Summary and Conclusions
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*Mycobacterium tuberculosis* (*M. tuberculosis*) is one of the most ancient human pathogens known to have co-evolved along with the human immune system to successfully persist within its host. *M. tuberculosis*, the etiological agent causing tuberculosis infects one third of the world’s population claiming 1.5 million lives annually. *M. tuberculosis* though capable of infecting almost any cell type, primarily infects and multiplies in macrophages and dendritic cells, the phagocytic antigen presenting cells of the immune system. In the normal course of infection, macrophages and the dendritic cells following contact with the pathogen, phagocytose mycobacteria and process and present antigens on class MHC molecules to T cells, thus initiating the adaptive immune response. During this process, activated macrophages and the dendritic cells also produce antimicrobial molecules such as nitric oxide, ROS and proinflammatory cytokines viz TNFα, IL-12, IL-6 and IL-1 etc. These molecules along with MHC class II presented antigens activate CD4+ T cells to produce IFNγ and drive Th1 helper responses. However, *M. tuberculosis* has evolved several mechanisms to evade this normal course of immune response. One such mechanism is to modulate host cell responses through production of secreted proteins/antigens an area that has been investigated by several researchers. In parallel, the modulation of host responses and their functions that contribute towards increased pathogenesis or protection has been less investigated.

Therefore, keeping the above in mind, in this study we investigated the role of host genes in regulating immune responses to *M. tuberculosis* and its antigens. To that end we used pathway specific siRNA libraries for the two key pathways- calcium calmodulin pathway and cysteine protease pathway- targeted by *M. tuberculosis* in dendritic cells. Our results indicate that a number of genes positively and negatively regulate mycobacterial survival and immune responses. A set of 13 genes was found that played a pivotal negative role such that a knockdown of the same resulted in the reduction of bacterial burden.
This was followed by detailed functional characterization of mechanisms that are employed by these genes to regulate the above responses. TLR2 and DC-SIGNR1 have been shown to play contrasting roles in mediating responses in DCs. For example, stimulation of TLR2 induces higher IL-12p40 production in DCs when compared with DC-SIGNR1 in the context of M. tuberculosis infection. These receptors were used in combination with two M. tuberculosis antigens Rv2463 (a day1 antigen) and Rv3416 (a day5 antigen) that are expressed as a function of infection and time. Characterization of these antigens revealed their role in suppression of TLR2 and M. tuberculosis mediated IL-12p40 production, M. tuberculosis mediated expression of surface T cell costimulatory and MHC molecules, surface expression of cytokine receptors, generation of Reactive Oxygen Species (ROS) and antigen specific T cell responses. Further, overexpression of these day1 and day5 antigens also resulted in increased survival of M. tuberculosis inside DCs as well as macrophages.

To begin with we investigated the ability of these genes to influence the production of IL-12p40 from DCs. Results indicated that knockdown of genes such as Usp25, Snp8 and Snrk significantly increased the levels of IL-12p40 expression following stimulation of TLR2 and DC-SIGNR1 in the presence of day1 and day5 antigens. In fact the increase in the levels was more significant with the day1 antigen when compared with day5 antigen, thus indicating a role of these genes in modulating early responses. This also indicated an antigen specific effect of these genes in mediating IL-12p40 production by TLR2. In contrast, no significant upregulation of IL-12p40 was observed following knockdown of Camkiia, Prkaa2 and Stk22a. These results indicate a differential role for these genes that is both antigen and receptor specific. Further, the fact that the increase was observed in the context of day1 antigen stimulation also indicates that these genes play a negative role in mediating early priming of Th1 responses.

We next investigated the ability of these genes to modulate the surface densities of key costimulatory molecules on DCs. Our results indicate that these genes differentially influenced the surface densities of key costimulatory molecules by showing an antigen
and receptor specific modulation of costimulatory molecules. Typically, stimulation of DC-SIGNR1 in the context of \textit{Rv3416} a day5 antigen was more effective in increasing the expression of most costimulatory molecules following knockdown of different genes when compared with similar stimulation of TLR2. This indicated that early priming of T cells by the day1 antigen (\textit{Rv2463}) was regulated by these genes via modulation of IL-12; the late or secondary priming of T cells by the day5 antigen (\textit{Rv3416}) was regulated at the level of costimulatory molecules by these genes. These results further indicate a role for these genes in influencing immune responses as a function of time and receptor triggering. These observations are consistent with the documentation that during \textit{M. tuberculosis} infections, TLR2 triggering occurs early on in the infection process and is followed by stimulation of DC-SIGN by soluble ManLAM secreted by infected macrophages as the infection proceeds with time.

Keeping the above results in mind, we next investigated the quality of T cell responses from ovalbumin stimulated DCs following knockdown of the genes. Since a potent Th1 response is a prerequisite and a marker for protective responses during \textit{M. tuberculosis} infections, we investigated if knockdown of the genes would skew the ovalbumin induced Th2 response to a Th1 response. Results revealed that ovalbumin induced a typical Th2 response with higher levels of IL-10 when compared with IFN-\(\gamma\). However, knockdown of some genes significantly skewed the Th2 response to a Th1 response by increasing the ratio of IFN-\(\gamma\) over IL-10. The results further indicated that these genes played a negative role in the priming of Th1 responses from infected or antigen stimulated DCs during \textit{M. tuberculosis} infections and inhibiting these genes could indeed potentiate Th1 responses.

In view of above results, we next investigated the role of these genes in cell defence mechanism as autophagy and ROS production in the context of \textit{M. tuberculosis} infection. Our results indicated that knockdown of these genes had a positive effect on autophagy, albeit at different levels, indicating a net negative role during mounting of autophagic responses during \textit{M. tuberculosis} infection. Similarly, knockdown of some of these genes
results into higher generation levels of ROS but in a differential activation status of these genes during a time dependent antigenic stimulation of different receptors. Since generation of ROS is often associated with modulations in the levels of the ROS quencher Super Oxide Dismutase 1 (SOD1), we monitored SOD1 levels in *M. tuberculosis* infected DCs following knockdown of the genes. Knockdown of many of genes resulted in downregulation of expression of SOD1 which is in agreement with the data on ROS.

Our collectively results indicate the identification of unique genes that play a significant role in modulating immune responses to *M. tuberculosis* from DCs by downregulating proinflammatory cytokine levels, Th1 responses, autophagy and oxidative burst during specific antigenic stimulation as well as whole bacterial infection. This point towards a unique strategy employed by *M. tuberculosis* targeting host genes leading to increased intracellular survival.