## SYNOPSIS

Mycobacteria are multifaceted pathogens capable of causing both acute disease as well as an asymptomatic latent infection. Host immune responses during mycobacterial infection involve potent cell effector functions including that of $CD4^+$, $CD8^+$ and γδT cells, macrophages and dendritic cells (DCs). Further, the critical regulators of protective immunity to mycobacterial infection include IFN-γ, IL-12, IL-23, TNF-α, lymphotoxins, CD40, nitric oxide and reactive oxygen species. However, the success of mycobacterial infection often relies in its ability to evade immune surveillance mechanisms mediated by sentinels of host immunity by modulating host signal transduction pathways and expression of immunoregulatory molecules. Therefore, the key to control mycobacterial growth and limit pathogenesis lies in the understanding the interactions between *Mycobacterium* and primary responders like macrophages and DCs. In this scenario, the role of pattern recognition receptors (PPRs) in orchestrating host immune responses assumes central importance.

The cell surface receptors play crucial role in influencing overall immune responses. Of the PRRs, the Toll-like receptors (TLRs) form key immune surveillance mechanisms in recognition as well as control of mycobacterial infection. Among them, TLR2 is the primary interacting receptor on antigen presenting cells that recognize the invading mycobacteria. Mycobacterial cell wall constituents such as LAM, LM, PIM and 19-kDa protein have been shown to activate TLR2 signaling leading to proinflammatory responses. Recent reports have suggested that PE_PGRS antigens of *M. tuberculosis* interact with TLR2. For example, RV0754, Rv0978c, RV1917c have been implicated in modulation of human DCs. The 19-kDa lipoprotein, LpqH (Rv3763) and LprG (Rv1411c) utilize TLR2 signaling to inhibit macrophage responsiveness to IFN-γ triggered MHC class II expression and mycobacterial antigen

presentation. Interestingly, recognition and amplification of pathogenic-specific signaling events play important roles in not only discriminating the invading microbes, but also in regulating explicit immune responses. In this context, integration of key signaling centers, which modulate host immunity to pathogenic mycobacterial infections, remains unexplored.

In accordance to above observations, signal transduction pathways downstream to TLRs play a critical role in modulation of battery of host cells genes in terms of expression and production of immune modulatory cytokines and chemokines, recruitment of cellular machineries to site of infections etc. This suggests the decisive role for TLRs in modulation of host cell fate decisions. However, during the ensuing immunity to invading pathogens, beside TLR signaling pathways, various other signaling molecules are thought to execute specific functions in divergent cellular contexts. Recent studies from our laboratory have clearly demarcated a novel cross talk of TLR2-NOTCH1 and TLR2-Wnt signaling pathways during mycobacterial infections. The current study primary focuses on the broad range of cross talk of TLR2 and Sonic hedgehog (SHH) signaling pathways and its functional significance.

The present investigation demonstrates that *M. bovis* BCG, a vaccine strain, triggers a robust activation of SHH signaling in macrophages compared to infection with diverse Gram-positive or Gram-negative microbes. This observation was further evidenced by the heightened SHH signaling signatures during *in vivo* scenario in cells /tissues from pulmonary tuberculosis (TB) individuals as well as tuberculous meningitis (TBM) patients. Furthermore, we show that the sustained TNF-α secretion by macrophages upon infection with *M. bovis* BCG is a critical necessity for SHH activation. Significantly, perturbation studies implicate a vital role for *M. bovis* BCG stimulated TLR2/PI3K/PKC/MAPK/NF-κB axis to induce TNF-α, that contributes to enhance SHH signaling. The TNF-α driven SHH signaling downregulates *M. bovis* BCG induced

TLR2 signaling events leading to modulation of battery of genes that regulate various functions of macrophages genes like *Vegf-a*, *Socs-3*, *Cox-2*, *Mmp-9* and M1/M2 genes. Importantly, utilizing whole-genome microRNA (miRNA) profiling, roles for specific miRNAs were identified as the molecular regulators that bring about the negative-feedback loop comprising TLR2-SHH signaling events. Thus, the current study illustrates how SHH signaling tightly regulates the kinetics and strengths of *M. bovis* BCG specific TLR2 responses, emphasizing a novel role for SHH signaling in host immune responses to mycobacterial infections.

