Summary and Conclusions
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Of the many dogmas occurring as a result of infection by Mycobacterium tuberculosis (M. tb), the causative organism for most forms of TB; is the ability of the microbe to survive in a hostile environment. Over the years, researchers around the world have been working to decipher various mechanisms and counter mechanisms this pathogen has evolved towards subverting a complex network of immune responses mounted by the mammalian host. The reason for the exceptional nature of the organism can be due to the fact that M. tb is typically not environmental in nature and, therefore, has co-evolved with the human host. The battle, thus, continues for understanding the immune evasive mechanism of M. tb that permits it to create a niche for its survival and to establish lifelong infections. A better understanding of host-pathogen interactions is required to study the holistic view of the complete infection process.

M. tb secretes a number of antigens, many of which serves as the players in survival tactics and help the bacteria to evade or modulate immune responses. Working on these lines, our lab has been providing clues by investigating the interactions of these M. tb antigens with dendritic cells (DCs) and essentially, the outcome of these interactions in mediating immunity to M. tb. Till now, we have been working on CFP-10 that is expressed exclusively by M. tb complex, to understand the function and possible roles of secretory antigens in host pathogen interactions. It has been found that CFP-10 (and also other M. tb antigens) differentiates bone marrow precursors into dendritic cells (CFP10-DCs). However, these DCs, unlike conventional GM-CSF derived DCs (GM-CSF-DCs) induce suppressor responses to M. tb thereby, providing first clue to the role of these antigens in immune suppression/evasion. Subsequent investigations into possible mechanisms highlighted the intricate complementation of intracellular and inter-cellular
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processes that culminate in the observed suppressive phenotype and T cell priming functions of these antigen differentiated DCs.

Thus, expression of antigens such as CFP-10, as the infection progresses in time, could be a strategy used by M. tb towards immune evasion and have an important bearing on immune responses mounted against tuberculosis early during the infection. In the light of the available reports, we argued that M. tb would express many more antigens, like CFP-10 and the 19-kDa antigen, as a function of time inside macrophages that would result in the continued suppression of macrophage and DC activation, creating a niche for the long-term survival of M. tb. This hypothesis formed the basis of the work presented in this thesis.

In view of the above hypothesis, we enriched and identified the genes that were expressed by M. tb inside the infected macrophages at different times post-infection and henceforth, characterized the immune responses mediated by these genes. Results indicated that M. tb expresses different genes at different times post-infection. While, the genes expressed early following infection (at day 1 of infection - referred as day 1 antigens) ensured the survival of M. tb inside macrophages, genes expressed later during infection (at day 5 of infection - referred as day 5 antigens) created conditions to induce persistence/latency. Also, the expression kinetics of day 1 antigens and day 5 antigens were essentially similar both in vitro and in vivo indicating that M. tb regulates the secretion of different antigens at different times post infection both in vitro (inside macrophages) as well as in vivo (in mice).

The functional studies were then carried out by expressing these antigens inside DCs and macrophages to study their effects on immune responses mediated by these host cells following M. tb infection. Day 1 antigens were found to enhance M. tb-mediated activation of dendritic cells (DCs), whereas day 5 antigens abrogated DC activation. However, all genes downmodulated the expression of MHC class I and II molecules on infected macrophages, thereby, compromising their ability to interact with antigen-specific T cells. Day-1 and day-5 genes also downmodulated pro-inflammatory cytokine
production from DCs, thereby, impairing signal 3 during DC–T cell cognate interactions. These results indicated that antigens expressed by M. tb at different times post infection differentially modulated the activation of DCs and macrophages. Consequently, T cells activated by antigen-experienced DCs secreted low levels of IFN-γ and IL-17 but maintained high IL-10 secretion, thus inducing suppressor responses. These results clearly indicated that by modulating the activity of DCs, these antigens ensured that subsequently elicited pro-inflammatory T cell responses were also abrogated.

The next phase of study involved the investigation of mechanisms that were exploited by these antigens to modulate various DC and macrophage functions. The results revealed that both day-1 and day-5 genes increased TLR2-induced expression of suppressors of cytokine signaling 1 (SOCS1) from DCs, which translated to the subversion of IL-12 levels thereby, influencing the induction of suppressor responses. However, day-1 antigen was observed to be more potent than a day-5 antigen, in downmodulating IL-12 levels. In addition, day-1 and day-5 genes prevented the generation of reactive oxygen species in DCs that might lead to better survival of the pathogen in these cells. In parallel to DCs; although day-5 genes increased TLR2-mediated SOCS1 expression in macrophages; day-1 genes downmodulated the expression of inducible nitric oxide synthase 2 (iNOS2). Also, day-1 genes downmodulated the nitric oxide (NO) produced from macrophages while day-5 antigens had minimal effects. This indicated that, in macrophages, TLR2 responses would get downmodulated at later times post infection by increased SOCS1 expression, while early responses were modulated by downregulating iNOS2 levels by day 1 antigens. Together, these results showed that day-1 and day-5 antigens display complementary roles towards evasion of protective immune responses from macrophages.

In parallel to the above studies, we recombinantly expressed and purified two day-1 antigens and two day-5 antigens and carried out the similar functional studies with purified proteins. A similar pattern of immune response modulation was observed upon exogenous stimulation with day-1 or day-5 antigens as observed in case of intracellular expression of these proteins. Finally, day 1 and day 5 antigens differentially modulated
the survival of *M. tb* inside DCs and macrophages. While a day 1 antigen promoted the survival of *M. tb* inside DCs and macrophages, a day 5 antigen promoted *M. tb* survival only inside macrophages with minimal effects on DCs. These results further validated our hypothesis that antigens expressed by *M. tb* as a function of time, collectively work towards the suppression of protective immune responses leading to increased survival of the pathogen in infected cells.

These results indicated that *M. tb* genes, expressed inside infected macrophages as a function of time, collectively suppress protective immune responses by using multiple and complementary mechanisms. Collectively, this point towards a unique survival strategy employed by mycobacteria towards immune evasion and increased intracellular survival in DCs and macrophages.