SYNOPSIS

The genus mycobacterium has more than 120 species of bacteria; one being *M. tuberculosis* (Mtb), the etiological agent of tuberculosis (TB). During infection, the host mounts a heightened immune response to contain the spread of the pathogen. Facilitating the response mediated by the host, PRRs (Pattern Recognition Receptors), upon their interaction with cognate ligands set forth a plethora of signaling cascades that effectuate responses like inflammation, apoptosis or autophagy to eradicate mycobacteria from the system. The early responses against Mtb are brought about by macrophages, dendritic cells (DCs) and neutrophils whereas the effector function of $CD4^+$ and $CD8^+$ T cells are critical for suppression of mycobacterial infection. Tumor necrosis factor (TNF)-alpha, Cytokines like interleukin (IL)-23, IL-12 and interferon (IFN)-gamma are secreted by type I T helper (Th1) subset of $CD4^+$ T cells that provides protective immunity against pathogenic mycobacteria. Inspite of these extreme measures taken by the host to contain the infection, afflicted individuals are unable to eliminate the pathogen. Over one-third of the world's population is infected with Mtb, which is a testimony of its success as a pathogen. This can be attributed to the various immune evasion strategies it employs like inhibition of phagosome-lysosome fusion, inhibition of antigen processing and presentation, suppression of pro-inflammatory cytokines, shifting the immune response towards Th2 type suppression of RNI and ROS, inhibition of apoptosis/autophagy and induction of regulatory T cells (T_{regs}) etc. Further, the emergence of MDR/XDR strains and co-infections has compounded the graveness of the disease. Understanding the mechanism underlying such immune evasion strategies will provide effective check on pathogenesis of mycobacteria.

In this regard, evaluation of the host-pathogen interactions during Mtb infection in terms of key the signaling pathway would provide us a critical insight into details of immune responses and its regulation. The present study addresses the role of WNT/ β-CATENIN pathway-dependent factors in modulating the host immune responses during Mtb infection. WNT/ β-CATENIN pathway-dependent G9a (histone methyltransferase) and Sirtuin6 (SIRT6; histone deacetylase) regulate cholesterol metabolism/homeostasis and mycobacterial survival (Chapter 3); AMPK- WNT/ β-CATENIN pathway-dependent COP1, an E3 ubiquitin ligase, skews the macrophage towards M2 phenotype hence aiding mycobacterial survival (Chapter 4) and finally the ability of mycobacterial surface antigen Ac2PIM in selectively suppressing NOD2- induced immunomodulators COX2, SOCS3 and MMP9 (Chapter 5).

Formation of highly structured aggregate of immune cells, called granulomas, is a hallmark of mycobacterial infection. One of the characteristic cells constituting the granulomas is the lipid-laden foamy macrophages (FM). These FMs serve as a source of nutrients as well as immune-modulators that aid in the survival of the pathogen. Amongst other lipids, cholesterol and cholesterol esters are significantly accumulated in the FMs. Further, it has been reported that cholesterol is one of the major carbon source for mycobacteria and helps in its persistence within the host. However, the molecular mechanisms that regulate intracellular cholesterol accumulation during the course of infection are not clear. Here, we analyzed the role of two histone modifiers G9a (histone methyltransferase; H3K9me1) and SIRT6 (histone deacetylase; H3K9Ac), which have been implicated in regulating immune responses. Mtb infection augments the levels of G9a and SIRT6 in macrophages as well as in the lungs of infected mice. Further, genes involved in cholesterol biosynthesis, uptake and efflux are differentially regulated upon mycobacterial

