U. Gowthaman (2011). *Targeting promiscuous peptides to dendritic cells through toll like receptor-2 for the elicitation of effective immunity against mycobacterium tuberculosis.* Ph.D. Thesis. CSIR-IMTECH, Chandigarh/ Jawaharlal Nehru University, New Delhi: India.

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SUMMARY OF THE THESIS

The immune system has robustly evolved to control a myriad of pathogenic organisms. However, some pathogens can outwit the defenses of the immune system to establish a chronic infection. *Mycobacterium tuberculosis (M. tb)* is one such pathogen. Tuberculosis (TB) continues to be a serious threat to public health due to the increased number of cases, latent infection, emergence of drug resistant strains of *M. tb* and association with diseases like AIDS.

The only vaccine for TB, BCG, has been a complete failure in TB endemic areas as it does not prevent TB in adults. Interference of environmental mycobacteria in antigen processing and presentation and inadequate induction of T cell memory upon BCG vaccination are thought to be the main reasons for the failure of BCG (Singh et al., 2010, 2011). Taking into consideration clinical trials conducted in endemic areas and experiments with animal models, we hypothesized that a vaccine formulation that requires minimal antigen processing, formulated with strong adjuvants that elicit robust and enduring Th1 memory response, will eventually be successful.

Peptide vaccines do not require extensive processing and therefore may be an alterative successful vaccination approach. However, they are poorly immunogenic and cannot be effective in genetically out bred populations due to HLA polymorphism. To overcome these caveats, we came up with a novel strategy of covalently linking the promiscuous peptide (F91) from 16 kDa antigen of *M. tb* to a TLR-2 agonist Pam2Cys (L91). L91has a self-adjuvanting property and can work irrespective of HLA barrier. This construct was immunogenic, as it could target DCs and elicit an enduring T cell response. We could showcase its vaccine potential against TB in both mice and Guinea pig models (Gowthaman et al., 2011). This vaccine has following advantages over the current BCG or other vaccines in clinical trials: (i) it induces a robust long lasting Th1 memory and reduces induction of Tregs. This gives a favorable immune response that ultimately leads to protection; (ii) it requires minimum antigen processing

and could be directly presented by APCs to T cells; (iii) it is promiscuous and could work in various genetically disparate strains of mouse and out bred Guinea pigs; (iv) it could also elicit a Th1 response in PBMCs, from individuals of TB-endemic areas; (v) it does not elicit anti-peptide antibody response and preformed antibodies were not present in TB patients or PPD^+ individuals; (vi) it circumvents the risks associated with BCG and many other vaccines in trials, which have danger of eliciting autoimmunity, risk of genetic integration, conversion to virulence, and disease in immunocompromised individuals. Therefore we envisage that this lipopeptide would make an ideal global TB vaccine, especially for the endemic world. Besides, vaccination studies, another interesting aspect that we focused was on exploiting the strategies that can help in rescuing T cells from exhaustion. Intriguingly, we showed for the first time that signaling through TLR-2 utilizing Pam2Cys could prevent T cells from exhaustion. This observation also has tremendous clinical potential in the future.

Further, we also explored the adeptness to use *in silico* tools to identify promiscuous peptides. Our laboratory had identified a promiscuous peptide p91-110 from 16kDa antigen of *M. tb* through wet-lab experimentation. However, to cross-check our findings, we employed *in silico* tools that predict T cell epitopes. Surprisingly, none of the *in silico* tools employed could testify p91-110 as a promiscuous peptide. This inspired us to do a "bottom up" evaluation study. We utilized peptides that had been validated by wet-lab experimentation, to confirm whether they could predict correctly HLA binding peptides by *in silico* programs. We could gain insights into the potential caveats of *in silico* tools and also could suggest some improvements (Gowthaman and Agrewala, 2008,