SYNOPSIS

The mammalian immune system consists of the innate and adaptive arm/s that protect the host against pathogenic infections in a highly co-ordinated process involving multiple steps. Innate immune cells such as macrophages and dendritic cells (DCs) are responsible for determining the initiation of specific events and thus they tailor specific immune responses to eliminate the invading pathogen. Host innate immune responses are triggered by sensing of PAMPs (pathogen associated molecular patterns) or DAMPs (damage associated molecular patterns) via pattern recognition receptors (PRRs) and these inturn facilitate adaptive immune responses. The four major families of PRRs including Toll- like receptors (TLRs), NOD-like receptors (NLRs), RIG-I like receptors (RLRs) and C-type lectin receptors (CLRs) recognize a wide range of PAMPs and DAMPs. Engagement of PRRs with these stimuli promotes the differential induction of PRRs-driven signaling cascades such as inflammation, apoptosis, and autophagy among others that result in organized actions of multiple immune cells to eradicate microbial infection. However, in spite of having such effective and efficient immune system, some of the pathogens are able to breach immune layers and establish a successful infection while escaping host key immune surveillance mechanisms. One such pathogen of rising concern, *Mycobacterium tuberculosis* (Mtb) causing tuberculosis (TB), has evolved with mankind and causes an alarming 1.4 million deaths annually, in accordance with recent WHO reports.

Mtb is an intracellular pathogen, whose primary target cells are macrophages. These are crucial effector immune cells that provide defense against a vast array of pathogens through the presentation of abundant cell surface receptors including TLRs that sensitize the host and execute the tailoring of immune responses during mycobacterial infection. In particular, TLR2 has been shown to elicit inflammatory responses including the increased expression of effector molecules such as tumor necrosis factor (TNF) α, *interferon gamma* (IFN-γ), xiinterleukins (ILs), chemokines and inflammatory cytokines in this process. Accumulating evidences indicate the role of phagosomal nicotinamide adenine dinucleotide phosphate (NADPH) oxidase (NOX)2 in producing oxidative stress in order to clear pathogen. Recent reports have suggested essential role of NOX2 in efficient killing of mycobacteria. For example, p47-phox (NOX2 subunit) null mouse showed increased susceptibility to mycobacteria infection. Interestingly, another recent study suggested the crucial role of host NAD(P)H quinone oxidoreductase 1 (NQO1) in promoting mycobacterial survival. Yet the contribution of NQO1 in regulating immune responses during pathogenic mycobacteria infection needs further investigation. Additionally, few reports indicate the role for hostderived reductases in regulating apoptosis. More importantly, several studies propose that mycobacteria can inhibit apoptosis and promote its survival. Therefore, dissecting the molecular mechanism in regulating host-derived reductases during mycobacterial infection will provide a further insight into comprehending the success of Mtb infection which depends on its ability to evade host immune responses by modulating host signaling and the related expression of immuno-regulatory molecules.

In line with the above observations, defined signaling downstream of TLRs plays a central role in generating effective immune responses at the site of infection. Several immunological modules act as important accessory events to TLR-triggered signaling. Among the many signaling pathways, the canonical WNT-β-catenin pathway has been recently shown to play a crucial role in controlling the expression of inflammatory molecules during infection. However, information on the roles of WNT signaling in mediating inflammatory responses remains scanty. In particular, a growing number of studies have indicated the potential involvement of epigenetic factors to determine the host cell inflammatory responses. Modifications by such factors including DNA methylation, histone modification and noncoding

RNAs are shown to be potential regulators of TLR mediated inflammatory responses. Histone methylation plays a crucial role in mediating TLR-triggered immune responses. For example, H3K27 demethylase Jumonji domain-containing 3 (JMJD3) was shown to determine the M2 macrophage development upon TLR stimulation during helminth infection. Furthermore, methyltransferases including SETDB2 and ASH1L, repress the expression of CXC-chemokine ligand 1 (CXCL1) and LPS-induced production of inflammatory cytokines respectively. However, it has not yet been determined, how pathogen modulates the recruitment of these epigenetic modifiers at specific promoters, thus orchestrating changes in complex phenomena such as inflammatory responses and apoptosis.