infection. G9a was found to regulate genes involved in uptake (*Lrp2*) and biosynthesis (*Aacs, Hmgcs1, Mvd, Dhcr24*) of cholesterol by recruiting at the respective promoters and bringing about H3K9me1leading to their enhanced expression. On the contrary, cholesterol efflux genes (*Abca1*, *Abcag1*) promoters were found to be enriched with SIRT6 with loss of H3K9Ac upon infection indicating their repression. Corroborating these observation mouse aerosol infection model of TB was utilized to ascertain the role of G9a and SIRT6 in mycobacterial survival wherein mice treated with G9a inhibitor or $Sirt6^{-/+}$ mice showed significant decrease in mycobacterial burden in organs such as spleen and lung. Immunofluorescence and transcript level analyses of the target genes in infected and uninfected lungs further substantiated our *in vitro* findings. Also, G9a and SIRT6 were found to be under the tight regulation of Mtb activated WNT/ β-CATENIN pathway. Thus our study unveiled the role of WNT/ β-CATENIN dependent G9a and SIRT6 in regulating cholesterol levels and consequently pathogen survival during mycobacterial infection.

The host induces the production of pro-inflammatory cytokines to counter any bacterial infection, including that by mycobacteria. However, mycobacteria skews the immune cells towards eliciting anti-inflammatory cytokine response, which prove more conducive for their survival. In this context, we investigated the role of AMPK, a crucial energy-sensing molecule that has been reported to play critical role in polarizing macrophage towards M2 phenotype and an E3 ubiquitin ligase COP1 in modulating immune responses during mycobacterial infection. AMPK is activated upon mycobacterial infection in macrophages through the action of upstream kinase PKCζ and LKB1. Activated AMPK cross-talk with WNT/ β-CATENIN signaling that leads to the expression of COP1, an E3 ubiquitin ligase. Chromatin immunoprecipitation analysis confirms the recruitment of β-CATENIN at the

promoter of COP1. Further, knockdown studies show that AMPK and COP1 are important for the expression of M2 cytokines/chemokines like IL-10, IL-4, CCL-4, CCL-17 and ARGINASE-1 that could aid the survival of mycobacteria in the host. Thus, our study highlights the activation of PKCζ-LKB1-AMPK-WNT/ β-CATENIN-COP1 pathway upon mycobacterial infection and postulates the essential role of the axis in modulating cytokine levels, thereby aiding in mycobacterial survival.

Specific and coordinated regulation of innate immune receptor-driven signaling networks often determines the net outcome of the immune responses. Here, we investigated the cross-regulation of toll-like receptor (TLR)2 and nucleotide-binding oligomerization domain (NOD)2 pathways mediated by Ac_2PIM, a tetra-acylated form of mycobacterial cell wall component and muramyl dipeptide (MDP), a peptidoglycan derivative respectively. While Ac_2PIM treatment of macrophages compromised their ability to induce NOD2-dependent immunomodulators like cyclooxygenase (COX)-2, suppressor of cytokine signaling (SOCS)-3 and matrix metalloproteinase (MMP)-9, no change in the NOD2-responsive NO, TNF-α, VEGF-A and IL-12 levels was observed. Further, genome-wide microRNA expression profiling identified Ac_2PIM-responsive miR-150 and miR-143 to target NOD2 signaling adaptors, RIP2 and TAK1 respectively. Interestingly, Ac2PIM was found to activate the SRC-FAK-PYK2-CREB cascade via TLR2 to recruit CBP/P300 at the promoters of miR-150 and miR-143 and epigenetically induce their expression. Loss-of-function studies utilizing specific miRNA inhibitors establish that Ac_2PIM, via the miRNAs, abrogate NOD2-induced PI3K-PKCδ-MAPK pathway to suppress β-CATENIN-mediated expression of COX-2, SOCS-3 and MMP-9. Our investigation has thus underscored the negative regulatory role of Ac_2PIM-TLR2 signaling on

NOD2 pathway, which could broaden our understanding of cross-regulation between two PRRs upon activation.

Altogether, we have established an implication of novel molecular players in the pathogenesis of TB. We have found that an intricately woven network of signaling pathways and epigenetic factors fine tune the execution of mycobacterial survival strategies such as accumulation of cholesterol and swerving of the inflammatory milieu. Further, we gained insights into the extensive implication of cross-talk between PRRs in coining the outcome of conditions where they are simultaneously stimulated. Such detailed investigations would confer a holistic perspective of host-pathogen interactions and would bear potential in effective disease control by aiding the search for efficacious therapeutics.