As described, variety of host factors contributes for ensuing effective host defenses and modulation of host cell fate decisions. Interestingly, avirulent pathogenic mycobacteria, including the vaccine strain *M. bovis* BCG, unlike virulent *M. tuberculosis*, cause extensive apoptosis of infected macrophages, which suggests a significant contribution of the apoptosis process to the initiation and subsequent amplification of innate as well as adaptive immune responses. Among various cues that could lead to apoptosis of host cells, the initiation of the apoptotic machinery by posttranscriptional mechanisms assumes significant importance. Among posttranscriptional control mechanisms, miRNAs are suggested to regulate several biological processes including immune responses. Various effectors of host immunity are known to be regulated by several miRNAs, and a prominent one among them, miRNA-155 (miR-155), often exhibits crucial roles during innate or adaptive immune responses. In this perspective, we identified a novel role of miR-155 during *M. bovis* BCG induced apoptosis of macrophages. The genetic and signaling perturbations data suggested that miR-155 regulates PKA signaling by directly targeting a negative regulator of PKA, protein kinase inhibitor alpha (PKI-α). Enhanced activation of PKA signaling resulted in induced expression of the apoptotic genes as well as Caspase-3 cleavage and Cytochrome c translocation. Thus, augmented PKA signaling by *M.*

*bovis* BCG-driven miR-155 dictates cell fate decisions of infected macrophages, emphasizing a novel role for miR-155 in host immunity to mycobacterial infections.

In perspective of these studies, important directives are often comprised of sequential and coordinated activation of TLR and NLR-driven signal transduction pathways, thus exhibiting foremost influence in determining the overall strength of the innate immune responses. As described, TLR2 exhibits dominant role in sensing various agonists including pathogen-associated molecular patterns (PAMPs) of microbes at the cell surface and generally considered as major effectuator of proinflammatory responses. Interestingly, NLRs like NOD1 or NOD2 often act in contrary, thus regulating anti-inflammatory responses as well as polarization of T cells towards skewed Th2 phenotype. This presents an interesting conundrum to functionality of DCs or macrophages in terms of effector functions during rapidly evolving immunological processes including effects originating from immunosuppressive effectors such as CTLA-4 or TGF-β. DCs like macrophages are important sentinels of innate immunity, possesses array of PRRs that include TLRs and NOD-like receptors (NLRs). Signaling events associated with innate sensors like TLRs and NLRs often act as regulatory circuits that modulate the overall functions of DCs in terms of maturation process, cytokine or chemokine production, receptor expression, migration to secondary lymphoid organs for antigen presentation for effectuating Th polarization. TLR2, while acting as sensors for extracellular cues or endocytic network, drives signaling events in response to recognition of PAMPs including mycobacterial antigens like ESAT-6, PE_PGRS antigens, while NOD1 and NOD2 operate as cytosolic sensors initiating signaling pathways upon recognition of diaminopimelic acid (DAP) and muramyl dipeptide (MDP), components of bacterial peptidoglycan. Thus, TLRs or NOD receptors could trigger similar or contrasting immune responses by cooperative or non-cooperative sensing,

consequently exhibiting immense complexity during combinatorial triggering of host DCs-PRR repertoire. In view of these observations, our current investigation comprehensively demonstrated that maturation process of human DCs were cooperatively regulated by signaling cascades initiated by engagements of TLR2, NOD1 and NOD2 receptors. Importantly, combined triggering of TLR2 and NOD receptors abolished the TGF-β or CTLA-4-mediated impairment of human DCs maturation, which required critical participation of NOTCH1-PI3K signaling cohorts. Thus, our data delineated the novel insights in modulation of macrophages and DCs effector functions by mycobacterial TLR2 or NOD agonists and broaden our understanding on the signal dynamics and integration of multiple signals from PRRs during mycobacterial infections.

Altogether, our findings establish the understanding of conceptual frame work in fine tuning of TLR2 responses by SHH signaling as well as potential co-operativity among TLRs and NODs to modulate NOTCH1 dependent DCs maturation. Importantly, our study provides mechanistic and functional insights into various molecular regulators of macrophage cell fate decisions like miR-31. miR-150 and miR-155, which can fuel the search for attractive and effective drug targets and novel therapeutics to combat diseases of the hour like tuberculosis.