Based on these evidences, the former part of the present study is focused on the expression of WNT-responsive epigenetic effectors which mediate the inflammatory responses and apoptosis during mycobacterial infection in mice. The investigation demonstrates that Mtb H37Rv triggers a robust activation of WNT-β-catenin signaling via its adaptor molecule protein kinase C (PKC) ζ. Indeed, emerging studies suggest a potential contribution of miRNAs in modulating immune responses during pathogenic infection. In line with these observations, we show that the WNT-responsive *Mir-30e-3p* stabilizes H4K20me1 methyltransferase SET8 by modulating the expression of its negative regulator, CDT2 (E3 ubiquitin ligase) during mycobacterial infection. Notably, stabilised SET8 leads to increased monomethylation of H4K20 on the promotors of *Nqo1* and *Trxrn1,* which reflects in their significantly high RNA and protein levels. Despite being bonafide antioxidants, NQO1 is known to play a crucial role in regulating inflammation and TRXR1 is considered as an apoptosis controller via the regulation of ASK1 activity. Thus the depletion of NQO1 and TRXR1 by using specific siRNA reveals the important roles for NQO1 and TRXR1 in regulating inflammatory responses and apoptosis respectively during mycobacterial infection. Furthermore, experiments based on the use of dicoumarol (NQO1 inhibitor) and auranofin (TRXR1 inhibitor) in macrophages followed by pathogenic mycobacterial infection suggested that inflammatory responses and apoptosis are dependent on reductase activity of NQO1 and TRXR1. Most importantly, these functions of TRXR1 and NQO1 were explored in an experimental mouse TB model. Interestingly, mice administered with dicoumarol and auranofin showed a significantly reduced lung and spleen Mtb CFU. This was also corroborated by lung histopathology which alluded the reduced severity of TB through the analysis of granuloma, underscoring the importance of NQO1 and TRXR1 in TB pathogenesis. Collectively, we suggest the crucial role for WNT-responsive *Mir-30e-3p* - histone methyltransferase SET8 axis in regulating host-derived antioxidants NQO1 and TRXR1 in suppressing host immune responses. Thus, epigenetic reprogramming of the host cell by SET8 promotes Mtb survival in macrophages by regulating inflammation and apoptosis.

Additionally, another histone methyltransferase, ASH2L, has been established as an immune regulator via facilitating activatory H3K4me3 (trimethylation) on the promoters of target genes at CG-rich DNA motifs. The participation of H3K4me3 performed by ASH2L methyltransferase, is highlighted in regulation of immune-related genes. However contribution of AHS2L in regulating host immune responses during mycobacterial infection has been not addressed. Based on these evidences, we established that Mtb-induced ASH2L, coupled with β-catenin directly regulates the expression of 5-LO and 15-LO. As a functional consequence, WNT/β-catenin and its responsive 5-LO and 15-LO were found to regulate expression of *Gpr18* transcript which in turn regulates host inflammatory responses.

In addition to membrane bound TLRs, emerging studies suggest the effective role for cytosolic NLRs in sensing PAMPs or DAMPs with great efficiency. Among the NLRs, nucleotide-binding oligomerization domain-containing protein (NOD) 1 and NOD2 are well characterized cytosolic receptors in determining inflammatory responses. Although,

inflammation is a highly regulated fundamental defensive host mechanism, its hyper and chronic activation in response to different stimuli is associated with serious inflammatory disorders. Importantly, recent reports implicated the close association of NOD2 and WNT signaling pathways during the development of Crohn's disease. In continuation, evidence suggests that NOD2-driven inflammatory disorders are associated with impairment of inflammasome function. Activation of WNT signaling is also implicated in the development of arthritis. However, precise mechanism of NOD2-WNT-inflammasome crosstalk or the pivotal role of WNT signaling during inflammatory arthritis requires extensive investigation. In this context, the present investigation demonstrates that upon activation with muramyl dipeptide (MDP, NOD2 specific agonist), NOD2 interacts positive regulator of WNT signaling Ly6/PLAUR domain-containing protein 6 (LYPD6) to stimulate and mediate WNT signaling activation. Strikingly, canonical adaptor molecules of NOD2 signaling, RIP2 and TAK1 were proven to be dispensable in NOD2-triggered WNT signaling activation. Furthermore, we found that WNT-responsive X-linked inhibitor of apoptosis (XIAP) leads to activation of NOD like receptors family pyrin domain-containing 3 (NLRP3) inflammasome complex. Further, NOD2-stimulated formation of active caspase-1 and secretion of IL-1β were found to be dependent on WNT and its responsive XIAP. Consistent with this *in vitro* data, mice administered with WNT-signaling inhibitor, XIAP inhibitor or Caspase-1 inhibitor displayed compromised ability to develop MDP-triggered acute arthritis. Taken together, our study contributes new biological insights towards understanding of NOD2-associated inflammatory responses.

Altogether, our findings lay the groundworks for comprehending conceptual framework in orchestrating TLR and NLR responses by WNT/β-catenin signaling. More importantly, our study pays a tribute to novel mechanistic and functional insights into Mtb pathogenesis and inflammatory diseases, which promises to provide important leads in diagnostic and therapeutic approaches for immune-associated disorder