, new vaccines with better protection than BCG are urgently needed.

The goal of a novel TB vaccine is to prevent the transmission of adult pulmonary tuberculosis. There are two main approaches to current vaccination strategies. First, improvement of current BGG vaccine because of its consistent effectiveness against severe childhood forms of meningeal TB (Colditz et al., 1994). Second, development of a new vaccine that can replace the BCG because of its variable protection in different geographical regions attributed to interference by environmental mycobacteria, lack of protective antigens, hindrance in TB diagnosis (Brewer and Colditz, 1995).

In the last decade, many vaccine candidates have been developed and tested in the experimental animal models. They include protein,

In the present study, we attempted to employ both the above mentioned approaches for the development of candidate vaccines. In the first approach, we employed a novel cell based vaccination strategy and a combination of memory enhancing cytokines to generate and sustain the long-lasting memory T cells. The rationale behind using infected macrophages was derived from the fact that a third of the world's population harbor mycobacteria but only 5%-10% develops active disease. Remaining 90%-95% develop effective and long-lasting immunity and remains protected throughout their life. This indicates that natural infection with viable mycobacteria develops an effective and long-lasting protective immunity. There may exists a possibility that growing bacilli express distinct molecules in macrophage during this encounter that may act as protective antigens necessary for the generation of effector and memory T cells. By utilizing this unique strategy, a substantial protection was observed in a standard short-term (30 days) mouse model of tuberculosis infection (Sharma and Agrewala, 2004). However, no remarkable difference was noticed in long-term (240 days) study.

T cell memory is dependent on crucial cytokines signal during priming phases (Dooms and Abbas, 2006; Schluns and Lefrancois, 2003). So, in the current study, we administered a combination of IL-1+IL-6+TNF-α with the infected macrophages vaccine to enhance long-term T cell memory response (Haynes et al., 2004; Pape et al., 1997) . The mice were rested for a reasonably long-period (240 days) to study the *bona-fide* T cell memory response before exposing them to aerosolized *Mycobacterium tuberculosis* (Orme, 2006). To our interest, vaccine significantly improved memory T cell response against *M. tuberculosis,* as evidenced by expansion in the pool of central as well as effector memory CD4 and CD8 T cells, elicitation of mainly Th1 memory response, significant reduction in the lungs mycobacterial load and alleviated lung pathology. Notably, the protection was significantly

better than BCG. Practical implications of this novel vaccination strategy by employing xenogeneic macrophages also demonstrated comparable outcomes. This study demonstrates that not only antigen-pulsed dendritic cells can be successfully used as vaccines against various malignancies but *M. tuberculosis* infected macrophages can also be utilized efficiently in protection against TB.

Finally, we elucidated the mechanism of protection imparted by infected macrophages vaccine. We hypothesize two possibilities which may explain generation of effective immune response by the infected macrophages vaccine. First, *M. tuberculosis* infected syngeneic macrophages may directly present antigens to T cells. Second, infected macrophages (syngeneic and xenogeneic) on γ-irradiation may undergo apoptosis (Hernandez-Flores et al., 2005; Sharma and Agrewala, 2004). Resulted apoptotic vesicles contain processed mycobacterial antigens (Schaible et al., 2003; Winau et al., 2006). Further, these apoptotic vesicles can be avidly taken up by dendritic cells (DCs), which then process and present antigens to CD4 T cells through MHC-II pathway or cross present to CD8 T cells (Schaible et al., 2003; Winau et al., 2006).

In second approach, we employed a different combination of memory cytokines for the improvement of conventional BCG vaccine. A combination of cytokines IL-7+IL-15 that are known to influence memory T cell generation were administered in mice along with BCG vaccine (Nanjappa et al., 2008; Pellegrini et al., 2009; Singh et al., 2010). The animals were rested for a period of 240 days before they were challenged with *M. tuberculosis* (Orme, 2006). Five weeks later, they were sacrificed to study T cell memory response. Co-administration of IL-7+IL-15 with BCG (BCG7.15) resulted in improved CD4 and CD8 T cell memory response. Mice injected with BCG7.15 illustrated enhanced T cell proliferation, Th1-type cytokines

production, and increased pool of multifunctional *M. tuberculosis* specific memory T-cells. Further, there was significant reduction in mycobacterial burden in the lungs of aerosol challenged mice. Our results indicate that supplementation of BCG with IL-7+IL-15 substantially improved its efficacy by enhancing T cell memory response.